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PROCEEDINGS

Institute of Experimental Morphology, Pathology and Anthropology with Museum

OF THE SEVENTH WORKSHOP ON EXPERIMENTAL MODELS AND METHODS IN BIOMEDICAL RESEARCH

MAY 16-18, 2016

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PROCEEDINGS

OF THE SEVENTH WORKSHOP ON EXPERIMENTAL MODELS AND METHODS IN BIOMEDICAL RESEARCH

May 16 – 18, 2016

Institute of Experimental Morphology, Pathology and Anthropology with Museum at the Bulgarian Academy of Sciences

Edited by: Dimitar Kadiysky and Radostina Alexandrova

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THE SEVENTH WORKSHOP

"EXPERIMENTAL MODELS AND METHODS IN BIOMEDICAL RESEARCH"

IS ORGANIZED BY THE INSTITUTE OF EXPERIMENTAL MORPHOLOGY, PATHOLOGY AND ANTHROPOLOGY WITH MUSEUM (IEMPAM)

UNDER THE AUSPICES OF

THE BULGARIAN ACADEMY OF SCIENCES

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The responsibility for the content of published papers/abstracts belongs entirely to their authors

THE PROGRAM OF THE WORKSHOP

Monday, 16 May 2015

9.00-9.20 OPENING CEREMONY

Session A

Chairpersons:
Prof. Reni Kalfin, PhD
Institute of Neurobiology, Bulgarian Academy of Sciences

Assoc. Prof. Andrey Tchorbanov, PhD
Institute of Microbiology, Bulgarian Academy of Sciences

Secretary: Desislav Dinev, MSc
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

9.20– 10.05
AO1. EXPERIMENTAL GENETIC MODELS FOR ELUCIDATION THE KEY ROLE OF TESTICULAR SOMATIC CELLS FOR MALE REPRODUCTION AND FERTILITY
Nina Atanassova
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

10.05-10.20
AO2. SPERM CHROMATIN ORGANIZATION GOVERNS SPERM QUALITY
Stefanova V[1], Georgieva M[1], Staneva D[1], Todorov P[2] and Miloshev G[1*]
[1]Laboratory of Yeast Molecular Genetics, Institute of Molecular Biology "R. Tsanev", Bulgarian Academy of Sciences
[2]Department of Reproductive Biotechnologies and Cryobiology of Gametes, Institute of Immunology and Reproduction "Acad. K. Bratanov", Bulgarian Academy of Sciences,

10.20 – 10.35
AO3. CHROMATIN MODULATES CELLULAR RESISTANCE TO ULTRAVIOLET LIGHT
Bela Vasileva[1], Milena Georgieva[1], Desislava Staneva[1], Plamen Zagorchev[2] and George Miloshev[1*]
[1]Yeast Molecular Genetics Lab, Institute of Molecular Biology "Acad. R. Tsanev", BAS, Sofia, Bulgaria
[2]Faculty of Pharmacy, Department of Medical Physics, Biophysics and Mathematics, Medical University, Plovdiv, Bulgaria

10.35 – 10.50
AO4. TARGETED ELIMINATION OF Der p1-SPECIFIC B CELLS IN HUMANIZED SCID MOUSE MODEL OF HDM ALLERGY
Kiril Kolev[1], Nikola Kerekov[1], Antoaneta Michova[2], Maria Nikolova[2], Andrey Tchorbanov[1]
[1] Laboratory of Experimental Immunology, Institute of Microbiology, Bulgarian Academy of Sciences
[2] National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria

10.50 – 11.10 Coffee Break

11.10 – 11.25

AO5. SELECTIVE ALTERATION OF ASELF-REACTIVE B AND T CELLS BY CHIMAERIC MOLECULES IN A HUMANIZED MOUSE MODEL OF TYPE 1 DIABETES (T1D)

Gabriela Boneva, Iliyan Manoylov, Andrey Tchorbanov

Department of Immunology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

11.25 – 11.40

AO6. SUPPRESSION OF LUPUS SYMPTOMS BY ANTI-C1Q scFv ANTIBODY THERAPY IN MRL/lpr MURINE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

Violeta Kostadinova[1], Silviya Bradyanova[1], Ventsislav Tcholakov[2], Nadezhda Todorova[2], Ivanka Tsacheva[2], Andrey Tchorbanov[1]

[1]Laboratory of Experimental Immunology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

[2]Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of Biochemistry, Sofia, Bulgaria

11.40 – 12.10

***AO7.* NEW APPROACHES FOR IMMUNOLOGICAL TESTING OF PATIENTS WITH SYSTEMIC SCLEROSIS**

E. Krasimirova[1], D. Kalinova[2], Ts. Velikova[1], K. Tumangelova-Yuzeir[1], E. Ivanova-Todorova[1], V. Reshkova[2], A.Kopchev[2], R. Rashkov[2], D. Kyurkchiev[1]

[1]University Hospital St. Ivan Rilski, Medical University of Sofia, Sofia, Bulgaria

[2]Clinic of Rheumatology, St Ivan Rilski Hospital, Medical University of Sofia, Sofia, Bulgaria

12.10 – 12.40

Poster Viewing and Discussion

Session B

Chairpersons:

Assoc. Prof. Dobroslav Kyurkchiev, MD, PhD
Laboratory of Clinical Immunology, University hospital "St. Ivan Rilski"

Assoc. Prof. Radostina Alexandrova, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Boika Andonova-Lilova, MSc
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

13.30 – 14.00

BO1. CULTURING AND CHRACTERISTICS OF CELLS ISOLATED FROM GLIOBLASTOMA MULTIFORME

Dobroslav Kyurkchiev
Laboratory of clinical immunology, University hospital "St. Ivan Rilski", Sofia, Bulgaria

14.00 – 14.30

BO2. BLOOD-CSF-BARRIER CHANGES IN EXPERIMENTAL HAMSTER GRAFFI TUMOR MODEL

V. Ormandzhieva, R. Toshkova
Institute if Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

14.30 – 15.00

B03. THE CHALLENGES OF TUMOR ANGIOGENESIS

Radostina Alexandrova[1], Tanya Zhivkova[1], Lora Dyakova[2], Abedulkadir Abudalleh[1], Boyka Andonova-Lilova[1], Desislav Dinev[1], Milena Glavcheva[1], Osama Azmi[3]
[1]Institute if Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[3]National Research Centre, Medical Research Division, Giza, Egypt

15.00 – 15.20 Coffee Break

15.20 – 15.35

BO4. LUNG CANCER

Stephanie Dimitrova[1], Nadezhda Stoyanova[1], Emil Belinski[2], Ivoslav Ivanov[3]
[1]Faculty of Medicine, Medical University Sofia, Bulgaria
[2]Department of Vascular Surgery, Tokuda Hospital Sofia, Bulgaria
[3]Fourth General Surgery Department, UMHATEM "N.I. Pirogov", Sofia, Bulgaria

15.35 – 15.50

BO5. GLYCOSYLATION CHANGES IN ASCITE HEPATOMA CELL LINES

J. Stoyloff

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

15.50 – 16.05

BO6. AMYGDALYN (VITAMIN B17) AND TUMOR CELLS

Vasil Boyanov[1], Liliya Lazova[1], Kiril Lazov[2]

[1]Medical University of Sofia, [2] Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences

16.05 – 16.35

BO7. REVIEW OF BALKAN ENDEMIC NEPHROPATHY HYPOTHESES

A. Damianova , Z. Tsolova

Institute for Nuclear Research and Nuclear Energy, Bulgarian Academy of Sciences

16.35 – 17.00

Poster Viewing and Discussion

Tuesday, 17 May 2016

Session C

Chairpersons:
Prof. Ivo Grabchev, PhD
Faculty of Medicine, Sofia University "St. Kliment Ohridski"

Assoc. Prof. Radostina Alexandrova, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Abedulkadir Abudalleh, MSc
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

9.00 – 9.15

CO1. ANTINEOPLASTIC AND ANTIMETASTATIC POTENTIAL OF THE NOVEL ALKILPHOSPHOHOLINE ERUFOSINE IN GRAFFI MYELOID TUMOR CELLS. CYTOMORPHOLOGICAL STUDY

Ani Georgieva[1], Reneta Toshkova[1], Veselina Uzunova[2], Martin Berger[3], Rumiana Tsoneva[2]
[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria
[3]German Cancer Research Center, Heidelberg, Germany

9.15 – 9.45

CO2. SCHIFF BASES

Katya Popova[1,2], Milena Glavcheva[1], Lora Dyakova[3], Desislav Dinev[1], Tanya Zhivkova[1], , Boyka Andonova-Lilova[1], Abedulkadir Abudalleh[1], Gabriela Marinescu[4], Daniela-Cristina Culita[4], Luminita Patron[4], Radostina Alexandrova[1]
[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, [2]Faculty of Biology, Sofia University "St "Kliment Ohridski", [3]Institute of Neurobiology, Bulgarian Academy of Sciences, [4]Institute of Physical Chemistry "Ilie Murgulescu", Bucharest, Romania

9.45 – 10.00

CO3. DO Cu(II) AND Co(II) COMPLEXES WITH SCHIFF BASES AFFECT VIABILITY AND PROLIFERATION OF CULTURED CANCER CELLS?

Desislav Dinev[1], Tanya Zhivkova[1], Lora Dyakova[2], Katya Popova[1,3], Boyka Andonova-Lilova[1], Abedulkadir Abudalleh[1], Gabriela Marinescu[4], Daniela-Cristina Culita[4], Luminita Patron[4], Radostina Alexandrova[1]
[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, [2]Institute of Neurobiology, Bulgarian Academy of Sciences, [3]Faculty of Biology, Sofia University "St "Kliment Ohridski", [4]Institute of Physical Chemistry "Ilie Murgulescu", Bucharest, Romania

10.00 – 10.15

CO4. Zn(II) COMPLEXES WITH SCHIFF BASES: INFLUENCE ON VIABILITY AND PROLIFERATION OF CANCER CELLS

Milena Glavcheva[1], Lora Dyakova[2], Desislav Dinev[1], Tanya Zhivkova[1], Katya Popova[1,3], Boyka Andonova-Lilova[1], Abedulkadir Abudalleh[1], Gabriela Marinescu[4], Daniela-Cristina Culita[4], Luminita Patron[4], Radostina Alexandrova[1]

[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, [2]Institute of Neurobiology, Bulgarian Academy of Sciences, [3]Faculty of Biology, Sofia University "St "Kliment Ohridski", [4]Institute of Physical Chemistry "Ilie Murgulescu", Bucharest, Romania

10.15 – 10.30

CO5. ВЛИЯНИЕ НА ВОДЕН ЕКСТРАКТ ОТ *MELISSA OFFICINALIS L.* ВЪРХУ ВИТАЛНОСТТА НА МИШИ ЕМБРИОНАЛНИ ФИБРОБЛАСТИ

М. Дерменджиева[1], Е. Стоянова[2], Т. Иванова[3], А. Александрова[4], М. Червенков[2]

[1]Биологически факултет, СУ „Св. Климент Охридски

[2]Институт по Биология и Имунология на Размножаването, БАН

[3]Институт по Биоразнообразие и Екосистемни Изследвания, БАН

[4]Институт по Невробиология, БАН

10.30-10.45

CO6. CHRONIC EXPOSURE TO COBALT(II) COMPOUNDS AND MURINE SPLEEN

Y. Gluhcheva[1], Ju. Ivanova[2]

[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

[2]Faculty of Medicine, Sofia University "St. Kl. Ohridski", Sofia, Bulgaria

10.35 - 11.00 Discussion

11.00 – 11.20 Coffee Break

Session D

Chairpersons:
Assoc. Prof. Evelina Shikova-Lekova, MD, PhD
National Centre of Infectious and Parasitic Diseases, Sofia

Assoc. Prof. Radostina Alexandrova, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Tanya Zhivkova, MSc
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

11.20 – 11.50
DO1. ANTIMICROBIAL ACTIVITY OF FLUORESCENT BENZANTHRONE DERIVATIVES
D. Staneva[1], S. Grabchev[2], E. Vasileva-Tonkova[3], P. Bosch[4], I. Grabchev[5]
[1]University of Chemical Technology and Metallurgy, Sofia, Bulgaria
[2]Sofia University "St. Kliment Ohridski", Faculty of Chemistry and Pharmacy, Sofia, Bulgaria
[3] Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[4]Institute of Science and Technology of Polymers, CSIC, Madrid, Spain
[5]Sofia University "St. Kliment Ohridski", Faculty of Medicine, Sofia, Bulgaria

11.50 – 12.05
DO2. INDUCED BY GLUCOSE FORMATION OF BIOFIMS BY *Bacillus subtilis* AND *Escherichia coli* STRAINS
Иво Тодоров Ганчев
Институт по микробиология „Стефан Ангелов", Българска академия на науките

12.05-12.20
DO3. INTERSPECIES RELATIONSHIPS IN THE STRUCTURE OF BIOFILMS
Иво Тодоров Ганчев
Институт по микробиология „Стефан Ангелов", Българска академия на науките

12.20-12.35
DO4. DETECTION OF BIOACTIVE PEPTIDES WITH IMMUNOMODULATORY PROPERTIES IN BULGARIAN CHEESE, RELEASED BY LAB DURING RIPENING
I.Gotova[1,2], Zh.Dimitrov[1], Hr.Naidenski[2]
[1]„LB Bulgaricum" Plc, 14 Malashevska Str.,Sofia, Bulgaria
[2]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

12.35 – 13.30 Lunch time

13.30 – 13.45

DO5. USAGE OF ELECTRON MICROSCOPY IMAGING FOR HHV-6 INFECTION DIAGNOSIS IN PATIENTS WITH AUTOIMMUNE THYROIDITIS IN HELP OF STANDARD CLINICAL PROCEDURES

Katerina Todorova[1], Alina Sultanova[2], Maksims Cistjakovs[2], Egils Cunskis[2], Boycho Nikolov[1], Rositsa Milcheva[1], Russy Russev[1], Modra Murovska[2]

[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

[2]Rīga Stradiņš University, Rīga, Latvia

13.45 – 14.15

DO6. НАШЕСТВИЕТО НА КОРОНАВИРУСИТЕ: CoV-SARS

Радостина Александрова

Институт по експериментална морфология, патология и антропология с музей – БАН

14.15 – 14.45

DO7. НАШЕСТВИЕТО НА КОРОНАВИРУСИТЕ: CoV-MERS

Радостина Александрова

Институт по експериментална морфология, патология и антропология с музей – БАН

14.45-15.05 Coffee Break

15.05 – 15.20

DO8. ALGORITHM FOR INVESTIGATION OF PREGNANT WOMEN EXPOSED TO THE VIRUSES THAT CAUSE NON-VESICULAR (MACULOPAPULAR) RASH

Stefka Ivanova[1], Silvia Voleva[2], Borislav Marinov[3], Stoian Shishkov[4]

[1]National Centre of Infectious and Parasitic Diseases, Department of Virology, Sofia, Bulgaria

[2]IInd UMHAT Sofia, Clinical Laboratory, Sofia, Bulgaria

[3]University Obstetrics and Gynecology Hospital "Maichin Dom", Sofia, Bulgaria

[4]Sofia University "St. Kliment Ohridski", Faculty of Biology, Sofia, Bulgaria

15.20 – 15.35

DO9. A POSSIBLE ASSOCIATION BETWEEN HPV16/18 AND LUNG CANCER?

Z. Ivanova[1], D. Metodiev[2], D. Alexandrova[3], M. Shindov[4], E. Shikova[1]

[1]National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria;

[2]5th MBAL – Sofia, Bulgaria;

[3]The National Specialized Hospital for Active Treatment in Haematological Diseases, Sofia, Bulgaria;

[4]Specialized Hospital for Active Treatment in Oncology, Sofia, Bulgaria

15.35 – 15.50

DO10. HIV-1 GENETIC DIVERSITY AMONG HETEROSEXUALS IN BULGARIA

Reneta Dimitrova[1], Asya Kostadinova[1], Anna Gancheva[1], Ivaylo Elenkov[2], Mariyana Stoycheva[3], Daniela Nikolova[4] and Ivailo Alexiev[1]

[1]National Reference Laboratory of HIV, National Center of Infectious and Parasitic Diseases, *Sofia, Bulgaria*.

[2]Hospital for Infectious and Parasitic Diseases, Sofia, *Bulgaria.*

[3]Medical University, Plovdiv, Bulgaria.

[4]Medical University, Clinic of Infectious Diseases, Varna, Bulgaria

15.50 – 16.05 Discussion

Session E

Chairpersons:
Prof. Margarita Gabrashanska, DVM, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Assoc. Prof. Radostina Alexandrova, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Lora Dyakova, MSc
Institute of Neurobiology Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

16.05 – 16.35
EO1. NEUROMARKETING. BUY-OLOGY IS A MASTERPIECE
Vera Kolyovska[1], Jane Maslarova[2], Dimitar Maslarov[3]
[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]University of Greenwich, London, UK
[3]Medical University of Sofia, Neurology Clinic, First MHAT-Sofia

16.35 – 17.05
EO2. МЕХАНИЗЪМ НА ВЪЗДЕЙСТВИЕ НА АКУПУНКТУРНИТЕ ИГЛИ ПРИ БОЛКА
Пол Джуров
Медицински университет София, катедра Фармакология

Wednesday, 18 May 2016

9.00-9.15
EO3. OPTIMIZED PCR METHODS FOR THE DETECTION OF INFECTIOUS AGENTS CAUSING FEVER AND RASH SYNDROME
Adelina Pavlova[1], Stefka Ivanova[2], Petia Genova-Kalou[2]
[1]Sofia University "St. Kliment Ohridski", Faculty of Biology, Sofia, Bulgaria;
[2]National Centre of Infectious and Parasitic Diseases, Department of Virology, Sofia, Bulgaria

9.15-9.30
EO4. ВИРУС ЗИКА: МОЛЕКУЛЯРНОБИОЛОГИЧНИ ХАРАКТЕРИСТИКИ, КЛИНИЧНА КАРТИНА, ДИАГНОСТИКА И ЛЕЧЕНИЕ
Георги Тошев, Хюлия Наил
Медицински факултет, СУ „Св. Кл. Охридски", София, България

9.30-10.00

EO5, СРАВНИТЕЛЕН АНАЛИЗ МЕЖДУ ТЕРЕННА И ЛАБОРАТОРНА ДИАГНОСТИКА НА НОЗЕМАТОЗАТА И ВАРОАТОЗАТА ПО ПЧЕЛИТЕ

Д. Салкова[1], К. Гургулова[2], С. Такова[2], М. Панайотова-Пенчева[1]

[1]Институт по експериментална морфология, патология и антропология с музей –БАН, [2]Национален диагностичен научноизследователски ветеринарномедицински институт „проф.д-р Г. Павлов“, София

10.00-10.30

EO6. ANTIPARASITE REMEDIES APPLIED IN MOUFLONS (*OVIS MUSIMON*) AND OTHER WILD SHEEP

Mariana Panayotova-Pencheva, Vasilena Dakova, Delka Salkova

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciencies

10.30-10.45

EO7. ANTIOXIDANTS IN THE LIVER OF *FASCIOLA HEPATICA* INFECTED RABBITS AFTER MIXED BASIC SALTS APPLICATION

M. Gabrashanska, N. Tsocheva-Gaytandzhieva, V. Nanev, I. Vladov

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciencies, Sofia, Bulgari,

10.45-11.00 Poster viewing and Discussion

11.00-11.20 Coffee-Break

Session F

Chairpersons:

Prof. Anna Tolekova. MD, PhD
Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

Assoc. Prof. Julia Radenkova-Saeva, MD, PhD
Toxicology Clinic, UMHATEM "N. I. Pirogov"

Assoc. Prof. Radostina Alexandrova, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Milena Glavcheva, MSc
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

11.20 – 11.50
FO1. CLINICAL CASE OF TOXIC EPIDERMAL NECROLYSIS
J. Radenkova-Saeva
Toxicology Clinic, UMHATEM "N.I.Pirogov", Sofia, Bulgaria

11.50-12.05
FO2. STUDY ON ACUTE POISONING WITH ANTIHYPERTENSIVE AND ANTIARRHYTHMIC MEDICINES
R. Stoyanova, J.Radenkova-Saeva
Toxicology Clinic, UMHATEM "N.I.Pirogov", Sofia, Bulgaria

12.05-12.35
FO3. ATOMIC ABSORPTION SPECTROMETRY – METHOD FOR ANALYSIS OF CD-INDUCED TOXICITY
K. Kamenova[1], Y. Gluhcheva[2], S. Arpadjan[1], Ju. Ivanova[3]
[1]Faculty of Chemistry and Pharmacy, Sofia University "St. Kl. Ohridski", Sofia, Bulgaria
[2]Institute of Experimental Morphology, Pathology and Anthropology with Museum, BAS, Sofia, Bulgaria
[3]Faculty of Medicine, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

12.35 – 13.30 Lunch Time

13.30 – 14.00
FO4. HAZARD AND IMMEDIATE MEASURES AGAINS THE PRODUCTION AND APPLICATION OF THE HERBICIDE GLYPHOSATE (ROUNDUP)
Yulia Karaivanova, *Institute of Biology and Immunology of Reproduction "Acad. K. Bratanov", Bulgarian Academy of Sciences*

14.00 – 14.15

FO5. VITAMIN D AS A PREVENTION FOR DIABETES

Daniel Addai, Jacqueline Zarkos, Petya Hristova, Ventsislava Dimitrova
Department of Physiology, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

14.15-14.30

FO6. GHRELIN AND ITS IMPLICATION ON DIABETES

Angel Todev, Niamh O'Donoghue, Aliza Fatima, Mohammed Channa
Department of Physiology, Faculty of Medicine, Trakia University, , Stara Zagora, Bulgaria

14.30-14.45

FO7. EFFECT OF MELATONIN ON TYPE 2 DIABETES

Abdurahman Moalin, Yaseen Hussain, Arsalan Bangash
Department of Physiology, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

14.45 – 15.00

FO8. TREATMENT OF DIABETES USING MORINGA OLEIFERA (LEAF)

Mustafa Mohammed
Department of Physiology, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

15.00 – 15.20 Coffee Break

15.20-15.50

FO9. MAXIMAL AEROBIC TEST AS A TOOL FOR ASSESSMENT OF FUNCTIONAL CAPACITY OF ATHLETES

Almira Georgieva[1], Elina Tzvetanova[1*], Lubomir Petrov[2], Rasho Makaveev[3], Albena Alexandrova[1,2]
[1] Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Department of Physiology and Biochemistry, National Sports Academy, Sofia, Bulgaria
[3]Department of Wrestling and Judo, Coaches faculty, National Sports

15.50 – 16.20

FO10. HOME DIAGNOSTIC AND SCREENING BY MEANS OF SMARTPHONES AND MEDICAL DEVICES - CLINICAL APPLICATIONS

Vera Kolyovska[1], Ivan Georgiev[2], Jane Maslarova [3], Dimitar. Maslarov[4]
[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. G. Bontchev Str., Bl.25, 1113 Sofia, e-mail: verakol@abv.bg
[2] NPHSPTO "M.V. Lomonosov" , XI z, Sofia
[3]University of Greenwich, London, UK
[4]Medical University of Sofia, Neurology Clinic, First MHAT-Sofia

16.20 – 16.50

FO10. ИНДИВИДУАЛИЗИРАНАТА ТАРГЕТНА ТЕРАПИЯ – НОВИ ИЗИСКВАНИЯ КЪМ ТЪКАННАТА ОБРАБОТКА НА БИОПСИЧНИТЕ МАТЕРИАЛИ

Здравка Петрова
Military Medical Academy; Faculty of Biology, Sofia University "St. Kl. Ohridski"; Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

16.50– 17.20 General Discussion and Final Remarks

POSTERS

BP1. GLIOBLASTOMA CELL CULTURES

Radostina Alexandrova[1], Abedulkadir Abudalleh[1], Orlin Alexandrov[2], Tanya Zhivkova[1], Lora Dyakova[3], Boyka Andonova-Lilova[1], Vladimir Kulchitsky[4].
[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria; [2]Health Service, Gorna Malina, Bulgaria; [3]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria; [4]Institute of Physiology, National Academy of Sciences of Belarus, Minsk, Belarus

CP1. THE METHOD OF COMPATIBILITY ASSESSMENT OF MESENCHYMAL STEM CELLS WITH TITANIUM IMPLANTS WITH DIAMOND-LIKE OR HYDROXYAPATITE COATINGS

Svetlana Alexandrova[1], Vladimir Kazbanov[2, 3], Murad Batalov[2], Nikolai Chekan[4], Tatiana Vinogradova[2], Olga Manicheva[2], Arcady Vishnevsky[2]

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[4]Physical Technical Institute, National Academy of Sciences, Minsk, Belarus
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CP2. THE METHOD OF NEUROTROPHIC EFFECT MODULATION AND ANTI-INFLAMMATORY EFFECT ACTIVATION IN BONE TISSUE AFTER IMPLANTATION OF DEVICES WITH DIAMOND-LIKE COATINGS

Vladimir Kazbanov[1, 2], Arcady Vishnevsky[1], Murad Batalov[1], Nikolai Chekan[3], Irina Kuznetsova[2], Tatiana Vinogradova[1], Marina Derevyanko[2], Olga Manicheva[1], Vladimir Kulchitsky[2]

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[2]Institute of Physiology, National Academy of Sciences, Minsk, Belarus
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EP1. EFFECTS OF NEMATODE INFECTIONS ON TUMORS IN ANIMALS AND ON TUMOR CELL CULTURES

N. Tsocheva-Gaytandzhieva
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

EP2. THE ROLE OF THE PARASITE VARROA DESTRUCTOR AS VECTOR OF VIRUSES ON HONEY BEE APIS MELLIFERA

Delka Salkova Salkova
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

FP1. ВИТАМИН Е – СТИМУЛАТОР И ИНХИБИТОР

Десислава Абаджиева
Институт по биология и имунология на размножаването „Акад. К. Братанов“, БАН, София, бул. „Цариградско шосе“ 73

FP2. TARGETED MIGRATION OF STEM CELLS IN THE MODEL OF BRAIN TRAUMA

Yulya Stukach[1], Stanislav Koulchitsky[2], Yuri Shanko[3], Svetlana Pashkevich[1], Vladimir Kulchitsky[1]
[1]Institute of Physiology, National Academy of Sciences, Minsk, Belarus
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[3]Republican Scientific and Practical Centre for Neurology and Neurosurgery, Minsk, Belarus
E-mail: vladi@fizio.bas-net.by

FP3. THE MODEL OF ENDOTOXEMIA IN RATS AFTER SUBDIAPHRAGMATIC VAGOTOMY

Stanislav Koulchitsky[1,2], Nina Netukova[1], Leos Navratil[3], Yana Pesotskaya[1], Pavel Kuna[3], Svetlana Pashkevich[1]
[1]Institute of Physiology, National Academy of Sciences, Minsk, Belarus
[3]GIGA-Neurosciences, University of Liege, Liege, Belgium
[2]University of South Bohemia, Ceske Budejovice, Czech Republic
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FP4. ON THE MANAGEMENT OF NEUROTRANSMITTER IMBALANCE AFTER THE USE OF NEUROTRANSMITTER LIGANDS OR DIELECTRICS IN HYPOXIA

Stanislav Koulchitsky[1], Svetlana Pashkevich[2], Nikolay Grinchik[3], Vladimir Kulchitsky[2]

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[2]Laboratory of Neurophysiology, Institute of Physiology, National Academy of Sciences, Minsk, Republic of Belarus
[3]A.V. Luikov Heat and Mass Transfer Institute of the National Academy of Science, Minsk, Republic of Belarus

FP5. IN THE WORLD OF BONES, CELLS AND MOLECULES

Radostina Alexandrova[1], Abedulkadir Abudalleh[1], Orlin Alexandrov[2], Tanya Zhivkova[1], Lora Dyakova[3], Boyka Andonova-Lilova[1], Virginija Jankauskaitė[4]

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FP6. НАКРАТКО ЗА СРЕБРОТО И НЕГОВАТА БИОЛОГИЧНА АКТИВНОСТ

Радостина Александрова[1], Таня Живкова[1], Абдулкадир Абудалех[1], Лора Дякова[2], Бойка Андонова-Лилова[1], Орлин Александров[3]
[1]Институт по експериментална морфология, патология и антропология с музей – БАН; [2]Институт по невробиология – БАН; [3]Здравна служба, Горна Малина

Session A

Chairpersons:
Prof. Reni Kalfin, PhD
Institute of Neurobiology, Bulgarian Academy of Sciences

Assoc. Prof. Andrey Tchorbanov, PhD
Institute of Microbiology, Bulgarian Academy of Sciences

Secretary: Desislav Dinev, MSc
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

AO1. EXPERIMENTAL GENETIC MODELS FOR ELUCIDATION THE KEY ROLE OF TESTICULAR SOMATIC CELLS FOR MALE REPRODUCTION AND FERTILITY

Nina Atanassova

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

AO2. SPERM CHROMATIN ORGANIZATION GOVERNS SPERM QUALITY

Stefanova V.[1], Georgieva M.[1], Staneva D.[1], Todorov P.[2] and Miloshev G.[1*]

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Infertility is one of the major problems of family planning in recent years. The causes of infertility can be identified as such caused by female or male factor. And though generally the problems related to the female reproductive system are far well-known than the male, according to recent studies in 20% of the infertility cases the dominant factor tends to be the male one. Therefore, it is of high priority the factors that lead to male infertility to be diagnosed promptly in order to be correctly and quickly treated. Evidence is accumulating suggesting a strong link between sperm DNA integrity and fertility. Spermatogenesis is a very complicated process. It lasts about 75 days and has to be finished properly to obtain a healthy and fertile sperm. The process itself is distinct from somatic cell differentiation. During it the majority of histone proteins are replaced sequentially, first by transition proteins, and then by protamines, facilitating chromatin hyper-compaction.
Here, we present the application of an innovative approach for assessment of sperm chromatin organization and its impact on male fertility. We have used a powerful technique for sperm higher-order chromatin structure organization studies, namely the method of Chromatin Comet Assay (ChCA) combined with the advantages of the Fluorescent Activated Cell Sorting (FACS). The application of both methods allowed a complex and exact evaluation of sperm chromatin compaction and sperm DNA

damage. Our results unambiguously show that chromatin compaction of sperm nucleus together with DNA damage index are parameters with utmost importance for the quality of sperm, and thus with a major impact on male fertility.

AO3. CHROMATIN MODULATES CELLULAR RESISTANCE TO ULTRAVIOLET LIGHT

Bela Vasileva[1], Milena Georgieva[1], Desislava Staneva[1], Plamen Zagorchev[2] and George Miloshev[1]*

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Chromatin-remodelling complexes (CRC) restructure chromatin in order to open/close it and thus finely regulate access to the densely packed DNA. CRCs play important roles in different cellular processes like transcription, chromosome segregation and DNA repair. Homologues of the human chromatin-remodelling complexes are found in yeast *Saccharomyces cerevisiae,* which is a perfect model organism for genetic and epigenetic studies. Actin-related proteins (Arps) serve as integral components of the chromatin-remodelling complexes providing scaffold but also managing CRCs functions. Recently, it was shown that the actin-related protein 4 (Arp4p) interacts with the builders of the higher-order chromatin structures – the linker histones.

This study was conducted using four different yeast strains - wild type, and three chromatin mutants. Our aim was to examine the role of the chromatin-remodelling complexes, and in particular Arp4p, in regard to the cellular response triggered by UV irradiation during cellular ageing. The results of our experiments unambiguously show that the preservation of genome stability during the process of cellular ageing, and the cellular response to stress caused by UV irradiation, are strictly regulated by the dynamics of the chromatin structure.

AO4. TARGETED ELIMINATION OF Der p1-SPECIFIC B CELLS IN HUMANIZED SCID MOUSE MODEL OF HDM ALLERGY

Kiril Kolev[1], Nikola Kerekov[1], Antoaneta Michova[2], Maria Nikolova[2], Andrey Tchorbanov[1]

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Objectives: Der p1 is allergenic molecule of Dermatophagoides pteronyssinus (Dpt) which causes house dust allergy. The pathological Der p1-specific B cells produce allergen-specific IgE antibodies that mediate most of the hypersensitivity allergic reactions.

Aim: It may be possible to influence Der p1-specific B cells by administrating to them a chimeric molecule, containing a monoclonal antibody against the inhibitory B-cell receptor CR1 coupled to a B

and a T cell epitopes from the Der p1 allergen. Co-crosslinking of the immunoglobulin receptors and CR1 by this molecule is expected to deliver suppressive signal selectively silencing these B cells only.

Methods: A synthetic peptide, Der p1 p52-71, and anti-CD35 monoclonal antibody 3D9 were used for the construction of Der p1 chimera. We analysed the effects of the chimeric molecule *in vitro* and *in vivo* using PBMCs from allergy patients. We measured Der p1-specific IgE and IgG antibody production by ELISA and determined the B-cell proliferation by ELISpot. We studied the effect of the constructed chimeric molecules on apoptosis by flow cytometry using AnnexinV-FITC/PI staining. We traced *Tyr*-phosphorylation of B-cell signaling molecules by chemiluminescent method.
We generated humanized SCID murine model of HDM allergy. Isolated PBMCs obtained from untreated allergic patients, sensitive to Dpt, or from healthy donors were transferred to female SCID mice. The successfully engrafted SCID mice were treated either with Allergen-peptide chimera or PBS, to assess *in vivo* the Dpt-specific B cell suppression. Eosinophil lung infiltration differences between chimera treated and untreated animals were examined by haematoxylin/eosin staining technique.
Results: We observed significant inhibition of allergen-specific cell proliferation and reduction of specific IgE antibody-production cells. Expression of phosphatidylserine on the outer layer of the cell membrane was changed in CD19+ and CD3+ cells from patients. We found that binding of the chimeric molecule to tonsillar B cells triggers the tyrosine phosphorylation of a protein of 30-32 kDa, which is most probably involved in the inhibitory process.
We demonstrate that administering the chimeric molecule to immunodeficient SCID mice transferred with PBMC derived from allergic patients results in reduction of allergen-specific IgE antibodies in the sera of the humanized SCID mice, and the lack of eosinophilic infiltration into the lung of the animals.

AO5. SELECTIVE ALTERATION OF ASELF-REACTIVE B AND T CELLS BY CHIMAERIC MOLECULES IN A HUMANIZED MOUSE MODEL OF TYPE 1 DIABETES (T1D)

Gabriela Boneva, Iliyan Manoylov, Andrey Tchorbanov

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Type 1 diabetes (T1D) is an autoimmune organ-specific disease, in which autoreactive immune cells target different autoantigens related to the blood sugar control. As a result hyperglycemia and destruction of pancreatic β cells are observed. Self-reactive B lymphocytes play an important role in the pathogenesis of T1D - they produce autoantibodies against several autoantigens. B cells act as antigen

presenting cells and activators of the T cells and can modulate the immune response via cytokine production. Therefore, B cells can be considered as a target for the potential treatment of T1D.

One of the main autoantigens in T1D is GAD65 (glutamic acid decarboxylase) - an enzyme, found in the pancreatic β cells that catalyzes the formation of γ-aminobutyric acid. CR1 (complement receptor 1) is a negative regulator on the surface of many cells in the blood. It is well known, that it can cause alteration of the cellular proliferation of several cell types, including B cells.

Based on this, we constructed chimaeric molecules, that consist of an antibody against CR1, conjugated to GAD65 B/T epitopes. Hence, we induce a selective suppression of disease-associated B cells by cross-linking their BCR and the negative receptor on their surface.

The results show that the chimaeric molecules predominantly target autoreactive B cells obtained from patients with T1D. We observed that the chimaeric molecules trigger a negative signal cascade and increase the percentage of apoptotic disease-associated, B lymphocytes.

AO6. SUPPRESSION OF LUPUS SYMPTOMS BY ANTI-C1Q scFv ANTIBODY THERAPY IN MRL/lpr MURINE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

Violeta Kostadinova[1], Silviya Bradyanova[1], Ventsislav Tcholakov[2], Nadezhda Todorova[2], Ivanka Tsacheva[2], Andrey Tchorbanov[1]

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Systemic Lupus Erythematosus (SLE) is a heterogeneous autoimmune syndrome characterized by chronic inflammation, B- and T-cell hyperactivity and generation of autoantibodies against self-nuclear antigens. One possible way of C1q contribution to onset of autoimmune disorder like SLE could be by the impairment of C1q mediated apoptotic clearance as part of human homeostasis. The capacity of C1q to bind early apoptotic cells could be decreased or even lost in the presence of anti-C1q antibodies which are specific for epitopes within gC1q.

An important tool for understanding human autoimmune diseases and specifically SLE is the use of different mouse models such as (NZBxNZW)F_1 and MRL/lpr mice. MRL/MpJ-Tnfrsf6^{lpr}/J (MRL/lpr) mice develop autoimmune symptoms closely resembling human SLE with multiple organ involvement. These include glomerulonephritis, vasculitis, splenomegaly, hypergammaglobulinemia, and the production of anti-dsDNA antibodies and autoantibodies to other nuclear components.

Material and Methods: A phage-displayed library expressing single-chain recombinant antibodies was screened to select scFv specific for anti-C1q autoantibodies from different groups of lupus sera. Selection of high affinity anti-idiotipic scFv was carried out using immunosorbent techniques. The selected clones were expressed in their soluble form and purified by affinity chromatography on Ni^{2+}matrix.

Seven-week old female lupus-prone MRL/*lpr* mice raised in controlled environment were used for *in vivo* experiments. At this age the animals show no clinical signs of autoimmunity, including autoantibodies and proteinuria. The MRL/lpr mice were treated weekly with 20 μg/mouse of scFv. A control group of mice was injected with PBS only. Blood samples were collected weekly and the sera were stored at -70

ºC for subsequent analyses. The levels of IL-4, IL-10, IFN-γ, anti-double stranded (ds)DNA and anti-C1q antibodies in the sera were quantified by ELISA.

Results: In the present study we have investigated *in vivo* the possibility to modulate autoimmune response in MRL/lpr mouse model of SLE using a neutralizing scFv, specific for anti-C1q antibodies. The data show that the scFv treatment decrease the levels of anti-dsDNA and anti-C1q antibodies in sera and appearance of proteinuria.

Conclusion: The generated scFv antibody could be used to down-regulate the auto-reactivity in MRL/lpr mice.

AO7. NEW APPROACHES FOR IMMUNOLOGICAL TESTING OF PATIENTS WITH SYSTEMIC SCLEROSIS

E. Krasimirova[1], D. Kalinova[2], Ts. Velikova[1], K. Tumangelova-Yuzeir[1], E. Ivanova-Todorova[1], V. Reshkova[2], A.Kopchev[2], R. Rashkov[2], D. Kyurkchiev[1]

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Introduction: Systemic sclerosis (SSc, scleroderma) is a complex connective tissue disease of unknown etiology with multiorgan involvement and heterogeneous clinical manifestations. Three subsets of SSc can be discerned, i.e., limited cutaneous SSc, diffuse cutaneous SSc, and SSc without skin involvement.
The detection of autoantibodies against cell nuclei (ANA) is an important diagnostic indicator in many autoimmune diseases, including SSc. The ANA prevalence in progressive SSc is 85%-95%. According to ACR/EULAR 2013 Classification criteria for systemic sclerosis considerable advances are made in the diagnosis of SSc. It includes the detection of specific serum autoantibodies such as anti–topoisomerase I (Scl-70), anticentromere (CENP A, CENP B), and anti–RNA polymerase III (RP 11, RP 155). The possibility to test patients for additional SSc autoantibodies, such as anti-Th/To, anti–U3 RNP, NOR90, Ku exists, but they are still not widely available in the clinical practice.
Materials and methods: selection of patients, ANA HEp-2 indirect immunofluorescence test, Systemic Sclerosis (Nucleoli) Profile (IgG) dot blot test
Aim: Until now, in Bulgaria, for immunological diagnostics of SSc, only ANA HEp-2 indirect immunofluorescence test, and detection of Scl-70, CENP B, nRNP/Sm, PM-Scl are used.
The aim of our study was to optimize the immunological testing of SSc using a large profile of autoantibodies and to establish their frequency distribution and diagnostic value.
Results: We collected the sera of 40 SSc patients and performed a qualitative and quantitative evaluation of the ANA fluorescence images using HEp-2 indirect immunofluorescence test. We also made a quantitative in vitro assay for human autoantibodies of the IgG class to 13 different antigens: Scl-70, CENP A, CENP B, RP11 and RP155 (RNA Polymerase III subunits), Fibrillarin (Fib), NOR90, Th/To, PM-Scl100, PM-Scl75, Ku, PDGFR (platelet-derived growth factor receptor) and Ro-52 in the sera. 5 patients were negative for all the 13 antigens and the remaining 35 were found positive for at least one antigen. 22 patients' tests were positive for Scl-70, CENP A, CENP B, Ro-52 or combination of these antigens. Respectively, 13 patients (37% of the 35 positive SSc profiles) were positive for RP 11, RP155, Fib, NOR90, Th/To, PM75, Ku (separately or in combination), missing the routinely

evaluated centromere and Scl-70. Some of these additional antibodies demonstrated a staining pattern neither specific for Scl-70 nor for centromere autoantibodies. Accordingly, in 37% of the patients miss a routinely performed diagnostic test proving the absence or presence of SSc.

Conclusion: The resulting data indicate the necessity of establishment of a large profile of antibodies against SSc-specific antigens in the routine clinical practice, which would facilitate the diagnostics of SSc patients.

Session B

Chairpersons:

Assoc. Prof. Dobroslav Kyurkchiev, MD, PhD
Laboratory of Clinical Immunology, University hospital "St. Ivan Rilski"

Assoc. Prof. Radostina Alexandrova, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Boika Andonova-Lilova, MSc
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

BO1. CULTURING AND CHRACTERISTICS OF CELLS ISOLATED FROM GLIOBLASTOMA MULTIFORME

Dobroslav Kyurkchiev
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Glioblastoma multiforme (GBM) is the most malignant tumor of the central nervous system (CNS) which causes the death of the patient shortly after the diagnosis. A lot of data has been published that gives a ground to accept that the major event in tumor development is the transformation of the normal neural stem cells located in their specific niches in CNS into cancer stem cells. Both neural stem cells and cancer stem cells express markers such as Nestin, Sox-2, CD133, CD44 and occasionally glial fibrillary acidic protein (GFAP). Both cell types have similar signal transduction pathways and a number of common properties as self-renewal, differentiation and proliferation capacity.

In vitro cultures of cells isolated from glioblastoma multiforme is one of the basic approaches to study the properties of the cancer stem cells. Cells isolated from GBM can be cultured in serum-free media containing epidermal growth factor (EGF) and beta fibroblast growth factor (bFGF) and in these conditions cells grow as neurosheres.

Alternatively, the glioblastoma cells can be cultured in media without EGF and FGF but in presence of fetal bovine serum (FBS) and they formed adherent cells. It is also possible to culture the cells in media combining these basic approaches. According to the concept that is predominant in the literature neurospheres represent the best *in vitro* model of cancer stem cels. Cells in neurospheres express Nestin, Sox-2, CD133, CD44; they have tumorigenicity and genetic alterations similar to the tumor *in vivo*. In contrast, adherent glioblastoma cells seem to lose the expression of Nestin and CD133 and start to express GFAP; they have disputable tumorigenicity and in the most cases possess have genetic changes different from those in the primary tumor. However, there are a lot of data which report that this concept is not absolutely true.

Moreover, culturing of adherent cells demonstrate that these cells have features of cancer stem cells but features of mesenchymal stem cells as well. Adherent cells isolated from glioblastoma multiforme share the same morphology, phenotype, self-renewal and differentiation capacity, and possibility to suppress the T cellular activation as mesenchymal stem cells.

However it is very important to be mentioned that there are a lot of factors which can influence the properties of the adherent cells in the course of their *in vitro* culturing.
The purpose of the present report is to describe the different models of culturing of cells isolated from GBM and their most important characteristics using data from the literature and our own experience as well.

BP1. GLIOBLASTOMA CELL CULTURES

Radostina Alexandrova[1], Abedulkadir Abudalleh[1], Orlin Alexandrov[2], Tanya Zhivkova[1], Lora Dyakova[3], Boyka Andonova-Lilova[1], Vladimir Kulchitsky[4]
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BO2. BLOOD-CSF-BARRIER CHANGES IN EXPERIMENTAL HAMSTER *GRAFFI* TUMOR MODEL

V. Ormandzhieva, R. Toshkova

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Abstract

Plexus choroideus is considered as a selective barrier between the blood and cerebrospinal fluid (CSF). It consists of epithelial cells, fenestrated blood vessels, and stroma, dependent on various physiological or pathological conditions.
In the present study were provided morphological investigations of the plexus choroideus in the experimental hamster Graffi tumor model. There are significant changes in the choroid plexus blood vessels on the 10th and 30th day after tumor implantation. The structural and morphometric changes in the plexus choroideus are evidence for alteration of the blood-CSF-barrier after tumor metastases in the brain.
Key words: blood-CSF-barrier, choroid plexus morphological and morphometric investigations, experimental hamster Graffi tumor model

INTRODUCTION

Brain tumors can be classified into two major classes, namely, primary brain tumors that start in the brain and secondary brain tumors that are generated by the cancer cells that migrated from tumors developed in other parts of the body. Secondary brain tumors are more common than primary ones and are the most common cause of tumors in the intracranial cavity.
The cerebrospinal fluid circulatory system is involved in the neuroimmune regulation, cerebral detoxification, and delivery of various endogenous and exogenous substances (2). The barriers of the brain play critical roles in controlling the movement of various methabolites, but also drugs, between the blood and the brain (Blood-Brain Barrier) and the blood and the CSF (Blood-CSF-Barrier). Fundamental

to all brain barrier mechanisms is the presence of intercellular tight junctions between intimately opposed cells comprising these interfaces (endothelial cells of the brain vesels - BBB and choroid plexus epithelial cells – B-CSF-B) (7).
Plexus choroideus is highly vascularised structure in the brain ventricles. It produces CSF and involves in the synthesis and transport of numerous CSF components. Choroid plexus has an important role in the homeostasis of nutrients in the CSF (1).
The ***aim*** of the present investigation is to study morphological changes of the B-CSF-B in hamster *Graffi* experimental tumor model.

MATERIAL AND METHODS

Experimental hamster Graffi tumor model: Golden Syrian hamsters, 2 months old, were used in experiments. The experimental Graffi tumor was primary created by the Graffi-virus in new-born hamsters, and maintained monthly *in vivo* by subcutaneous transplantation of live tumor cells (2×10^6/ml PBS) in the interscapular area of hamsters, for keeping the tumor's survival. The tumor is 100% cancerous, and the animals die usually up to the 30th day after transplantation. The animals were kept under standard conditions with free access to food and water.
Histopathologycal examination: Brain samples from control (healthy) and tumor bearing hamsters (TBH) were taken, fixed in Carnoy's solution and embedded in paraffin using routine histological practice. Tissue sections (5-7μm) were stained by hematoxylin-eosin and examined under light microscope Leica DM5000B.
Morphometric analysis: We obtained morphometric data from the light microscope Carl Zeiss Jena at 1000x magnification using a square grid system. The luminal diameter was measured as perpendicular distance across the maximum chord axis of each vessels of control and TBH.
Statistical analysis: Results are reported as mean values ± SEM and statistically analyzed by Student's t-test using statistical package (STATISTICA, ver.6, Stat-Soft Inc., 2001), and differences were regarded as significant at $p<0.05$.
All studies were performed in accordance with the Guide for Care and Use of Laboratory Animals, as proposed by the Committee on Care Laboratory Animal Resources, Commission on Life Sciences and National Research Council, and a work permit No. 11130006.

RESULTS AND DISCUSSION

The histopathological and morphometric studies of the brain were carried out on the 10th and the 30th day after tumor implantation. At the same time samples were taken from control hamsters. The changes in luminal diameter (μm), cross-sectional area (Sd in μm^2) and numbers (relative part in %) of the choroid plexus blood vessels divided in four subgroups in control and TBH are studied and shown on Fig. 1, 2 and 3. Significant changes were observed in the number of the blood vessels with luminal diameter of the 3.75-7.5 μm (reduction) and 8.0-15.0 μm (increase) in TBH on the 10th and 30th day of examination in comparison with control hamsters. The number of the blood vessels with luminal diameter of the 15.5-30.0 μm and > 30.0 μm was not statistically changed in TBH in comparison with control. Significant increase were observed in the luminal diameter and cross-sectional area of capillaries (vessels <15.0 μm in diameter) and large vessels (>30.0 μm in diameter) in TBH on the 10th and 30th day of examination.
The choroid plexus lies in the brain ventricles and consists of single layer of large cuboidal light and dark epithelial cells, connective tissue elements and fenestrated capillaries (Fig. 4). It is known that tumor cells spread to other parts of the body in the lymphatic and blood transfer. In the present investigation the remarkable histopathological changes of the brain on the 10th and the 30th day after

tumor implantation are presented in Figure 5 and 6. In some of the blood vessels near by the lateral ventricles as well as the choroid plexus blood vessels are observed destructive changes and damaged endothelial cells.There were many macrophages in the apical part of the choroid plexus epithelial cells and CSF and many dark epithelial cells. The observed morphological changes are due to violations in functional activity of the plexus choroideus and represent a compensatory reaction as a result of tumor metastasis in the brain. Similar changes we observed in previous our investigation after low doses ionizing iradiation (3, 4). Massive accumulation of tumor metastatic cells were detected in the brain tissue, lateral ventricle and under ependyma in TBH on the 30-th day of examination. Tumor cells in metastatic lesions have a similar morphological characteristic to that described in the primary tumor, reported in our previous studies (8, 9). The histopathological and morphometric changes of the brain are in support of our previous investigation in which we found metastatic lesions in the brain ventricles and brain tissue (5, 6). Experimental study of the TBH revealed that the tumor appearance and development led to the morphological damages of the choroid plexus and violation of the permeability of the B-CSF-B.

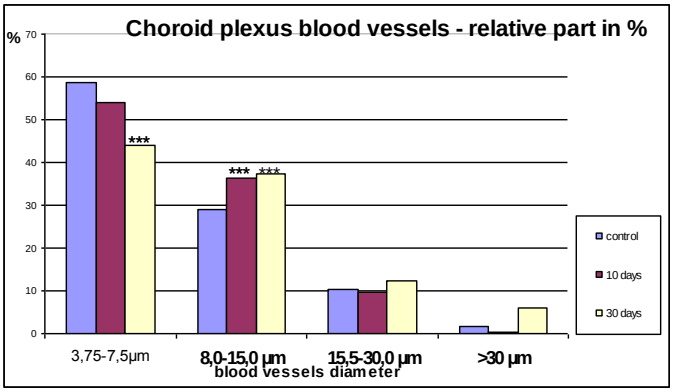

Fig. 1.

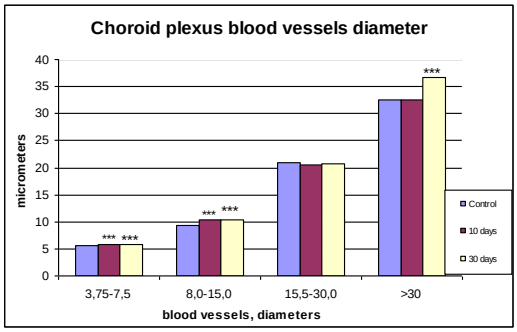

Fig.2.

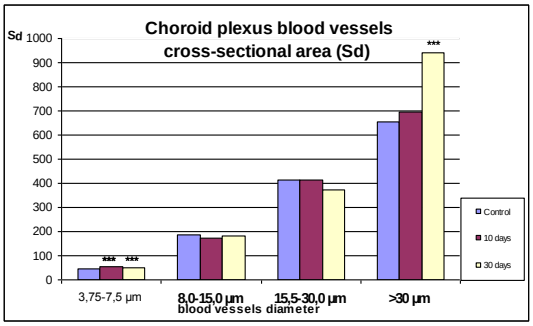

Fig. 3.

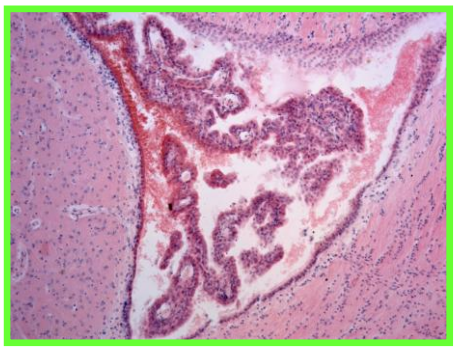

Fig. 4. Light microscopic micrograph of the plexus choroideus of control hamster in the lateral ventricle. H&E stain. X 10

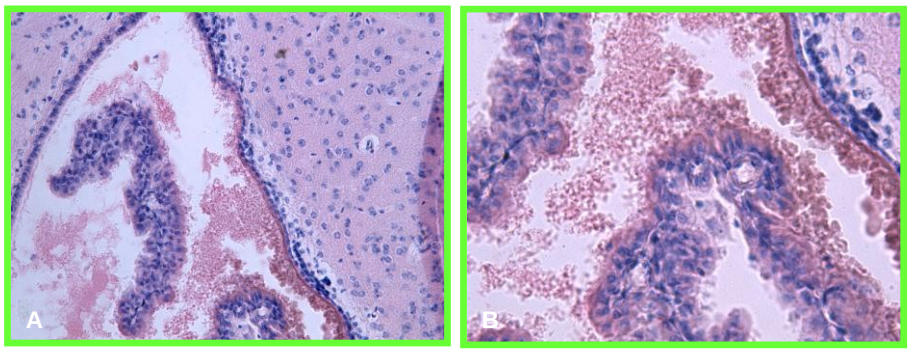

Fig. 5. Light microscopic micrograph of the plexus choroideus of tumor bearing hamster (10 days after tumor implantation). H&E stain. A X20; B X40

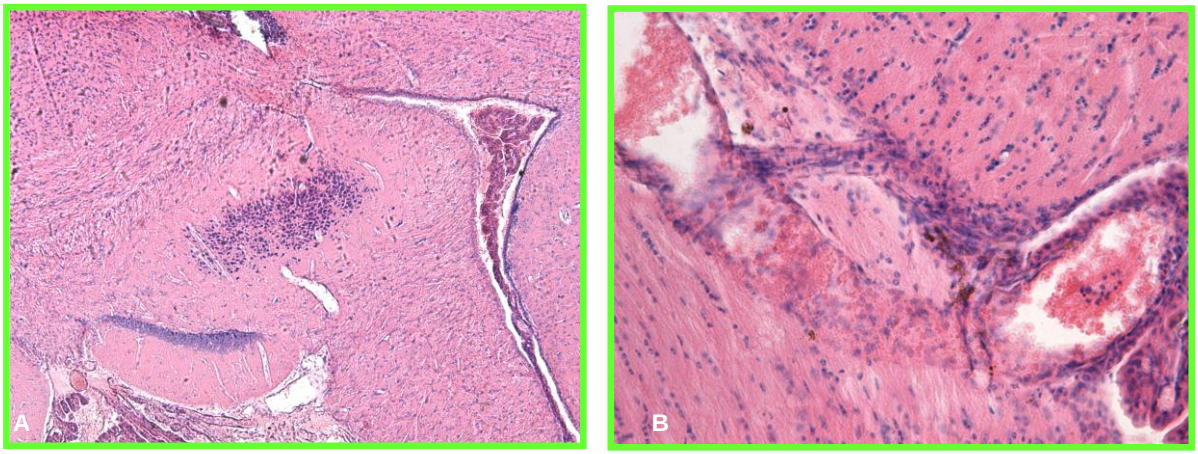

Fig.6. Light microscopic micrograph of the plexus choroideus of tumor bearing hamster (30 days after tumor implantation). H&E stain. A X5; B X20

Conclusion:

The obtained structural and morphometric changes in the plexus choroideus in the experimental hamster Graffi tumor model are evidence for alteration of the blood-CSF-barrier.

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B03. THE CHALLENGES OF TUMOR ANGIOGENESIS

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BO4. LUNG CANCER

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Lung cancer which is a worldwide malignant disease is a leading cause of high mortality in both men and women. Its prevalence is estimated at 25/100000 people a year. The disease occurs between the age of 55 and '60, but there are cases diagnosed before the age of 40. As with all malignancies specific cause of the degeneration of malignant cells has not been established. Recognized and proven risk factors are:

- Smoking / passive smoking;
- ionizing radiation - radon, uranium;
- Nickel;
- A family history of lung cancer;
- Emphysema and other inflammatory - destructive diseases of lungs

As in the beginning there are not any specific visible symptoms, when they become apparent, the disease is already advanced. The most common symptoms include: persistent cough refractory to treatment, blood in sputum, wheezing, pain, chest tightness, shortness of breath, change in normal voice, headache, fever, weight loss, pleural effusion, pneumonia.
Scientists have found that perhaps a gene is associated with the development of lung cancer. The discovery of the gene dmp1 is an important step to better clarify the issues that arise at the cellular level, leading ultimately to cancer. Scientists at the University of Medicine Wake Forest analyzed 51 samples of non-small cell cancer and found that the gene dmp1, which normally suppresses tumor formation is dysfunctional in 35% of cases. Previous studies with mice have established that dmp1 activates tumor suppressors called p53 and Arf. When the dmp1 does not function, these suppressors are not produced and are not able to destroy cancer cells.
According to its location lung cancer is divided into:
• Central (krayhilusen) cancer - usually small or squamous;
• Peripheral kartsinom- particular form is a tumor of Pancoast-Tobias - a top location;
• Diffuse Lung Cancer
Lung cancer is divided into four main histological types that have a large number of varieties: small- cell carcinoma, squamous, large-cell and adenocarcinoma.
Lung cancer has bad prognosis. It quickly metastasizes to the brain, liver, bones and adrenal glands, blood and lymph path.
The conducted studies are radiography, CT scans, biopsy, laboratory tests, examination of tumor markers and etc. The treatment is surgery, radiotherapy and chemotherapy, immunotherapy, symptomatic treatment.

BO5. GLYCOSYLATION CHANGES IN ASCITE HEPATOMA CELL LINES

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Abstract:
Tumor cells can undergo rapid growth, adhere to a variety of other cell types and invade tissues. In contrast to normal cells tumor ones have unique glycosylation pattern. Changes range from loss of expression or excessive expression of certain structures, the persistence of incomplete structures or appearance of new structures. Most commonly we observe altered branching of N-glycans, changes in the amount and acetylation of sialic acids and appearance of sialylated Lewis structures. Tumor growth, invasion, and metastasis is connected with survival of the fittest cells. Tumor cells glycotype, reflecting the highly selective changes seen in tumor cell glycosylation, have great functional consequences in tumor survival and metastases.
The aim of this paper is to review glycosylation changes in ascite hepatoma cell lines.

Introduction:

Malignant tumors arise from normal cells in a multi-step process of progressive accumulation of genetic and epigenetic DNA alterations. Tumor cells have disturbed cellular signaling pathways and regulatory circuits leading to growth advantages. In this process the micro-environment of the tumor cells change under chemical signals emitted from developing tumor. In further stages of tumor development new blood vessels are formed, inflammatory cells are recruited, and fibroblasts are activated in the surrounding tumor stroma.

Development of some tumors includes formation of malignant ascites, which are cancer-associated accumulation of fluids in the peritoneal cavity. The neoplasms most frequently associated with ascites are these of ovarian, breast, colon, pancreas and liver. In general, the prognosis of patients with malignant ascites is poor. Most cases have mean survival time between 20 to 58 weeks, depending on the type of malignancy. Ascites typically develops in the setting of advanced cancer. Patients may have a history of metastases to the peritoneum and enlarged abdominal lymph nodes prior to the development of ascites. In people with ovarian and liver cancer, the fluid contains cancer cells that have disrupted the normal absorption and production of fluid in the abdomen. The origin of the primary tumor has an impact on the sites of abdominal metastases and the etiology of subsequent ascites [1]. Some diagnostic tests are developed distinguishing malignant ascites caused by hepatocellular carcinoma from ascites associated with cirrhosis [2].

Models of cancer come in a variety of forms, from cancer cell lines in culture to genetically modified mice. Ascite cancer cells can be grown and experimented with *in vitro* in cell culture conditions. Same cancer cells can be grown *in vivo* in host animals. I*n vivo* model of ascite tumor cell lines is a better choice as we may obtain information on host response to developing tumor.

Ascite tumor cell lines are established from different tumor types, such as: Ehrlich ascite cell line, Krebs ascites cells, ovarian cancer ascite model, Dalton's ascites lymphoma, ascites sarcoma 180, L1210 ascitic tumor. Hepatoma ascites cell lines are one of the most studies ascite cell lines, most notably murine H 22-F 25/L, P388 cell lines, mouse Hca-F, Hca-P cell lines and rat AH-13, AH-34, AH-41B, AH 44, AH-60C, AH-64B, A66, AH66F, AH-109A, AH 130, AH 130FN, AH130 W1, AH136B, AH7974, AH 7974F, AS-30D, L-1210, LC-AH, MM1, P230, Zajdela and Novikoff hepatoma.

Key words: cancer, ascite tumor cell lines, glycosylation, ascite hepatoma.

Glycosylation of hepatoma ascite cell lines

Glycosylation of O-linked glycoproteins is not complete as shown from investigations on UDP-GalNAc: polypeptide N-acetylgalactosamine transferase in AH -66 hepatoma ascite tumor line. The transfer reaction terminates at the level of glycosylation of from only a few to at most about 40% of the serine plus threonine residues from which mucin-type oligosaccharides had been removed [3]. InAH-66 hepatoma the liver enzyme has asparagine-linked sugar chains with complete outer chain, NeuAc α Gal β 1 4GlcNAc, while hepatoma enzyme has sugar chains incomplete in their outer chain moieties. On the other hand Gal β 1 4GlcNAc β 1 4GlcNAc group is found in the sugar chains of liver enzyme, but not in those of hepatoma enzyme. A third feature is that more than 40% of the sugar chains of hepatoma enzyme contain bisecting N-acetylglucosamine which is not found in those of liver enzyme [4].

In AH-109A hepatoma sialyltransferase is more active than liver enzyme, due to an increased activity of Gal (β1 4) GlcNAc (α 2 6) sialyltransferase. Gal (β 1 3, 4) GlcNAc (α 2 3) sialyltransferase and the sialyltransferase acting on asialo-bovine submaxillary mucin are decreased in the hepatoma. A similar pattern of sialyltransferase alterations is observed in regenerating liver. AH-109A hepatoma is higher in lactosylceramide- and lower in GM3-sialyltransferase activity [5]. In rats bearing a solid form of AH-109A hepatoma, serum asialofetuin sialyltransferase activity is significantly increased, but this serum sialyltransferase originate mainly from the liver of host animals. This result indicates host response to tumor growth [6].

Electrophoretic analysis of A 130 hepatoma Golgi membranes show 23 protein bands, which are different from the electrophoretic profile of Golgi membranes from rat liver [7].

AH7974 hepatoma have reduced production of extracellular matrix proteins, decreased expression of cell surface integrins, or lack of heparan sulfate proteoglycans on its cell surface. These cells express also 56 and 62 kDa laminin-like substances, containing *Griffonia Bandeirea simplicifolia* isolectin B4-binding carbohydrates [8]. Expression of 62 and 56kDa laminin-like substances with α-galactose residues on tumor cell surface is one of the determinants associated with the lung-colonizing

potential of these cells. Carbohydrate antigen expressions in cancer cells are related to metastatic spread and the organ distribution pattern of metastasis. Lymphatic metastasis is related to the expression of mucin core type carbohydrates (Tn antigen and Tn-like antigens). These results suggest that carbohydrates are one of the determinants of cancer metastasis [9]. Asialo GM1 is found to be widely distributed in rat ascites hepatomas AH7974. Treatment of host animals with anti-asialo GM1 antiserum is found to be effective for prolonging their survival [10]. AH7974F also expresses asialo-GM2 [11].

In motility assay of MM1 hepatoma cells on glass coated with fibronectin, lysophosphatidic acid could induce phagokinetic motility which is accompanied by transformation of these cells to fusiform-shape. Fibronectin is glycoprotein from the extracellular matrix, mediating adhesion through its carbohydrate part [12].

Rat Zajdela ascite hepatoma (ZAH) is a malignant cell type with some properties in common with rat hepatocytes. ZAH have the presence of highly sialylated O-linked glycosylation, absent in normal liver cells [13]. The electrophoretic profile of serum glycoproteins from ZAH is significantly altered. There is approximately a 2.5 times increase of [^{3}H] fucose incorporation into serum glycoproteins from rats with an ascitic form of hepatoma, compared with serum from normal rats and serum from animals bearing the solid form of the tumor [14]. A glycoprotein carrying lactosaminoglycan (GPIII) is identified in hepatoma by its affinity for *Datura stramonium* lectin. This glycoprotein is absent from normal hepatocytes. The fact that this lactosaminoglycan-carrying glycoprotein is not expressed in hepatocytes suggests its expression to be linked to the malignant state of this hepatoma [15]. Comparison of Asn-linked glycans between ZAH and normal liver show that: 1. Zajdela hepatoma cells express tri- and tetra-antennary complex N-linked glycan chains, whereas hepatocytes display large amounts of bi-antennary N-linked structures; 2. 20% of the glycan chains in hepatoma cells contain a bisecting GlcNAc residue which is β (1, 4)-linked to the β-mannosyl residue of the core and is not detected in the hepatocytes; 3. Hepatoma cells express a high proportion of the fucosylated GlNAc β (1, 6) Man α -1branch, whereas hepatocytes contain a little of this branch; 4. Hepatoma cells have a repeating (Gal β (1, 4) GlcNAc β (1, 3)) sequence characteristic of poly-N-acetyllactosaminoglycans; 5. Alpha (2, 3)/α (2, 6)-linkage ratio of sialic acid is significantly higher in hepatoma cells [16]. In serum of Zajdela bearing rats, host liver and Zajdela ascitic cells, galactosyltransferase activity towards ovomucoid is elevated, compared to control serum and liver. In ZAH cells α(1-3) galactosyltransferase activity is 3 times higher than that in liver [17]. There are differences in expression of β-galactoside α2, 6-sialyltransferase in Zajdela hepatoma cells, compared to normal liver cells [18]. It was shown that activities of α -2, 3- and α -2, 6-sialyltransferases are higher in hepatocytes, compared to Zajdela hepatoma [19]. Serum from rats with acute or chronic experimental fascioliasis show specific ^{3}H-fucose labeled glycoproteins [20]. Still in a model of acute and chronic experimental fascioliasis in rats bearing Zajdela hepatoma a new and specific ^{3}H-fucose labeled glycoproteins were found in sera from these animals [21] and [22].

New glycosylated structures may appear in host animals directly from tumor cells, as in case of shedding of cell surface molecules. Thus glycopeptides present on the surface of Novikoff hepatoma cells are shed into the ascitic fluid and may be distinguished from components in normal serum by their Con A receptor activity [23]. Rats bearing Novikoff hepatomas exhibit elevated serum levels of fetuin: N-acetylneuraminic acid transferase activity. The serum transferase activity could be correlated with the growth rate of the tumor. These results suggest that tumor-bearing animals release large quantities of this enzyme into the serum of host animals [24].

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BO6. AMYGDALYN (VITAMIN B17) AND TUMOR CELLS

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Amygdalin (vitamin B17) is natural cyanide-containing substance abundant in the seeds of rosaceous plants such as apricots, almonds, peaches, apples and others.
Since early 1950s it have been promoted as alternative cancer treatment. At this stage amygdalin is not improved by European Medicines Agency, nor Food and Drug Administration for use and the anticancer effect has admitted controversy.
However there are studies that concluded it has selective and dose dependent cytotoxic effect. It upregulates pro-apoptotic Bax protein and downregulates anti-apoptitic Bcl-2 protein. It also downregulates cell-cycle related genes such as EXO1, APCF2, MRE11A, TOP1 and FRAP1.
This information reveals vitamin B17 as a substance which can be used as auxiliary therapy of patients with cancer.

Key words: amygdalin, vitamin, B17, apoptosis, cancer
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BO7. REVIEW OF BALKAN ENDEMIC NEPHROPATHY HYPOTHESES

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Introduction

Balkan Endemic Nephropathy (BEN) is non-inflammatory, slowly progressing, tubulo-interstitial kidney disease (Tanchev, Y. et al., 1956) citated by [14]. It occurs with a high rate of prevalence in Serbia, Bulgaria, Romania, Bosnia and Herzegovina, and Croatia. The geographical distribution of the disease has not changed significantly since it was first described. BEN was almost simultaneously recognized in the three countries involved as well as each major affected region of these countries by Tanchev at al., in Bulgaria (1956), by Danailovic and others in 1957 in Yugoslavia, and by Fortza and Negoescu in Romania in 1961 [19].

The most prominent features of the disease are its endemic nature, long incubation period, the familial clustering of the disease and, remarkably, an unusually high incidence of upper urothelial tumour (UUT) associated with BEN or in the population [15].

The etiology of BEN has been the topic of many studies producing the publication of several hypotheses.

Characteristics of BEN

Epidemiology

Balkan Endemic Nephropathy is observed most frequently between the ages of 40 and 60. It has been diagnosed only among people living (or those who used to live) in more or less well defined areas of the Balkans. Affected areas are in Bulgaria, Romania, Serbia, Bosnia and Herzegovina, and Croatia (Fig. 1).

As, recently summarized [7], the affected territory has a shape of a rhomboid. Its longer diameter spreads over 500 km (from the Vratza municipality in Bulgaria to villages west of Slavonski Brod in Croatia), while its transversal diameter has about 300 km (from endemic foci in eastern Romania to Vitina municipality in Kosovo). The disease affects individuals who live (or used to live) in rural environment. There are some spared households even in the most affected areas, leading to frequently cited remarks on mosaic distribution of the disease.

Fig. 1 Distribution Of Balkan Endemic Nephropathy

Clinical features

The first clinical classification of Balkan endemic nephropathy was elaborated by A. Puhlev et al. in 1960, according to the stages of the disease. During, the past more than fifty years, the clinical course of BEN has extended and has moved towards the older age in all endemic foci. The clinical symptoms and signs of BEN are characterized by the initial long-lasting asymptomatic period. There is no fever, severe dysuria or other diseases preceding the onset of symptoms [15].

Balkan Endemic Nephropathy develops with a normal arterial pressure and thus does not affect the cardio-vascular system. The disease is always combined with aneamia. Renal function is disturbed at an early stage of the disease because the proximal and distal tubules are affected as well as the collecting ducts. The glomeruli are affected in the late stage of the disease. The amino-acid metabolism of the patients suffering from BEN is disturbed and the liver function is also affected [8].

Pathology

The pathological picture of the kidney, shows a bilateral, mostly smooth nephrocirrosis, with strongly reduced dimensions – the weight being between 30 and 40 g at the terminal stage. Very specific is the reduction in thickness of the cortex. This is sharp contrast to the relatively well preserved renal pyramids [8]. Tubular and interstitial changes without any inflammatory cellular infiltration have been histologically established, and characterized BEN as tubulo-interstitial nephropathy of a non-inflammatory nature.

Many factors (ecological and genetic) have been accused as a starting point of BEN. The possibilities of the etiology include genetic or environment only, environmentally in induced disease with genetics playing role, and genetically induced disease, with the environment playing role. For the purposes of this article, we will look at popular hypothesis for the etiology of the disease.

Overview of the hypotheses

Lead Intoxication

In the first paper on BEN by Danilović et al. (1957) lead was considered to be responsible for BEN, being found in flour in affected settlements. At the time lead was used to repair cracks in the mill wheels, contaminating the flour. However, this hypothesis was not substantiated by further studies. In patients with BEN lead excretion was not found different from urinary excretion in control population. Gaon et al. (1962) performed $CaNa_2$ EDTA lead mobilization tests in 31 patients with BEN. The maximum daily leadchelate excretion was 530 μg and the mean three-day lead-chelate excretion was 192 μg, being in the limits of normal [9]. Urine and blood concentrations may be inadequate measures of low-level environmental exposure. However, BEN and lead nephropathy have several different features. Similar to BEN lead nephropathy is characterized by a latency of 3-30 years, however marked hypertension, gout and neurobehavioral disturbances are characteristic for lead nephropathy, and not observed in BEN. The absence of familial occurrence, low-molecular weight (LMW) proteinuria or association with urinary tract tumours in lead poisoning further distinguishes these two nephropathies [3].

Multi-element impact

There are, however, sufficient reasons for accepting the idea that Balkan Endemic Nephropathy is caused by the impact of one or a group of metals. These reasons are: the endemic character of the disease; the presence of lead, copper, uranium, chromium and other metals in the vicinity of endemic villages; The frequent combination of BEN with tumors of the urinary ducts; The spectrographic determination of a higher content of chromium, nickel, tin and aluminum in organs of patients who have died the disease; The tubule-interstitial disorders in the kidneys of deceased patients without the presence of inflammatory cellular infiltration; The presence of anemia and affected liver function [8].
Donev et al., investigated blood, nails and hair samples form patients with Balkan endemic nephropathy in District Hospital of Vratsa and from out-patients form Montana (former name Mikhailovgrad), villages of Belotintsi and Nikolovo. They also analysed organs from patients who had died from the disease.

They obtained, that the amount of chromium in the blood of patients is twice that of the control group, besides, chronic intoxication does not fit in with the clinical picture of the Balkan endemic nephropathy.

Cadmium content increment is reported to be possible reason for the disease, as well. Other investigations shows that cadmium was found increased in soil, water and food from endemic regions. Cadmium produced a chronic Fanconi Syndrome, characterized by LMW, proteinuria, and urinary calcium wasting. Renal failure is uncommon but severe osteomalacia characterized by painful bone lesions/pseudofractures and renal stone diseases develop [16]. Cadmium has affinity for the kidney [10]. The increased concentrations of Cd in kidneys are the result of industrial development and increased application of this element, as well as of smoking.

As known, the half-period for the elimination of cadmium from the blood is approximately 10 years [10]. One can assume that such a period is sufficient even for minimal increases in concentration in the heart and kidneys to give rise to alterations similar to those of endemic nephropathy. Besides its affinity for the kidney, cadmium also accumulated in the gonads, a fact which could explain the importance of the family factor for this disease [8].

Chronic Intoxication with *Aristolochia Clematitis*

Aristolochia clematitis, (European) Birthwort, is a twining herbaceous plant in the Aristolochiaceae family, which is native to Europe (Fig.2). The leaves are heart shaped and the flowers are pale yellow and tubular in form. The plant seeks light by ascending the stems of surrounding plants.

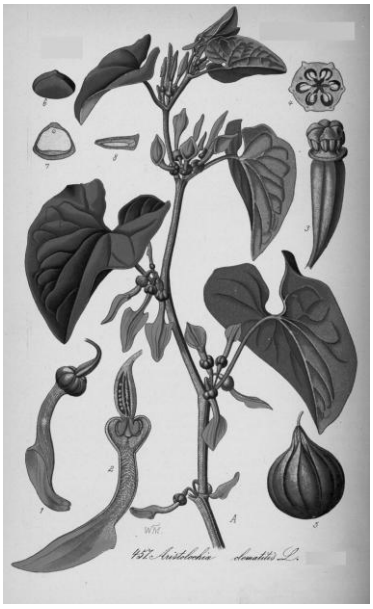

Fig. 2 *Aristolochia Clematitis*

It was formerly used as a medicinal plant, though it is posonous and is now occasionally found established outside of its native range as a relic of cultivation. It is now thought to be the cause of thousands of kidney failures in Romania, Bulgaria, Serbia, Bosnia and Herzegovina, and Croatia where the plant is thought to be unintentionally consumed through contaminated flour. Urinary tract malignancies among those who have consumed the plant are also reported. The link between renal failure and aristolochic acid (AA) (Fig. 3), which the plant contains, was discovered after a clinic for obesity in Belgium used herbal products based on another plant of the same genus as a diuretic. After a few months, some of the patients experienced kidney failure.

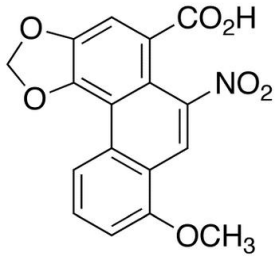

Fig. 3 Aristolochic acid (AA)

AA is a family of carcinogenic, mutagenic, and nephrotoxic compounds commonly found in the Birthworth (Aristolochiaceae) family of plants. These plants are widely associated with kidney problems and urothlial cancers. Since AA is a mutagen, it does damage over time. However, urothelial cancer is only observed long after consumption. A study estimates that it takes on average ten years from the start of daily AA consumption for detectable cancer to develop [2].

The exact mechanism of action of AA is not known, especially in regards of nephropathy. It is thought that the carcinogenic effects of AA are a result of mutation of the tumor supressor gene TP53, which seems to be unique to AA associated carcinogenesis [11]. Nephropathy caused by AA consumption is not mechanistically understood, however DNA adducts characteristic of AA induced mutations are found in the kidneys of AA nephropathy patients, indication that these might play a role [11].

Historically *Aristolochia clematitis* was a well known as a healing plant and used by the ancient Egyptians and Greeks [14].

Peters and Hedwall in 1962 indicated that AA was known to be nephrotoxic in the rabbit as early as in 1892, in the horse in 1893 and in the rabbit and the mouse in 1958 (Peters, G. et al, 1963) citated by [14].

Later, in 1969, Ivic proposed that the aetiology of BEN could be related to this plant. He concluded that on the basis of geographical, epidemiological and laboratory investigations (Ivic M., 1969) citated by [14].

Ochratoxin A

Ochratoxin A, (Fig.4) a toxin produced by *Aspergillus ochraceus*, *Aspergillus carbonarius* and *Penicillium verrucosum* (Fig. 5, 6) is one of the most-abundant food-contaminating mycotoxins [1]. It is also a frequent contaminant of water-damaged houses and of heating ducts [15].

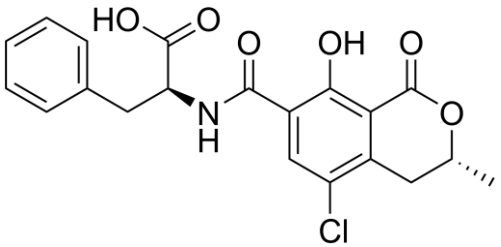

Fig. 4 Ochratoxin A

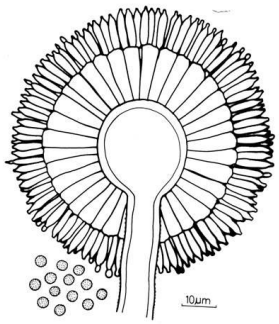

Fig. 5 *Aspergillus ochraceus*

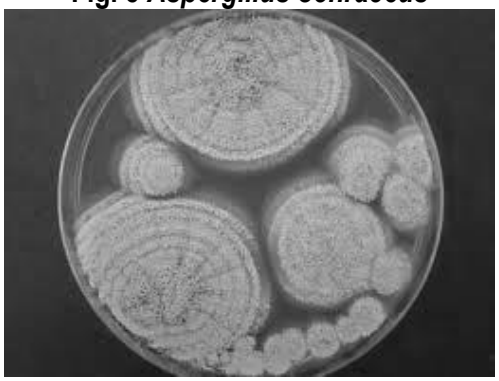

Fig. 6 *Aspergillus ochraceus*

Human exposure can occur through consumption of contaminated food products, particularly contaminated grain and pork products, as well as coffee, wine grapes and dried grapes. The toxin has been found in the tissues and organs of animals, including human blood and breast milk [5].

A number of descriptive studies have suggested a correlation between exposure to ochratoxin A and BEN, and have found a correlation between its geographical distribution and a high incidence of, and mortality from, urothelial urinary tracy tumours [4]. However, insufficient information is currently available to conclusively link ochratoxin A to BEN [12].

Pliocene Lignite

This hypothesis was proposed in 1991 [13] based on the geographical overlapping between the location of Pliocene lignite deposits in the Balkans and the location of endemic areas, as well as analyses of well water from endemic villages in former Yugoslavia, which showed the presence of organic compounds in higher concentrations than in well water from nonendemic villages (Feder G.L., et al., 2001) citated by [15].

The lignite hypothesis, is based on the assumption, that toxic organic compounds in lignite or in weathered lignite, may be leached, by groundwater and thus, contaminate drinking water wells, supplied by this groundwater. Although the concentrations of these organic molecules in the well water may be low, long exposure and/or accumulation in body tissues over time may lead to kidney lesions. The development of urothelial carcinomas in some individuals can also be explained by this hypothesis because most of these toxic organics are well known carcinogenic factors (Orem, W., et al., 2007) citated by [15].

Conclusion

Based on the examined hypotheses, the hallmark of the disease is its multifactorial nature, both genetic and environmental. Genetic predisposition is more or less proven in numerous studies, and it is presumably responsible for providing the circumstances for the action of environmental endogenous etiology factors.

Of all hypotheses, that associated with Aristolochic acid, stand out. Chronic poisoning with AA, provide evidences related to BEN etiology, distribution and clinical characteristics.

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Session C

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CO1. ANTINEOPLASTIC AND ANTIMETASTATIC POTENTIAL OF THE NOVEL ALKILPHOSPHOHOLINE ERUFOSINE IN GRAFFI MYELOID TUMOR CELLS. CYTOMORPHOLOGICAL STUDY

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Abstract

Alkylphosphocholines (APCs) are phosphocholine esters of long-chain aliphatic alcohols with a pronounced antineoplastic activity. The class of APCs comprises of various compounds, one of which, the hexadecylphosphocholine (miltefosine) has found application in medical oncology practice. Other APCs are currently at different stages of preclinical and clinical studies.

The purpose of the present study is to assess the in vitro antineoplastic and antimetastatic effects of the novel alkilphosphoholine erufosine (EPC3) on cells from Graffi myeloid tumor in hamsters (GMTH) by cytomorphological methods.

Our results indicate that EPC3 significantly reduces the viability and proliferative activity of the GMTH cells after 24 hours of exposure. Fluorescent microscopy of EPC3- treated cells, double stained with acridine orange/propidium iodide revealed typical morphological features of apoptotic cell death – membrane blebbing, chromatin margination, nuclear fragmentation and formation of apoptotic bodies. The proapoptotic activity of erufosine was confirmed by annexin V/propidium iodide and 4',6-diamidino-2-phenylindole (DAPI) staining. The effect of erufosine on the tumor cell cytoskeletal organization were examined by immunofluorescent visualization of the of the actin, tubulin and vimentin microfilaments. The migratory activity of EPC3- treated GMTH cells was evaluated by wound healing assay (scratch assay). The results indicate that EPC3 induces reorganization of the cytoskeleton and reduces the migration activity of the tumor cells.

The results presented confirm the previously reported data about apoptogenic potential of EPC3 in leukemic tumor cell lines and point it out as a promising drug candidate for treatment of hematological malignancies.

Acknowledgement

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CO2. SCHIFF BASES

Katya Popova[1,2], Milena Glavcheva[1], Lora Dyakova[3], Desislav Dinev[1], Tanya Zhivkova[1], , Boyka Andonova-Lilova[1], Abedulkadir Abudalleh[1], Gabriela Marinescu[4], Daniela-Cristina Culita[4], Luminita Patron[4], Radostina Alexandrova[1]

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CO3. DO Cu(II) AND Co(II) COMPLEXES WITH SCHIFF BASES AFFECT VIABILITY AND PROLIFERATION OF CULTURED CANCER CELLS?

Desislav Dinev[1], Tanya Zhivkova[1], Lora Dyakova[2], Katya Popova[1,3], Boyka Andonova-Lilova[1], Abedulkadir Abudalleh[1], Gabriela Marinescu[4], Daniela-Cristina Culita[4], Luminita Patron[4], Radostina Alexandrova[1]

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CO4. Zn(II) COMPLEXES WITH SCHIFF BASES: INFLUENCE ON VIABILITY AND PROLIFERATION OF CANCER CELLS

Milena Glavcheva[1], Lora Dyakova[2], Desislav Dinev[1], Tanya Zhivkova[1], Katya Popova[1,3], Boyka Andonova-Lilova[1], Abedulkadir Abudalleh[1], Gabriela Marinescu[4], Daniela-Cristina Culita[4], Luminita Patron[4], Radostina Alexandrova[1]

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СО5. ВЛИЯНИЕ НА ВОДЕН ЕКСТРАКТ ОТ *MELISSA OFFICINALIS L.* ВЪРХУ ВИТАЛНОСТТА НА МИШИ ЕМБРИОНАЛНИ ФИБРОБЛАСТИ

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Резюме

Melissa officinalis L. (маточина) е лечебно растение, принадлежащо към семейство *Lamiaceae*. То е с доказани антиспастични, седативни, антиоксидантни, антибактериални, антигъбни и антивирусни свойства.

В научната литература има ограничени данни за действието на воден екстракт от маточина върху първични клетъчни култури. Целта на настоящата работа беше изследване на действието на воден екстракт от маточина, върху виталността на първични клетъчни култури от миши ембрионални фибробласти. Клетките бяха третирани с различни разреждания на екстракта (1:4; 1:8; 1:16; 1:32; 1:64). Тяхната виталност беше определена на 24, 48 и 72 час след третирането, чрез прилагане на 3-(4,5-диметилтиазол-2-ил)-2,5-дифенил-тетразолиум бромид (MTT) анализ. Ядрата на клетките бяха оцветени с Hoechst 33258. Промените в клетъчната морфология бяха проследени чрез светлинна и флуоресцентна микроскопия.

Установено беше, че разреждания на екстракта в съотношение 1:64 и 1:32 не влияят негативно върху виталността на мишите фибробласти. При повишение на концентрацията на екстракта (разреждания 1:16; 1:8; 1:4) беше отчетено понижаване на клетъчната жизненост. В клетките третирани с концентрации на екстракта, по-високи от 1:32, беше наблюдавана кондензация на ядрото и намаляване на клетъчния обем.

От получените резултати може да се заключи, че 1:32 е най-високото разреждане на екстракта от маточина, което не влияе негативно върху виталността на първични култури от миши ембрионални фибробласти. Концентрации на екстракта над 1:32, водят до понижаване на жизнеността на клетките и до апоптотични изменения в тях.

Ключови думи: *Melissa officinalis L.*, миши ембрионални фибробласти, виталност.

CO6. CHRONIC EXPOSURE TO COBALT(II) COMPOUNDS AND MURINE SPLEEN

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Spleen is very sensitive to metal treatment. It is also a place for storage of iron, erythrocytes and platelets, therefore alterations in spleen morphology and functions will affect iron metabolism and hematopoiesis.

The aim of the study was to determine the effect of chronic exposure to Co(II) compounds – cobalt chloride ($CoCl_2$) and Co-EDTA on the spleen of mice from different age groups. Pregnant ICR mice (placed in individual cages) were treated with daily doses of 75 mg/kg b.w. or 125 mg/kg b.w. $CoCl_2$ or Co-EDTA. On day 25 pn the newborn mice were placed in individual cages and treatment continued until day 90. At different ages – days 25, 30, 45, 60 and 90 the experimental animals were sacrificed, their spleens excised and processed for histological studies. The number of megakaryocytes was determined under a light microscope. The results showed diverse and dose-dependent effects of both compounds. Exposure to $CoCl_2$ increased the number of megakaryocytes (except for d90 mice) while treatment with Co-EDTA reduced it compared to age-matched untreated controls. Comparative assessment of the effects of both compounds revealed ~1,2 to 5,7-fold increase of the number of megakaryocytes in the spleen of mice treated with the low dose $CoCl_2$ compared to those obtaining the same dose Co-EDTA. Analyses of the high doses showed the same tendency – the number of the cells was increased ~1,3 to 1,6-fold after treatment with 125 mg/kg $CoCl_2$.

The results indicate that the effects of chronic exposure of cobalt on murine spleen depend on the type of compound as well as on the applied dose. Applied as chloride, cobalt stimulated megakaryocyte formation.

CP1. THE METHOD OF COMPATIBILITY ASSESSMENT OF MESENCHYMAL STEM CELLS WITH TITANIUM IMPLANTS WITH DIAMOND-LIKE OR HYDROXYAPATITE COATINGS

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Abstract

Objective: to assess *in vitro* compatibility of mesenchymal (stromal) stem cells of rabbit bone marrow with titanium implants.

Methods: Mesenchymal stem cells of bone marrow of newborn rabbits (n=5) were cultivated in αMEM medium using 24- or 96-well plate. Formation of monolayer was observed during 2-7 days outside and

at the surface of small implant fragments placed in wells. Scanning electron microscopy was used for 3D shooting besides visual control and light microscopy.
Results: The peculiarities of adhesion of bone marrow mesenchymal stem cells at the surface of titanium implants with different coatings were established. The best formation of stem cells monolayer was observed at the surface of titanium implants with diamond-like and hydroxyapatite coatings.
Conclusion: Obtained results indicate high level of biocompatibility of titanium implants with hydroxyapatite or diamond-like coatings.
Key words: titanium implants, hydroxyapatite, diamond-like coating, mesenchymal stem cells, adhesion

Introduction

The problem of compatibility of heterogenous implants with biological tissues still remains unsolved [1, 2]. The process of "foreign" material rejection is observed after inflammatory processes development, which sometimes transform from local to system processes followed by failure of different functional systems of the organism [3, 4]. Another one task is the use of implants in patients with local and system inflammatory processes, for example tuberculous etiology [5]. In this regard the search of methods for experimental substantiation of ability of titanium implants with different coatings use in patients with infectious inflammation of bone tissue is one of the primary tasks.

Methods

Mesenchymal (stromal) stem cells (MSCs) of bone marrow of newborn rabbits (n=5) were cultivated using 24- or 96-well plate with cell density of 40 thousand cells per 1 well, in 1 ml of medium (αMEM (Sigma), 10% fetal calf serum (HyClone, USA), 1% antibiotics solution (PEN-STREP additive, Lonza, Belgium), and 2 mM of glutamine (Ultraglutamine 1 additive, Lonza, Belgium). Implant samples 1.5x1.5 mm in size were placed in wells. The fraction of viable MSCs was determined by colorimetric method using MTT assay. Osteogenic medium was added to wells for assessment of osteogenic differentiation of cells, and alizarin red was used for staining. Scanning electron microscopy was used for detection of cells monolayer at implant surface at different periods of observation.

Results and Discussion

Fixation of certain cells at the surface of titanium samples without special coatings was established in 1 and 7 days of observation (Fig. 1). Figure 1 shows how MSCs acquire elongated form and do not form monolayer at the surface of titanium.

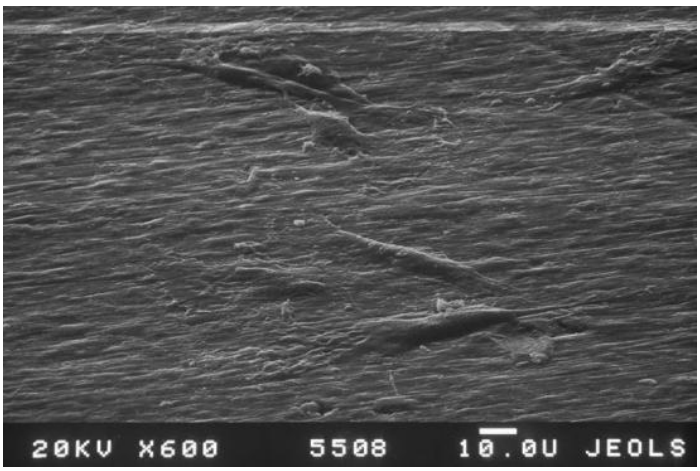

Fig.1. Distribution of bone marrow mesenchymal stem cells of newborn rabbits at the surface of titanium samples (scanning electron microscopy).

Scanning electron microscopy revealed increase of MSCs density at the surface of titanium samples with diamond-like coatings (Fig. 2) compared to Figure 1. Figure 2 shows formation of original network of MSCs.

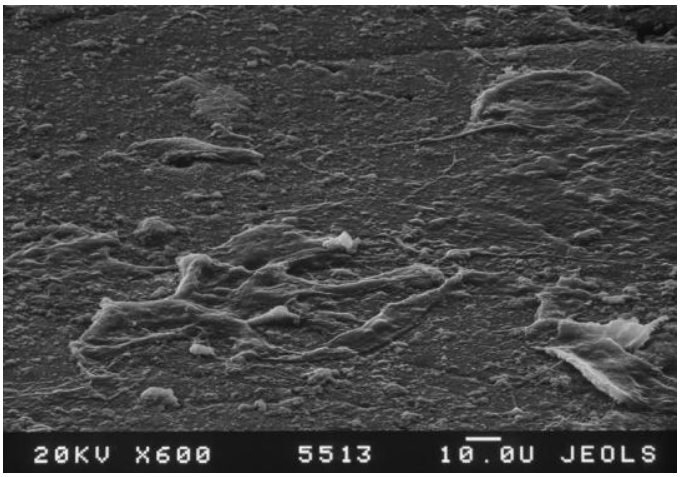

Fig. 2. Distribution of bone marrow mesenchymal stem cells of newborn rabbits at the surface of titanium samples with diamond-like coatings (scanning electron microscopy).

The most effective picture of adhesion and MSCs network formation is shown at Figures 3A and 3B. Density of cells distribution far exceeds two previous series of observations (Fig. 1 and Fig. 2).

A B

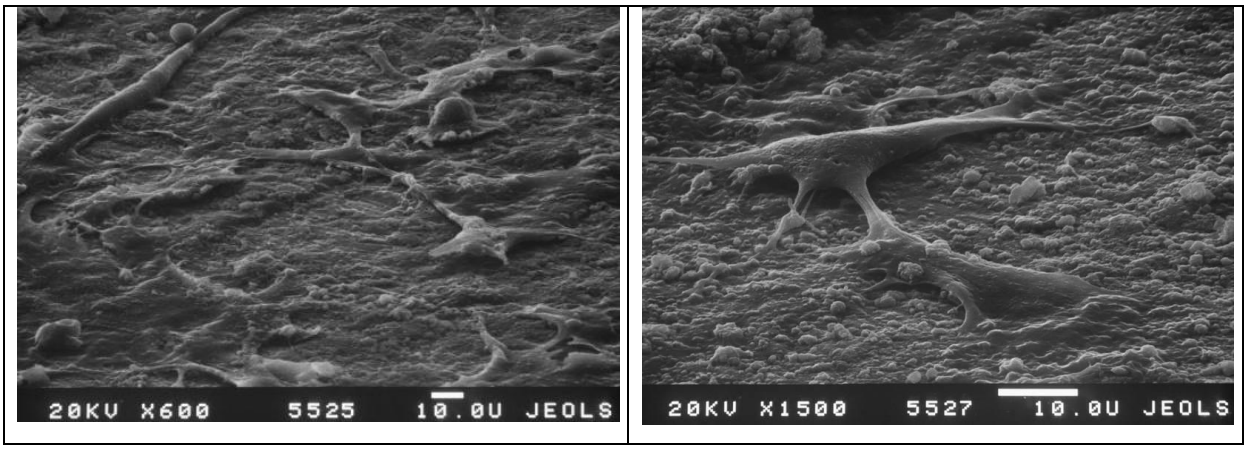

Fig. 3. Distribution of bone marrow mesenchymal stem cells of newborn rabbits at the surface of titanium samples with hydroxyapatite coatings: horizontal (A) and side (B) surfaces (scanning electron microscopy).

Conclusion

Obtained results indicate high level of biocompatibility of titanium implants with hydroxyapatite or diamond-like coatings.

Acknowledgments: We express our highest esteem and thank Scientific Enterprise “Medbiotech” (Minsk, Republic of Belarus) for cooperation and sampling of standardized titanium screws BT-16.

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CP2. THE METHOD OF NEUROTROPHIC EFFECT MODULATION AND ANTI-INFLAMMATORY EFFECT ACTIVATION IN BONE TISSUE AFTER IMPLANTATION OF DEVICES WITH DIAMOND-LIKE COATINGS

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Abstract

Objective: to perform comparative analysis of bone tissue nociceptive reactions state in male Chinchilla rabbits after fixation of titanium implant with diamond-like coating in femoral bone.
Methods: 18 anesthetized rabbits underwent fixation of 1-2 titanium screws into distal metepiphysis of femoral bone. First group of rabbits (n=9) had titanium screws' surface covered with diamond-like coating, and the second one (n=9) had uncoated screws. All the animals were sacrificed at 4, 12 и 24 weeks. Structural features of bone tissue were studied after hematoxylin-eosin staining.
Results: All the animals had no signs of surgical wound infection after the implantation procedure. Histological examination of the 1st group (implants with diamond-like coating) revealed preservation of femoral bone periosteum at the site of implantation and normal state of bone marrow in 4, 12 and 24 weeks. Histological examination of the 2nd group (uncoated implants) revealed signs of local inflammation in bone marrow, multiple sequestrate in bone tissue and destruction of periosteum in 12 and 24 weeks.
Conclusion: Diamond-like coatings on titanium implants provide leveling of adverse effects in bone tissue in post-operative period. Preservation of periosteum with nerve endings at the sites of

implantation is one of the key conditions of protective (nociceptive) reactions realization because of adverse effects leveling and retention of bone tissue structure.

Key words: implant, diamond-like coating, bone, inflammation, rabbit

Introduction

Destruction of bone tissue is accompanied with violation of bone single structure. Different implants are used to restore destroyed structure of bone tissue. Any implant is foreign substrate that is why protective (nociceptive) reactions are a priori initiated in the living organism in order to eliminate negative effects of implantation. What are the consequences of modern implants use in the comparative aspect? How effective is modern techniques of implantation? Two variants of titanium implants – diamond-like coated and uncoated – were chosen to answer these questions in the experimental study. Fixation of implants is always accompanied with additional injury of bone tissue. First of all structure and functions of periosteum are violated. Periosteum is a thin layer of connective tissue covering external surface of all bones except articulations. There are many nociceptive nerve endings in periosteum [1]. It also provides bone perfusion. Periosteum is fixed with Sharpey fibers to the bone. Cambial layer of periosteum contains progenitor cells becoming osteoblasts and chondroblasts. Neurotrophic role of periosteum manifests due to presence of such involved in nociceptive reactions neuropeptides as substance P (SP), calcitonin gen-related peptide (CGRP) [2], vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY) and tyrosine hydroxylase (TH). Large amount of nerve endings are found near epiphyseal plate and in periosteum, i.e. in the regions with high osteogenic activity. The role of bone tissue in the calcium level control in the organism and the presence of systems of intra- and extracellular calcium level control give the evidence of important neuroendocrine [3, 4] and nociceptive significance of bone tissue. The task of the study was to perform comparative analysis of nociceptive reactions state bone tissue of male Chinchilla rabbits after fixation of titanium implant with diamond-like coating in femoral bone.

Methods

All procedures involving experimental animals (18 rabbits) were conducted using a Protocol approved by the Ethics Committee of St. Petersburg Research Institute of Phthisiopulmonology, Ministry of Health of the Russian Federation. All steps of investigation were carried out in accordance with International guidelines to minimize animal suffering. Animal experiments were conducted at the St. Petersburg Research Institute of Phthisiopulmonology. Surgical procedures were performed under aseptic conditions with the rabbits under intravenous (into ear marginal vein) anesthesia with Zoletil («Virbac Sante Animale», France) (6.6 mg/kg of body mass). Operation was performed without endotracheal intubation and did not exceed 20 minutes. Premedication was made with intravenous injection of Diazepam (1.0 mg/kg, Sigma). 18 anaesthetized rabbits underwent skin and soft tissues dissection in femoral area in distal metaepiphysis of femoral bone. Then fixation of 1-2 titanium screws 2 mm in diameter and 6 mm in length was performed. Eighteen adult male rabbits weighing 2.10± 0.14 kg were randomly divided into two groups. First group of rabbits (n=9) had titanium screws' surface covered with diamond-like coating [5], and the second one (n=9) had uncoated screws. All incisions were closed using interrupted silk sutures after the operation. Rabbits postoperatively received 1 g of amoxicillin once per day for 5 days. Sutures were removed in 10 days after surgery.

Every animal underwent X-ray examination in 1 week after the implantation and at the last day of the experiment in order to control the state of bone tissue around the implant. Animals were euthanized using intravenous injection of Pentobarbital (100 mg/kg, Sigma). Titanium screws were carefully extracted after the euthanasia. Then the bone fragment with the site of implantation was retrieved. 18 bone blocks with bone fragments were immersed in a solution of 4 % formaldehyde, dehydrated in graded ethanol and acetone. Nondecalcified bone specimens were infiltrated and embedded in glycol

methacrylate resin. For each sample, 7-μm serial sections were cut and fixed in buffered isotonic formaldehyde and embedded in paraffin. After 24 h, samples were immersed in 70 % alcohol, stained with hematoxylin-eosin and examined using a light microscope (Leica DM-RBE) equipped with a high-resolution video camera (Q-500 MC; Leica). Analogue image was converted into digital one with x40 zoom. The region of "bone-implant" border was shot.

Results and Discussion

All the animals had no signs of surgical wound infection after the implantation procedure. Cicatrix was formed and hair coat partially restored against 7 days of antibacterial treatment. Histological examination of the 1st group (implants with diamond-like coating) revealed preservation of femoral bone periosteum at the site of implantation and normal state of bone marrow in 4, 12 and 24 weeks. The region of normal bone tissue structure near bone marrow with lipocytes is marked with arrow at Figure 1.

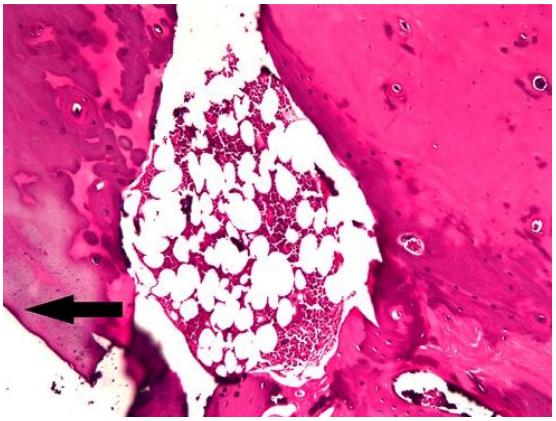

Fig.1. Histological specimen of "implant-bone" division border fragment in 6 months after implantation of titanium implant with diamond-like coating into distal metaepyphisis of rabbit femoral bone (hematoxylin-eosin staining, slice thickness 7 micron, x40 zoom). The region of bone tissue around bone marrow is marked with arrow.

Figure 2 shows the absence of local inflammation signs in bone marrow and preserved canicular structure of bone tissue (central part of the Figure 2).

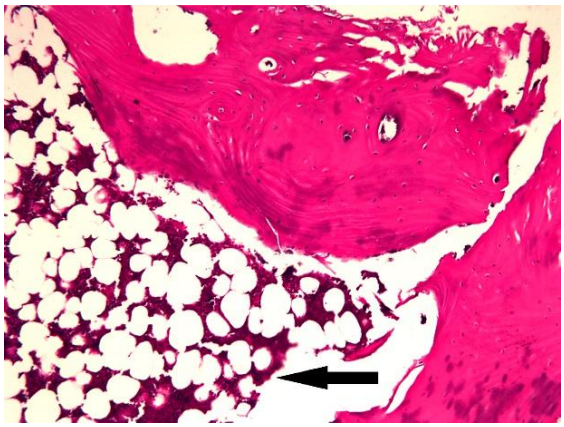

Fig. 2. Histological specimen of "implant-bone" division border fragment in 6 months after implantation of titanium implant with diamond-like coating into distal metaepyphisis of rabbit femoral bone (hematoxylin-eosin staining, slice thickness 7 micron, x40 zoom). The bone marrow is marked with arrow.

Histological examination of the 2nd group (uncoated implants) revealed signs of local inflammation in bone marrow, multiple sequesters in bone tissue and destruction of periosteum in 12 and 24 weeks (Figures 3 and 4).

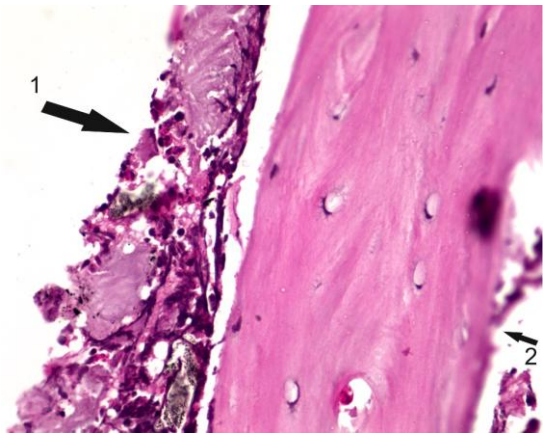

Fig. 3. Histological specimen of "implant-bone" division border fragment in 6 months after implantation of uncoated titanium implant into distal metaepyphisis of rabbit femoral bone (hematoxylin-eosin staining, slice thickness 7 micron, x40 zoom). The zone of bone tissue infiltration with leukocytes is marked with arrow 1. Arrow 2 is near the damaged periosteum.

The region of bone tissue with multiple leukocytes and destructive processes is marked with arrow 1 at Figure 3. Bone tissue sequesters heavily infiltrated with leukocytes (neutrophils, mononuclear) at the "implant-bone" division border is also shown at Figure 3. Damaged periosteum is shown with arrow 2 at Figure 3. Figure 4 shows multiple necrotic bone and tightly packed trabecular bone. Figure 4 shows severe destruction of bone tissue in 6 months after uncoated titanium implants fixation in femoral bone.

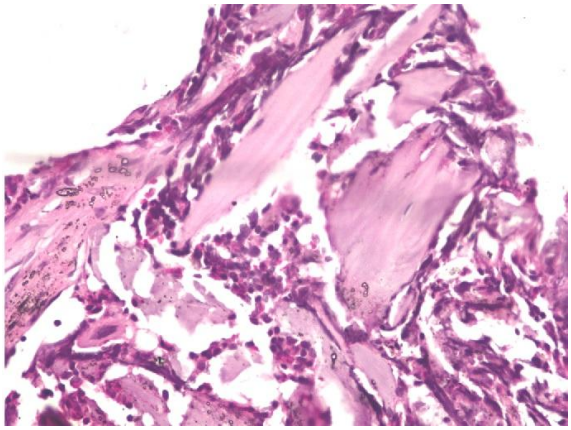

Fig. 4. Histological specimen of "implant-bone" division border fragment in 6 months after implantation of uncoated titanium implant into distal metaepyphisis of rabbit femoral bone (hematoxylin-eosin staining, slice thickness 7 micron, x40 zoom).

Conclusion

Performed experimental studies showed significance of different moments of constructions implantation into bone tissue. The choice of implantable material was the key point in implantation technology [5]. Diamond-like coatings on titanium implants provide leveling of adverse effects in bone tissue in post-operative period. Preservation of periosteum with nerve endings at the sites of implantation is one of the key conditions of protective (nociceptive) reactions realization because of adverse effects leveling and

retention of bone tissue structure. The high importance of nerve tissue in the development of systemic and local protective (nociceptive) reactions accompanied with inflammation was shown [6]. This process is of high relevance in age aspect [7], when the incidence of bone fractures increases. Due to actuality of bone tissue functions preservation in any age [6], it is necessary to focus full attention on the choice of devices and techniques of implantation [8, 9], because this issue aspect may become critical in the treatment outcome.

Acknowledgments: We express our highest esteem and thank Scientific Enterprise "Medbiotech" (Minsk, Republic of Belarus) for cooperation and sampling of standardized titanium screws BT-16.

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Session D

Chairpersons:
Assoc. Prof. Evelina Shikova-Lekova, MD, PhD
National Centre of Infectious and Parasitic Diseases, Sofia

Assoc. Prof. Radostina Alexandrova, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Tanya Zhivkova, MSc
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

DO1. ANTIMICROBIAL ACTIVITY OF FLUORESCENT BENZANTHRONE DERIVATIVES

D. Staneva[1], S. Grabchev[2], E. Vasileva-Tonkova[3], P. Bosch[4], I. Grabchev[5]

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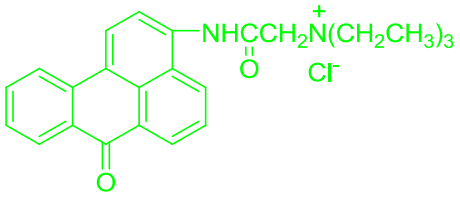
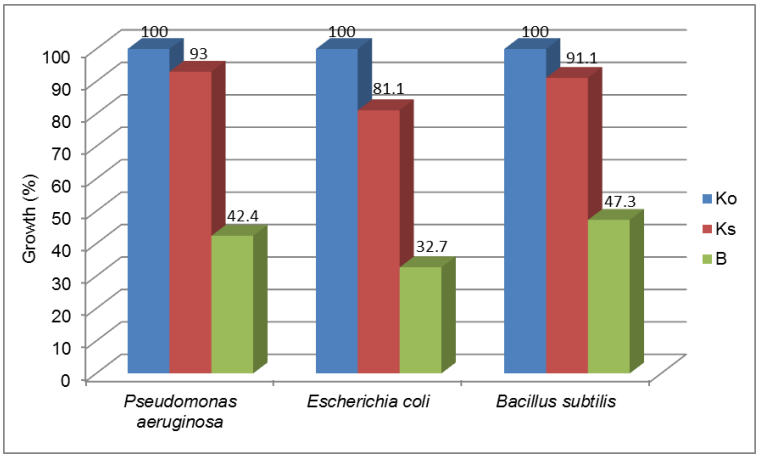
[2]Sofia University "St. Kliment Ohridski", Faculty of Chemistry and Pharmacy, 1164 Sofia, Bulgaria
[3]Institute of Microbiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[4]Institute of Science and Technology of Polymers, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain
[5]Sofia University "St. Kliment Ohridski", Faculty of Medicine, 1407 Sofia, Bulgaria

The antimicrobial activity of newly synthesized benzanthrone derivatives with biocide quaternary ammonium group has been investigated *in vitro* against various Gram-positive and Gram-negative indicator bacteria and yeasts. The results revealed good inhibitory activity of the novel benzanthrone compounds against the tested microbial cultures. Thin polylactide film



Benzanthrone (**B**)

(PLA) with compound **B** into the polymer matrix has been prepared and its antimicrobial ability in aqueous solution has also been evaluated. The gradual release of the substance with antimicrobial activity has been investigated. The results showed that the compound B may be released slowly into the water solution and the polymer film exhibits a prolonged antibacterial activity which guaranties the very good activity against P. aeruginosa, E. coli and B. subtilis.



Effect of PLA film (K_S) and PLA-**B** film on the growth of *P. aeruginosa*, *E. coli* and *B. subtilis* strains tested in nutrient broth (NB). K_O, NB without added PLA film

Acknowledgment: The authors, acknowledge with thanks to COST-CA15114: Antimicrobial coating innovations to prevent infectious diseases (AMICI)

DO2. INDUCED BY GLUCOSE FORMATION OF BIOFIMS BY *Bacillus subtilis* AND *Escherichia coli* STRAINS

Иво Тодоров Ганчев
Институт по микробиология „Стефан Ангелов", Българска академия на науките

DO3. INTERSPECIES RELATIONSHIPS IN THE STRUCTURE OF BIOFILMS

Иво Тодоров Ганчев
Институт по микробиология „Стефан Ангелов", Българска академия на науките

DO4. УСТАНОВЯВАНЕ НА БИОАКТИВНИ ПЕПТИДИ С ИМУНОМОДУЛИРАЩИ СВОЙСТВА В БЪЛГАРСКО СИРЕНЕ, ОСВОБОДЕНИ ОТ МКБ ПО ВРЕМЕ НА ЗРЕЕНЕТО

И. Готова[1,2]; Ж. Димитров[1]; Хр. Найденски[2]
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DETECTION OF BIOACTIVE PEPTIDES WITH IMMUNOMODULATORY PROPERTIES IN BULGARIAN CHEESE, RELEASED BY LAB DURING RIPENING

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Abstract

The immunomodulatory properties of bioactive peptides released during the cheese ripening were studied. The main object of the present study was the evaluation of cytotoxic immunomodulatory potential of bioactive peptides in cheese produced with a new starter, in comparison to the control

cheese produced with traditional starter containing L. bulgaricus and S. thermophilus only. Lactic acid bacteria with strong proteolytic abilities were selected in order to obtain higher quantity of low- molecular weight peptides in Bulgarian white brained cheese. The bioactive peptides were separated and purified to single peptides so they can be evaluated for their immunomodulatory properties. Interferon-gamma was used as the main marker of cytotoxic effect, induced by LAB in mouse splenocyte model. It was proven that the low-molecular weight peptides from cheese produced with the new developed starter induce the production of Interferon-gamma. Such production was not detected by the peptides in cheese produced with regular starter. The mean value of Interferon-gamma in splenocyte model, induced by certain peptides in cheese with the new starter, was 12,5 pg/ml. The developed starter can increase the beneficial properties of Bulgarian white brined cheese.

Key words: bioactive peptides, immunomodulatory properties, splenocyte model

Въведение

С помощта на богатия си протеолитичен комплекс определени щамове при условията на зреене на сиренатa могат да освобождават биоактивни пептиди, разграждайки протеиновите фракции на казеина и на суроватъчните протеини [1, 4]. Основна цел в това изследване бе да се открият такива щамове и да се докаже тяхната способност за продуциране на биоактивни пептиди с имуномодулиращо действие. За всичките изследвани изолати от млечнокисели бактерии (50 на брой) се колекционираха и изследваха супернатанти за оценка на имуномодулиращите активности, използвайки спленоцитна система. За разлика от целите бактериални клетки оценката на имуномодулирането от биоактивни пептиди не може да се проведе директно чрез използването на антиген-представящи имунокомпетентни клетъчни линии (макрофаги и дендритни клетки) [2]. Това е така, понеже антиген-представящите клетки след стимулиране от определени пептиди излъчват слаби сигнали към Т-хелперни клетки от 1-ви или 2-и класове. Именно тези стимулирани Т- клетки екстпресират сигнални пептиди, стимулиращи цитотоксичния (стимулиране на цитотоксичния ефект чрез индукция на клетките натурални убийци, цитотоксичните Т-клетки и т.н. с помощта на цитокините IFγ и др.) или хуморалния (стимулиране пролиферацията на В-клетки чрез експресия на цитокините IL4, IL5 и др.) имуноотговори [3, 5]. Тези цитокини IFγ, IL4, IL5 вече могат да се измерват достатъчно точно. Изобщо, ясно е че трябва да се разполага със система от имунокомпетентни клетки за обхващане на комплексността на различните имуноотговори. Такава подходяща за изпитване на имуномодулиращата пособност на биоактивните пептиди система е съвкупността от спленоцитни клетки. Като мярка за имуностимулирането бе използвано количественото определяне на експресията на сигналния пептид IFγ от система пречистени спленоцити. Това е първото изследване на имуномодулиращо действие на биоактивни пептиди, образувани при зреенето на българско бяло саламурено сирене, произведено със специално разработена закваска.

Материали и методи

1. Получаване и поддържане на спленоцитната система.

Спленоцитите се получават и пречистват с помощта на специални аналитични комплекти по инструкции на производителя (R&D SYSTEMS Inc.). Поддържането на спленоцитната система от имунокомпетентни клетки се извършва при 37°C и 5% CO2 на среда RPMI 1640 (ATCC 30-2001), съдържаща 10% фетален говежди серум (ATCC 30-2020), 4.5 g/l глюкоза, 10mM HEPES, 1mM натриев пируват, 2mM L-глутамин, 1.5 g/l натриев бикарбонат, 100 U/ml пеницилин, 100 μg/ml стрептомицин. Спленоцитните клетки с концентрация 10^6 клетки на кладенец бяха култивирани в 96 ямкова платка в присъствие на пептидни екстракти (получени след ферментация на мляко) от различни щамове, които се приготвят по следния начин: от 10-те щама с най-висока протеолитична способност бяха ултрафилтрирани супернатанти през макроцентрофужни патрони (50 мл, 5000 Da cut-off). Ултрафилтратите бяха дозирани на обратнофазови миниколони за еднократна употреба Chromabond HLB 1g. След промиване с вода, пептидите бяха елуирани с

0.1% трифлуороцетна киселина в 60% ацетонитрил. След изпаряване в ротационен вакуум-изпарител пробите бяха реконституирани с 1 мл 0.1% солна киселина.

Така приготвените пептидни екстракти се инкубират за 24 часа в кладенците със спленоцити. Следва центрофужно отделяне на клетките и получената супернатанта се използва за оценка на индукцията на IFγ.

2. Оценка на индукцията на сигналния пептид IFγ от пептидни фракции чрез сандвичова ELISA

След финала на инкубирането супернатантата се събира и се центрофугира за елиминиране на спленоцитните клетки. 200µl от получените супернатанти се използват за определяне на IFγ чрез специален комплект на R&D Systems (IFγ Immunoassay), следвайки инструкциите на производителя. Накратко: имуноплатките са покрити с моноклонално антитяло срещу IFγ, което задържа специфично IFγ и след промиване кладенците се третират с поликлонално антитяло срещу IFγ, свързано с ензима Horseradish Peroxidase; след промиване се добавят субстратите водороден пероксид и тетраметилбензидин и след провеждане на ензимната реакция на тъмно при стайна температура за 20 min следва стопиране на реакцията с 2N сярна киселина и измерване абсорбцията при 450 nm. За изчисляване концентрацията на IFγ се използват стандарти и построена стандартна крива.

3. Фракциониране на пептидната материя.

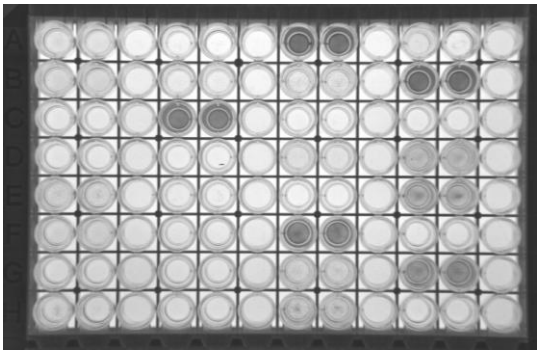
Пречистване и секвениране

Нови супернатанти, след ферментация на подбраните щамове в мляко, бяха частично пречистени чрез обратно-фазови патрони (C18ec, Waters) и подложени на центробежна ултрафилтрация с мембрана 5000 Da. TFA се добавя към пробите до 0,1% концентрация и 1 мл се инжектира в RP-HPLC колона Nucleosil C18. Градиентът на ацетонитрил в 0.1% TFA е от 0% до 80% в продължение на 45 минути. Пептидите се детектират при 210 нанометра с помощта на UV-детектор (Shimadzu) и елуатът се колекционира във фракционен колектор по 1 мл за всяка фракция. Тези фракции се очистват от ацетонитрил (чрез ротационно вакуум изпарение) и реконституирани, се оценяват за имуномодулираща активност. Фракциите демонстриращи най-висока активност се фракционират отново на HPLC колона Nucleodur Sphinx в специфични условия на градиент за съответната фракция. Последният стадий на пречистване се извършва с помощта на йонообменна HPLC колона SCX Shimadzu съдържащ бензен-сулфонови катионобменни групи в литиева форма. Елуирането се извършва чрез градиент на pH 3.0 до 9.0 в цитратни буфери. В края на пречистването се достига до единични пептиди с имуномодулираща активност. Пречистените пептиди се секвенират след фиксирането им чрез свързване на техния C-край към arylamine-PVDF мембрана. Секвенирането на пептидите откъм N-края е по метода на Edman.

Резултати и обсъждане

На фигура 1 е представена снимка от 96-ямковата платка, съдържаща пептидни фракции, някои от които индуциращи IFγ. Останалите изследвани пептидни фракции на други платки не водят до индукция на IFγ. Колкото по-интензивно е оцветяването, толкова по-значимо е индуцирането на IFγ. На всеки две съседни колони отгоре- надолу са повторени анализите на определена пептидна фракция от съответните щамове. Така в два съседни кладенеца по посочената вертикала на всеки щам, се намират еднакви пептидни фракции. За прегледност е оставена по една празна колона между значещите колони.

hV28 cas 3 h A1 b J24



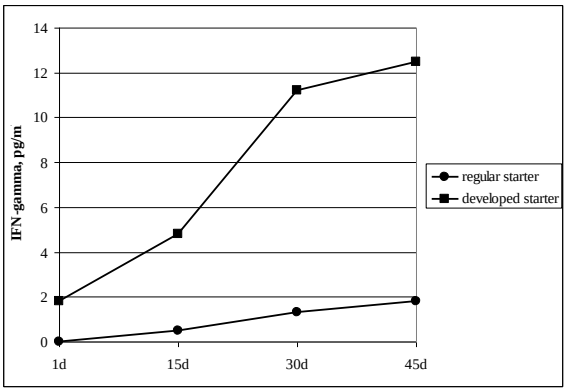
Фиг. 1. Снимка на оценката на IFγ при стимулиране с различни пептидни фракции.

Интензитетът на оцветяването е правопропорционален на експресията на IFγ.

Резултатите сочат откриването на 3 пептидни фракции със значима индукция на IFγ (позиции: A7/8, B10/11, C4/5), още 2 пептидни фракции с интермедиерна индуктивна сила (F7/8, G10/11), и още 4-5 пептидни фракции със слаба индуктивна сила. Всички останали пептидни фракции, намиращи се в кладенци без оцветяване, не притежават индуктивна сила по отношение на IFγ.

На колони 4/5 на фигура 1 са нанесени пептидни фракции от щама *L. casei* 3, като един от значимите протеолитици и един от кандидатите за разработка на закваска за сирени продукти с биоактивни функции. На колони 7/8 са оценени пептидни фракции на щама *L. helveticus* A1, откроил се като най-силния протеолитик. Този щам особено след анализа на имуностимулиращите пептиди се превръща във фаворит за включване в състава на сиренарски закваски. Прави впечатление, че при *L. helveticus* A1 активните имуномодулиращи пептидни фракции са две, а при *L. casei* 3 – една. На колони 10/11 от платката на фигура 1 са нанесени пептидни фракции от щама *L. bulgaricus* J24. Пептидните фракции от този щам проявяват значителна сила. Голямо предимство на този новооткрит щам е неговата видова принадлежност - *L. bulgaricus*, понеже поначало сред този вид много рядко се срещат щамове способни да образуват биоактивни пептиди. Щамове от вида *L. bulgaricus* се използват заедно със *Str. thermophilus* при производтвото на йогуртови продукти, включително и на Българското кисело мляко. Тоест, щамът *L. bulgaricus* J24 е много подходящ кандидат за разработка на закваска не само за сирени продукти, но и за киселомлечни такива. От фигура 1 се вижда и способността на *L. bulgaricus* J24, подобно на *L. helveticus* A1, да образуват повече от една активна пептидна фракции.

Важно е да се отбележи добрата повторяемост на резултатите, която е видна при сравнение на интензитетите на оцветяване на всеки две последователни кладенчета от две съседни колони, на които са нанесени две еднакви пептидни фракции. Тоест, възпроизводимостта на анализа е много добра.



Фиг. 2. Индукция на IFN-gamma от пептиден извлек на сирена, произведени с конвенционална и новоразработена закваска.

На фигура 2 са представени резултатите от индукция на IF-gamma от пептиден извлек на сирена, произведени с конвенционална и новоразработена закваска. Конвенционалната закваска

е 189, която от дълги години се използва при производство на бяло саламурено сирене. Новоразработената закваска съдържа двата щама с доказана способност да освобождават биоактивни пептиди с имуномодулиращи свойства - *L. bulgaricus* J24 и *L.helveticus* A1. Останалите два щама в закваската са *S.thermophilus* tN1 и *Lc.lactis* 310. Разликата в индукцията на IF-gamma, тоест и на имуномодулиращия ефект, между новоразработената и конвенционалната закваска е много висока в полза на новоразработената закваска. При 45-я ден от зреенето на моделните сирена общият пептиден извлек индуцира 12,5 pg/ml IF-gamma в спленоцитната система.

Заключение

Резултатите показват, че е разработена закваска за бяло саламурено сирене, водеща до образуването на биоактивни пептиди със значима имуномодулираща способност. Закваската се състои от щамовете *L. bulgaricus* J24, *S.thermophilus* tN1, *Lc.lactis* 310, и *L.helveticus* A1.

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DO5. USAGE OF ELECTRON MICROSCOPY IMAGING FOR HHV-6 INFECTION DIAGNOSIS IN PATIENTS WITH AUTOIMMUNE THYROIDITIS IN HELP OF STANDARD CLINICAL PROCEDURES

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Abstract

Human herpesvirus-6 (HHV-6) infection is very common and has a worldwide distribution. HHV-6 is a betaherpesvirus related to several chronic autoimmune inflammatory processes including Hashimoto's Thyroiditis (HT) - the most frequent of all autoimmune thyroid disorders. According to available literature data and our own researches, HHV-6 increased viral loads were often exhibited in HT biopsies,

compared to controls (benign follicular epithelial lesions), which indicates the virus participation and facilitator role in the development of immunologic attack to thyroid gland and inflammation.
The aim of this study was to examine and evaluate the particular involvment of HHV-6 presence as predisposing factor for autoimmune thyroiditis and to evaluate its relation to thyrocytes injury by electron microscopy, based on the visualization and morphological identification of virus particles.
Seven female patients in age between 28 and 65 years, with histologically confirmed autoimmune thyroiditis and positive HHV-6 genomic sequence detection (nPCR), were involved in this research. BD FACSAria II flow cytometer and BD FACSDiva software were used for sorting and analysing of peripheral blood mononuclear cells (PBMC). DNA from the main lymphocyte subpopulations was also examined for HHV-6 load by RT-PCR.
Our results showed that in two of the patients enrolled in the study, HHV-6 genomic sequence was detected using RT-PCR in thyroid gland tissue (355 copies/1x10^6cells) and in whole blood (46 copies/1x10^6cells). HHV-6 loads were detected in two of the patients – in NK and $CD95^+$ cells (24 and 100 viral copies/1x10^6cells, respectively for the first patient) and in $CD95^+$ cells - (40 copies/1x10^6cells for the second patient).
The light microscopic examination of thyroid glands showed different stages of follicular damage, expressed in alteration of follicular shape and cell appearance, decreased intensity of colloid staining, extensive lymphoid cell infiltration, coalescence of adjacent follicles and atrophy, and slight to moderate fibrosis of the interstitial tissues. At ultrasrtuctural level, the thyroid follicular cell damage was manifested in moderate margination of the condensed chromatin and changed shape of nuclei (differ from typically round), swollen mitochondria and vacuolization in the cell cytoplasm. Virus-like particles, morphologically identical to HHV-6 nonenveloped nucleocapsids and multivesicular bodies (MVB), associated with HHV-6 infection, were observed predominantly in the cytoplasm and membranous organelles of thyrocytes by means of electron microscopy.
All presented data show that thyrocytes act as target cells for HHV-6 amid the progression of an autoimmune inflammatory process.
Key words: *Human herpesvirus-6, Hashimoto's Thyroiditis, electron microscopy*
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DO6. НАШЕСТВИЕТО НА КОРОНАВИРУСИТЕ: CoV-SARS

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DO7. НАШЕСТВИЕТО НА КОРОНАВИРУСИТЕ: CoV-MERS

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DO8. ALGORITHM FOR INVESTIGATION OF PREGNANT WOMEN EXPOSED TO THE VIRUSES THAT CAUSE NON-VESICULAR (MACULOPAPULAR) RASH

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Abstract

Introduction:

Viral infections during pregnancy, along with some form of accompanying pregnancy diseases such as diabetes, cardiovascular, gastrointestinal, kidney and others, are a major cause of arising complications and mortality of mother and fetus.

This study **aims** analysis of samples from immunocompetent pregnant women exposed to non-vesicular (maculopapular) rash, and can be used to study the possible contact (search for the etiological agent) with the following viral agents: measles, rubella and parvovirus B19.

Materials and methods: The total 53 serum samples collected January 2015 - April 2016 from pregnant women were tested in NRL Measles, Mumps, Rubella, NCIPD. The serological (indirect ELISA IgM and IgG test) and molecular methods (PCR and RT-PCR test) were used.

The study included the pre-analytical (clinical syndrome) stage, the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Results: The algorithms assume that immunization status for measles and rubella has been determined from the vaccination record and/or serological results (ELISA IgG). A person is considered immune if they are immunocompetent and either have had two doses of a vaccine (MMR) that protects against rubella and measles or have laboratory evidence of prior immunity. Where immunization history or prior tests suggest no immunity, or immune status is unknown, proceed to testing algorithms (ELISA IgG). The positive ELISA IgM results and evidence of viral DNA/RNA are indicator for acute viral infection. About parvovirus B19 they were detected in 6/53 (11.32%) pregnant women. About measles and rubella markers for recent infection were not found. Monitoring of pregnancy and the requirement to provide tracking serum samples (1-3-6 months) were recommended.

Conclusions: A specific cause for maculopapular rash illness is rarely investigated or confirmed. Therefore, for pregnant contacts of maculopapular rash illness, investigation of parvovirus B19, rubella and measles immunity may be considered in parallel if clinically indicated and especially when epidemiological linked were detected. For detected congenital infection a stored antenatal booking sample may be used for convenience.

Key words: pregnant women, maculopapular rash, measles, rubella, parvovirus B19, ELISA, PCR

DO9. A POSSIBLE ASSOCIATION BETWEEN HPV16/18 AND LUNG CANCER?

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Introduction: The role of human papillomavirus (HPV) in the development of lung cancer is still unclear. One of the reasons for this is the wide variation (0 to 100 %) of reported HPV infection rates in these cancers. This may be explained by the geographic differences in HPV prevalence or/and by the different detection techniques employed. There are no data about the link between HPV and lung cancer for Bulgaria. The objective of our study is to investigate the prevalence of HPV16/18 in specimens from lung cancer cases from Bulgaria using two detection systems.

Materials and Methods: We analyzed a total of 142 biopsy materials from patients with lung cancer and other chronic pulmonary diseases. Of all materials 119 were from patients with histologically proven lung cancer - cases with squamous cell carcinomas, adenocarcinomas, adenosquamous carcinomas, small cell carcinomas. The biopsy materials from patients with other lung diseases were used as negative controls.

Detection of HPV was based on two PCR systems: consensus PCR system GP5+/6+ and type-specific (TS) PCR system. Each sample was subjected to three parallel PCRs using broad spectrum GP5+/6+ primers and TS primers for HPV types 16 and 18.

Results: Of the 102 β-globin positive lung carcinoma samples 33 (32.4 %) were positive for HPV16 and/or HPV18 DNA by TS PCR and only 4 (3.9%) samples were HPV positive by consensus GP5+/6+ PCR system. HPV 18 was more prevalent, found in 15 (14.7%) of β-globin positive samples. HPV16 was detected in 12 (11.8%) of β-globin-positive samples. 5.9% of β-globin-positive lung cancer samples were positive for both, HPV16 and HPV18. All patients with other lung diseases were HPV negative. The correlation of HPV status with histopathological diagnosis revealed the highest rate of HPV16/18 positivity in adenocarcinoma samples.

Conclusions: Our study shows a high HPV16/18 prevalence in lung carcinoma samples from Bulgarian patients when TS PCR as detection method is used. There is significant difference in sensitivity between the two PCR systems used in this study for detection of HPV16/18 in lung cancer samples. HPV18 was more prevalent than HPV16, contrary to the cervical cancer, where HPV16 is more common.

DO10. HIV-1 GENETIC DIVERSITY AMONG HETEROSEXUALS IN BULGARIA

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Background

From 1986 until 2012, 1606 HIV/AIDS cases were registered in Bulgaria, 911 (56.7%) of them were heterosexuals (HET). The aim of the present study was to determine HIV-1 diversity and among HET individuals in Bulgaria.

Materials & Methods

322/911 (35.3%) HIV-1 *pol* gene sequences from HET individuals were analyzed. HIV-1 subtypes were classified using internet based tools COMET v1.0, REGA v3, manual phylogenetic analysis with ML in FastTree 2 program and recombinant analysis using bootscan with SimPlot.

Results

47/322 (14.6%) of HET were migrants, including 5 foreigners diagnosed with HIV-1 in Bulgaria and 42 Bulgarian citizens infected abroad. 40.7% from studied HET were from the capital city of Sofia, the mean age was 33.4. The most common subtype was B 38.8%, followed by CRF01_AE 19.6%, subtype C 6.8%, CRF02_AG 6.5% and a big variety of 29 HIV-1 subtypes, CRFs and unique recombinant forms.

Conclusions

We found high genetic diversity among HET with domination of non-B subtypes. Non-B subtypes were more common in HET individuals compared to non-HET transmission groups. Our study indicated that providing of molecular epidemiological surveillance of HIV-1 diversity and resistance mutations is of importance to better control HIV-1 epidemic in Bulgaria.

ГЕНЕТИЧНО РАЗНООБРАЗИЕ НА HIV-1 СРЕД ХЕТЕРОСЕКСУАЛНИ ЛИЦА В БЪЛГАРИЯ

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Цели

От 1986 до 2012 г. 1606 случая на HIV/СПИН са регистрирани в България, от тях 911 (56.7%) са хетеросексуални лица (ХЕТ). Целта на настоящето проучване е да бъде установено генетичното разнообразие на въведените субтипове сред ХЕТ в България.

Материали и Методи

HIV-1 субтиповете бяха анализирани при 322/911 (35.3%) HIV-1 *pol* секвенции и бяха класифицирани с интернет базираните инструменти COMET v1.0, REGA v3, мануален филогенетичен анализ с ML във FastTree 2 и анализ за наличие на рекомбинации чрез bootscan със софтуер SimPlot.

Резултати

47/322 (14.6%) от ХЕТ са мигранти, включително 5 чужденци диагностицирани в България и 42 български граждани инфектирани в чужбина, 40.7% от изследваните ХЕТ са от София, а средната възраст е 33.4 години. Най-често срещания субтип е B 38.8%, следван от CRF01_AE 19.6%, субтип C 22 6.8% и още над 29 различни HIV-1 субтипове, циркулиращи рекомбинантни форми (CRFs) и уникални рекомбинантни форми.

Заключения

Беше установено голямо генетично разнообразие сред ХЕТ с доминиращи не-B субтипове. Не-B субтиповете са по-често срещани в тази трансмисионна група в сравнение с други трансмисионни групи. Резултатите от нашето изследвани показват необходимостта от провеждането на молекулярно-епидемиологични проучвания на HIV-1 епидемията в страната сред различните трансмисивни групи, за по-доброто контролиране на инфекцията в България.

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Session E

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EO1. NEUROMARKETING. BUY-OLOGY IS A MASTERPIECE.

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"Products are made in the factory, but brands are created in the mind"
Walter Landon
(Martínez 2012, p. 115)

Abstract

Neuromarketing is an important development in the field of understanding how the subconscious mind helps the consumer to make decisions. This article investigates the literature regarding the neuromarketing tools, methods and specific techniques. In recent years, long-standing science, advanced technology, and complex management have come to a common ground that researchers and marketers are able to map science to marketing. Marketers sublimely attract the customer towards their product. For this ground-breaking research has taken place in various areas of studying the human brain. Innovation in techniques is taking place by neuroscientists, and the field of neuroscience is being tapped into extensively. Neuromarketing is expected to be used broadly by marketers. Neuromarketing is believed to provide more accurate insight into people's psychological reactions to stimuli and, hence, their actions in buying situations because people cannot always verbalize their feelings and thoughts in accurate, unambiguous ways.

This encouraged marketers to use the neuro-imaging techniques to identify decision making actions among shoppers to help companies directly click the "buy button" on the customer's brain to boost sales. Given that neuromarketing has borrowed a series of neuroscience-specific methods and techniques, the neuromarketing or consumer's neuroscience represents a passing from marketing specific instruments, such as the focus group, the observation, the direct enquiry, the interview to a series of instruments, which record both the brain's electrical activity and the metabolic activity. There are several ways to measure physiological responses to advertising. Still, there are currently three main well-established and non-invasive methods for measuring and mapping brain activity in this relation:

functional magnetic resonance imaging (fMRI), *electroencephalography* (EEG) and *magnetoencephalography* (MEG). The other methods are: *positron emission tomography* (PET), *eye-tracking* (ET), *facial recognition* (or electromyography), *galvanic skin response* (GSR), *cardiovascular parameters, transcranial magnetic stimulation* (TMS). Due to their non-invasive nature, these methods represent the majority of studies conducted within the field of neuromarketing.

Key words: neuromarketing, brain mapping, consumer behaviour, marketing, neuroscience, advertising, decision making, body language

What is Neuromarketing?

Where brain science meets brand architecture, where neurons meet new products-that is where you will find neuromarketing. However, names can be deceiving: it is not a new type of marketing, but it is rather a measurable, empirical way to study the impact of marketing and advertising on consumers. The techniques that fall under the umbrella of neuromarketing are based on scientific principles about how humans really think and decide, which relies on a host of brain processes of which we are mostly unaware. Marry these principles with smartly designed experiments and you have an unparalleled peek into the mind of the modern consumer [1].

What are the tools of the trade?

Measuring consumer response and behaviour in a Neuromarketing experiment might include any of the following:

- Eye tracking experiments (measuring eye gaze patterns, say, on a landing page)
- Analysing facial expressions
- Behavioural experiments (for example, seeing how changes in the color of a product impact a customer's opinion of it)
- Biometrics (body signal measures) that measure perspiration, respiration, heart rate, and facial muscle movement (electromyography [EMG])
- Neuromeric (brain signal measures) that measure electrical activity (electroencephalography [EEG]), and blood flow (functional magnetic resonance imaging [fMRI]) in the brain [6].

The application of neuroscience to marketing has gained popularity over the last decade. The birth of the nascent field of consumer neuroscience generated wide–ranging, ongoing debates what benefits they take. A primary and critical distinction is between "consumer neuroscience" should be understood as the scientific study of biological bases of cognitive and affective processes underlying consumer behaviour.

Ale Smidts first coined the word neuromarketing in 2002. He studied consumers' sensorimotor, cognitive, and affective response to marketing stimuli [1].

Author and marketing guru **Martin Lindstorm's** bestselling book "Buyology - Truth and Lies About Why We Buy" (2010) [6] claims from his experimental studies that subconscious mind plays a major role in people's buying decisions. The author seem to be mystified while the marketers still try to unravel the gap between the consumer intention and action. Neuromarketing is not a new kind of marketing - it is a new way to study marketing, so it is part of the field of market research. Here are six major areas where neuromarketing is used today: **Branding, Product design and innovation, Advertising effectiveness, Shopper decision making, On-line experiences, Entertainment effectiveness.**

Neuromarketing involves application of cognitive neurosciences in the field of marketing and marketing research. It uses a brain mapping medical technology known as fMRI to study blood flow and blood oxygenation in the neuron activity of consumers at the time of selecting and buying a product. Though it started with the application of neurosciences, over the years it gained entry into the traditional methods of doing marketing research. As research proceeded, it was applied to promote sales and

research organizations such as Bright House Institute was set up to serve corporations eager to reap the nascent developments in the field.

Branding expert Dr. Peter Steidl says neuromarketing will change the face of marketing, and without it, campaigns will lag behind competitors that have embraced this new way of thinking about consumer behavior and branding.

He is not talking about lab tests that deliver reliable but limited information about how consumers process marketing stimuli such as ads, logos or package designs. Rather, he is referring to the application of neuroscience concepts in a strategic context. **In other words, how marketers can benefit from the latest insights into how consumers think, feel and, and most importantly, make purchase decisions.**

The application of neuroscience lifts the effectiveness of marketing, brand, communications, and shopper marketing, pricing and innovation strategies – including, of course, social and other digital media strategies.

Brief overviews on three important neuroscience insights, highlighting a number of important marketing implications are as follows:

Insight 1: Consumers have two parallel circuits in their mind, one for thinking and one for doing - Nobel Prize Laureate Daniel Kahneman simply called them System 1 and System 2, although marketers may want to think about these circuits as the consumer's 'doing' and 'thinking' minds, respectively. Harvard professor Gerald Zaltman suggests that 95% of purchase decisions are made by the non-conscious 'doing' mind.

Insight 2: The brain is designed to avoid thinking by using shortcuts to make purchase decisions – The 'doing' mind influence extends even further. Having been designed to help us survive in a hostile natural environment, the 'doing' mind has developed energy preservation strategies. In addition, as the brain – which accounts for only some 3% of body weight – accounts for approximately 20% of all energy consumed, the 'doing' mind seems to have focused on finding ingenious decision shortcuts that eliminate the need for thinking.

Insight 3: the marketer's challenge is to shape the consumer's brand memory - a marketer needs to invest into developing a positive, emotionally strong brand memory that is linked to one or more of the consumer's goals. However, current practice is often not aligned with this principle. It is important to ensure at all touch points that the brand plays a central, emotionally engaging role. If that is not the case, you may get consumers involved but they will not end up buying your brand. Adopting a neuroscience perspective, it can be said that the principle task is not marketing management, but memory management. The good news is that applying neuroscience insights in marketing is neither complicated nor difficult. It is just a matter of integrating our understanding of how consumers think, feel and make decisions with well-established marketing concepts and tactics to lift their effectiveness.

Neuromarketing Techniques

This bridge between several lines of neuroscience and marketing allows each of these techniques to have enhanced or reduced applicability to respond to different problems that traditional marketing techniques cannot respond to, or respond to only partially. Among all of the techniques, new and old, the ones that drew the most attention were the techniques that used brain imaging, and the results of these studies had great impact in both academia and business. One of the first studies that demonstrated this potential was conducted in Harvard in the late 1990s using some invasive equipment called PET-SCAN (Zaltman, 1997). Another important milestone project used another technique that also draws considerable attention, functional magnetic resonance, which is costly but not invasive equipment (McClure et. al, 2004).

If no area of the brain underwent changes after having been exposed to an advertising stimulus, then this stimulus was not successful. However, if the stimulus caused organic modifications in a region, it can be inferred that there is a correlation between advertising and the activated emotion (Fugate, 2007). It is flippant, though, to say that a specific area of the brain that lights up during the investigations does so because an image triggered certain emotions and patterns of

consumption, as different emotions depend on different combinations of activations of neural substrates (Marcus, 2012). Each of the techniques has advantages and disadvantages, often measuring variables that are complementary to better understand a marketing problem. Therefore, to obtain an effective result with Neuromarketing technologies, it is advisable and productive to use combined techniques whenever possible. There are three types of Neuromarketing techniques: those that measure the metabolic activity in the brain or related to it, those that measure electrical activity in the brain and those that do not measure brain activity. The main Neuromarketing techniques are, currently, the following:

• **Functional magnetic resonance imaging (fMRI)** is a technology which uses basic Physics and Biology. The advantage of this technique is its ability to measure deeper and smaller structures of the brain, i.e., with high spatial resolution. Along with the technique of electroencephalography, it is the most employed technique to measure brain activity in the field of neural science. However, the use of this technique is expensive, the equipment is not portable, and the environment makes it difficult to conduct the experiment. This technique requires a delay of 6 to 10 seconds to record the processing of neurons, which represents a great disadvantage with respect to several marketing stimuli because these numbers constitute low temporal resolution (Ariely & Berns, 2011).

It uses a powerful magnet and radio waves to create high-resolution image of the living brain. It draws on the fact that the Red Blood Cells(RBC) in the blood contain iron in the oxygen- carrying- part of the hemoglobin and these atoms create distortions in the magnetic field around them. While any part of the brain becomes active, the blood vessels in the specific region dilate causing more blood to flow in that region to supply the additional oxygen and glucose required by the more active brain cells to do their work. This large amount of freshly oxygenated blood in to the region causes a small change in the magnetic field.

The result is displayed as a patchy area of colour, amidst the high-resolution grey background of the brain. The coloured area represents the active region as opposed to the grey background, which represents the inactive region of the brain. Armed with such high-resolution 3D images of the brain on a real time basis, one can pinpoint exactly which part of the brain is active.

This area-specific knowledge plays a significant role in the utility of fMRI. Several parts of our brain work together. Even as you read this article, the connectomes related to your visual sense along with the ones responsible for reading and understanding the material are working. Each region with a rich intermesh of neurons is responsible for a certain activity. The more you stress on any activity, the more the work done by that part of the brain and more the blood flow in that region. The interesting part is, the region responsible for each activity is well demarcated in the human brain. While the whole brain is yet to be mapped by scientists, yet various centres of the brain are already known for various though processes such as reward centre, face-recognition centre, self-referencing centre, liking centre, anticipation centre etc.

• **Electroencephalography (EEG):** in this technique, electrodes that measure the brain waves associated with different states of stimuli are placed on an individual's scalp through bands or helmets, and these waves can be measured at small intervals up to 10,000 times per second [7]. In addition to having the advantages of being more widely available, being less invasive and cheaper, EEG presents greater validity in the measurement of emotional styles and the detection of psychopathologies (Kline, 2004). This technique features the possibility of synchronization with the stimuli, and the equipment is portable. The weakness of this technique concerns the measurement of deeper brain structures; it can only record more superficial electrical signals. Therefore, in contrast to fMRI, EEG features high temporal resolution and low spatial resolution.

• **Positron emission tomography (PET):** a technique with validity and spatial resolution similar to those of fMRI. However, radioactive particles (positrons) must pass through the participant for the collection of results, making this technique highly invasive and difficult to use in neuromarketing (Lin, Tuan & Chiu, 2010).

• **Magnetoencephalography (MEG):** this technique is based on the expansion and mapping of the magnetic field created through neural activities, electrochemical signals between neurons. Similarly,

to electroencephalography, magnetoencephalography has excellent temporal resolution; however, its spatial resolution, while not ideal for measuring subcortical areas and deeper areas in the brain, is superior to that of EEG [7]. In contrast to EEG, when conducting MEG research, individuals use hypersensitive sensors to measure the electromagnetic field without contact with the scalp. The cost of the acquisition of the necessary equipment and of the magnetoencephalography session is very high, which contributes to the greater popularity of EEG than MEG (Crease & Robert, 1991).

• **Eye tracking (ET)**: increasingly used along with other techniques such as EEG and fMRI. Advantages, this method is able to measure the focus of consumers' attention, the pattern of visual behavior of fixations of the gaze, dilation of the pupils, focus, and microfocus; in addition, the equipment is portable. Among the main disadvantages is the fact that it is not possible to understand what emotions are associated with the areas that were the focus of attention, and not deducting, automatically, that focus necessarily represents higher visual attention. Some of the data of interest to marketing in relation to this technique are the time that the subject spends focusing on the object of study, the measurement of the pupils, the areas and the frequency of observation of users in the stimuli presented (Nenad, 2011).

ET measures where the person is looking (gaze or fixation point), the time that this person looked at this certain point, the movement of his eyes in relation to his head, pupil dilation, and the number of blinks (Zurawicki, 2010). In addition to the fixation, the sequence in which his or her eyes shift from one location to another (saccade) can also be evaluated (Chae & Lee, 2013). There are different ET technologies to measure eye movement and the most common are those that measure the observation of controlled stimuli at fixed points in videos, photos, and user's interaction with a computer screen. There are more advanced devices that also automatically track the head position in three-dimensional space in relation to the camera (Zurawicki, 2010).

This makes the measurement process more subtle, with very little or no interaction between the researchers and their subjects. Studies with ET equipment, although not new, have offered new perspective within neuromarketing. These studies, and the potential of ET, have gained relevance in today's world of the visual pollution that is vying for consumers' attention. Understanding the mechanisms that guide consumers to select certain points of interest in an image (attention standards and forecasting the places of greatest interest) have many applications for the business world. Therefore, ET can provide information on what is more relevant to the involvement of attention, as it is related to patterns of visual fixations, in many different marketing issues. In addition, ET can also be used with other equipment to measure cognitive responses, lead synergy for new insights, particularly in relation to consumer behaviour and marketing communications. When connected to facial coding, the results show the precise amount of visual activity (exactly where people are looking) associating specific emotional responses to different elements of a stimulus (how people felt about what they saw). The synchronisation between emotional response and visual focus provides a reliable method for understanding what is driving the reactions to a given stimulus. This is of inestimable value, especially for TV advertisements, in which lots of information is generated every millisecond, possibly hindering the identification of what the viewer really liked, or what actually called his attention in a positive or negative way. The typical model of eye movements applied to the use of eye-tracking consists of two concepts: fixations and saccades (Velásquez, 2013) [8].

• **Facial recognition (or electromyography):** a technique still not largely used in Neuromarketing that consists of measuring facial muscle movements that are imperceptible to the human eye through electrodes placed on the muscles of the mouth (zygomaticus minor and major) and on the occipitofrontal and orbicularis muscles to check the type of emotion (happiness, sadness, indifference, pain, etc.) (Melillo, 2006). Following can be emphasised: high spatial resolution, growing credibility for use in the analysis of different affective reactions to visual stimuli, reactions of taste, smell and hearing, human interactions and behaviors. One disadvantage is that the electrodes fixed on the face may inhibit some facial movements. Another important disadvantage for use in Neuromarketing is the double meaning of certain expressions, which invalidates a standardisation of single expressions

correlated with specific emotions, restricting studies of some more specific emotional reactions (Jones & Beer, 2009).

• **Cardiovascular parameters**: this approach records the heart rate and its variability, blood pressure, interaction between heartbeats and pulse transition time to infer emotional and attention states of the research subjects [7].

• **The galvanic skin response (GSR):** Founded in 2006 by neuroscientist Dr. Carl Marci and MIT alum Brian Levine, Innerscope uses biometrics such as brain scans and galvanic skin response to measure subconscious emotional responses to media and marketing. This technique measures the objective excitation caused by an emotionally relevant stimulus. The central nervous system is directly connected to the reactions recorded on individuals' hands, and this method is able to identify the neural responses that precede certain emotions, such as happiness, sadness, fear, anger, disgust and indifference (Banks et al., 2012) [3]. Galvanic skins response is a method of measuring the electrical conductance of the skin, which varies with its moisture level. This is of interest because the sweat glands are controlled by the sympathetic nervous system so skin conductance is used as an indication of psychological or physiological arousal. Therefore, if the sympathetic branch of the autonomic nervous system is highly aroused, then sweat gland activity will also increase, which in turn increases skin conductance. In this way, skin conductance can be used as a measure of emotional and sympathetic responses.

It should be noted that neuromarketing is not exactly the same as subliminal marketing. The latter is only a subset of the former and focussed on the application part as implemented by the marketers. Neuromarketing involves much more such as involving the test subjects, using the biometric and physiological sensors to carry out experiments, studying the brain's reaction (sometimes also heart rate, breathing, and skin response) to the social triggers and so on. Application in the real world to boost sales or acceptability (say, of presidential speech) is the end result of neuromarketing. An important part of neuromarketing which is more related to subliminal messaging is "priming" which refers to subtle suggestions made deliberately to the subconscious mind, without the subject's knowing, which could influence his/her subsequent behaviour.

So how is Neuromarketing implemented in real life? Starting with, say the fMRI scanners (other technologies are used too), the consumer's brain is scanned which help the marketers to find out how consumers react subconsciously to advertising, brand and products. This will tell the marketers what the consumer reacts to, whether it was the shape of the packaging, the color of the packet, the sound the box makes when shaken, and so on [6]. This rare ability to watch inside the mind of consumers and noting how sensory inputs like image, smell and touch culminate to reach decisions enables the advertisers and marketers to optimise their advertisement, campaigns and product or service features to make them more acceptable. fMRI is not the only technology that is used. While fMRI is chiefly used for refining the product attributes, Electroencephalography (EEG) measures fluctuations in response to advertisements, Magnetoencephalography (MEG) measures the fluctuations but with greater accuracy than EEG and Transcranial magnetic stimulation (TMS) is used to measure causal roles [4].

Neuromarketing World Companies: Brighthouse, NeuroFocus, Millward, Brown, Neurosense, Neuro-Insight [2]. In a bid to further hack its way into the consumer psyche, Nielsen has completed its acquisition of the Boston-based neuroscience firm Innerscope Research [3].

Neuromarketing and Advertising Research. Neuromarketing research can predict the effect of TV ads, even before the commercial is made. Effective TV commercials evoke a unique pattern of brain activity, the scientists from Neurosense concluded after studying over 150 commercials, including award-winning ads like Effies, humorous and even irritating ads. Functional magnetic resonance imaging makes it possible to read what is going on in people's brains while seeing certain stimuli. By measuring emotions in the brain of the consumer with an MRI-scanner, the researchers discovered a 'neural signature' that predicts how effective a commercial is with a stunning accuracy up to 82% [4]. If you know how an effective commercial evokes activity in the brain, you can also determine how

effective a storyboard is to the final production. This was the goal of a recently completed follow-up study, which showed that indeed the same brain pattern is activated when seeing storyboards and the final production [1].

The results are amazing! With a correlation of 80% between the storyboard and final production, the researchers could see whether the concepts had the effect promised by the creators. The study additionally revealed something beneficial for advertisers: even simple storyboards, where the imagination of the consumer is put to the test, gave the same high correlation rate with the final production. For the first time in history, companies are able to test propositions and concepts in advertising with Neuromarketing techniques [1]. Considering this new form of marketing research was only really started in 2004, most opponents are worried about where the industry might be in a few more years. Scientists have already mapped the entirety of the brain and know exactly what parts light up when we make the decision to buy a product in certain industries. The field of Neuromarketing is still in its embryonic stage, but is developing with more and more new studies being conducted each year [5].

The utility of Neuromarketing is of course dependent on the development of Neuroscience. Our present day knowledge of the functioning of the 'neuronal geography' in the brain is very similar to the late eighteenth century map of the world, hand-drawn by cartographers [3]. However, with more and more studies and the development of sophisticated technologies like Brainbow, which can map individual neurons with 90 odd fluorescent colours, we might soon have the 'Google Earth' of our brain. The analogy shows that the implications are tremendous. If a better knowledge of the world geography was instrumental in colonizing half the planet, imagine the immense possibility that Neuromarketing has for the marketers in influencing the consumers' psyche [7].

The effect that certain publicity campaigns, brands and products have upon us, from a cognitive and emotional point of view, is being assessed, by measuring the attention, the encoding and the emotional engagement [9].

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https://www.academia.edu/969187/Neuromarketing
http://www.stateofdigital.com/crossroads-ethics-of-Neuromarketing/

http://343f11.pbworks.com/w/page/47862658/Neuromarketing.
https://www.blinkist.com/page19/page19-primer-how-to-use-neuromarketing-to-get-inside-your-customers-heads
http://adage.com/article/media/nielsen-buys/298771/
http://www.forbes.com/forbes/2009/1116/marketing-hyundai-neurofocus-brain-waves-battle-for-the-brain.html
http://theoldspeakjournal.wordpress.com/2011/08/23/neurofocus-uses-Neuromarketing-to-hack-your-brain/
http://www.neurosciencemarketing.com/blog/articles/neuromarketing-soup-nuts.htm

ЕО2. МЕХАНИЗЪМ НА ВЪЗДЕЙСТВИЕ НА АКУПУНКТУРАТА ПРИ БОЛКА

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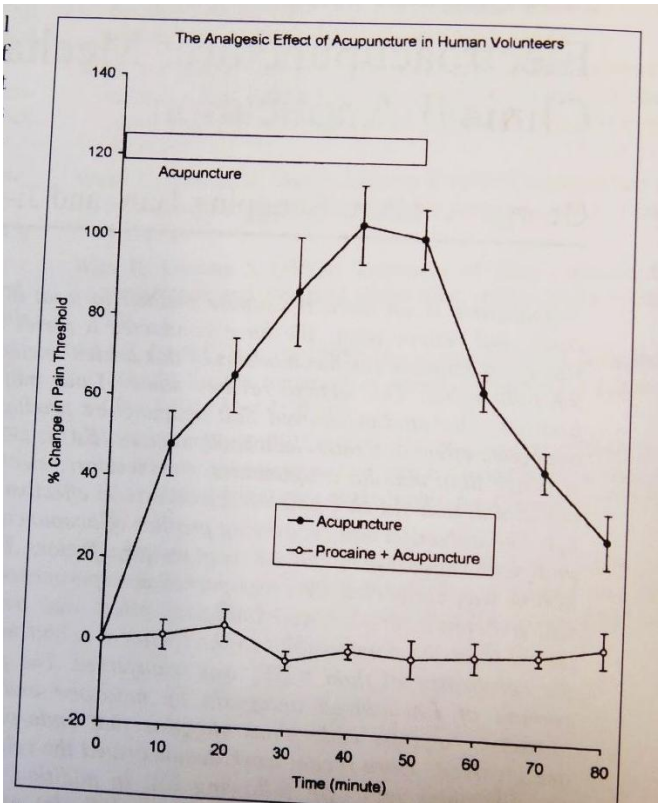
Един от основните проблеми пред акупунктурата е изясняването на въпросът за начина на въздействие върху човешкия организъм.

За пръв път този въпрос се поставя след посещението на президента Никсън в Китай през 1971 г , което отваря пътя на акупунктурата към западния свят. Първоначално се е приемало като метафизика цялата дейност (St. Louis Post Dispatch, August 4, 1974 –call it “quackery”). Обяснението, което се е предлагало е било “oriental hypnosis”.

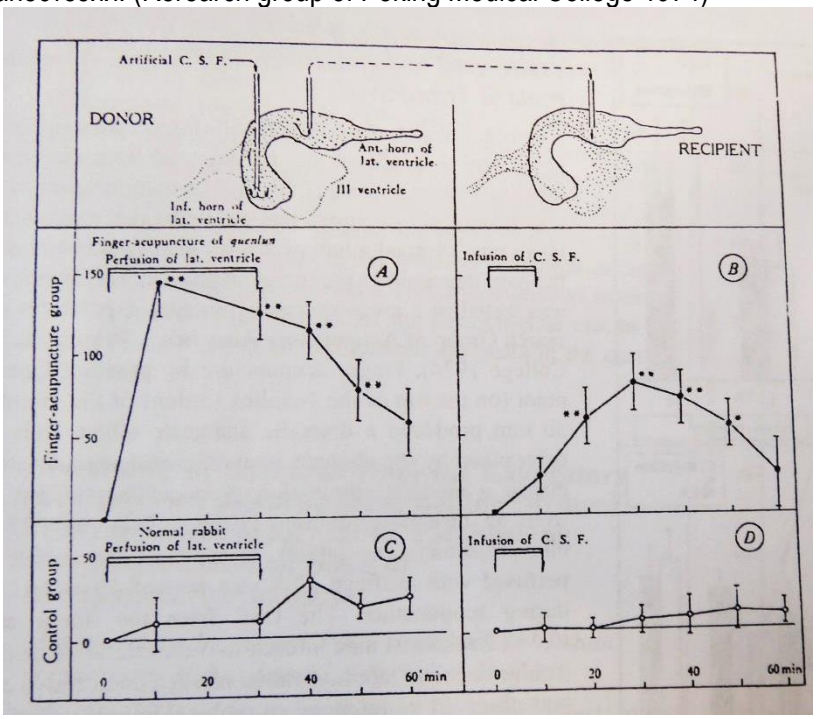
След проучванията на Parwaiticar at al 1979, Ulett at al 1979, Ulett 1983 е установено със сигурност, че това не е хипноза. Публикациите в това направление продължават и Pomeranz and Stux 1979 намират физиологично обяснение. Най-подробно физиологичните изследвания на механизма на акупунктура са направени от prof. Ji Sheng Han (Beijing Medical University), който през 1990 демонстрира диференциалното отделяне на специфични невропептиди в зависимост от различната честота на електрическото стимулиране.

Направен е опит върху студенти доброволци в медицинския университет като са поставяне игли във две точки – Hegu and Zusanli като мануално се е осъществявало twistling - twitling stimulation .



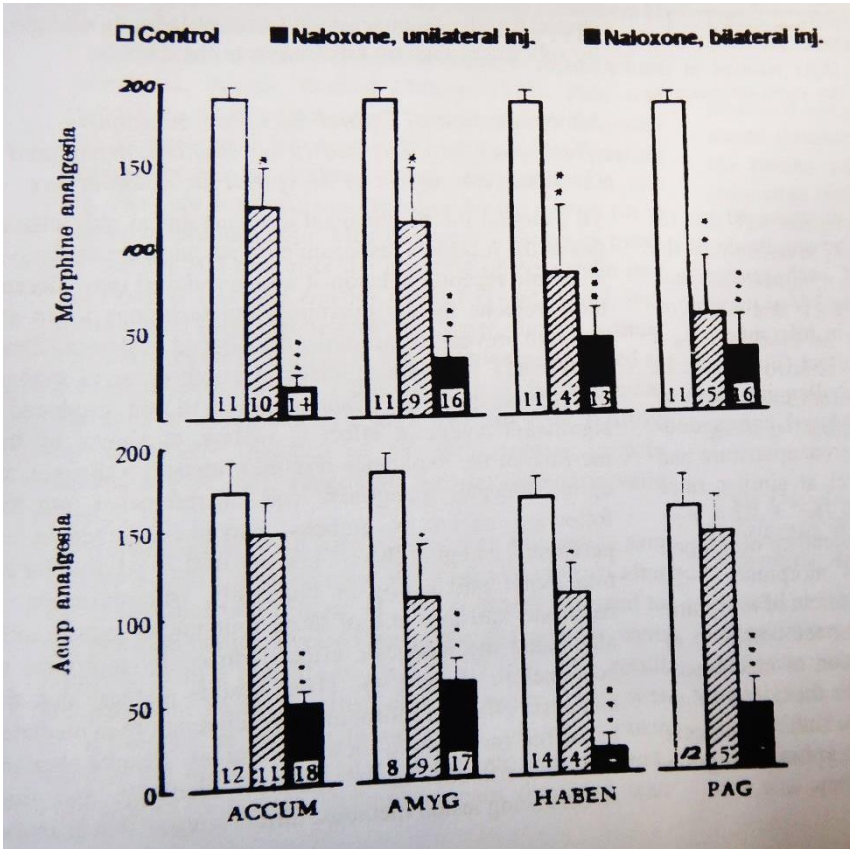
Ефектът на аналгезия спрямо токов удар чрез йонофореза с калий и постепенно увеличаване на тока в точката Hegu показва тези резултати – фиг. 1. Аналгетичният ефект е бил подтиснат при инжектиране на новокаин.

За да се докаже неврохимичния механизъм на аналгезията е бил направен много интересен опит. В мозъка на плъх е поставена микроканюла и след предизвикване на анестезия, често от цереброспиналната течност е прехвърляна в мозъка на друг плъх, като доказано е предизвиквала анестезия. (Rerearch group of Peking Medical College 1974)

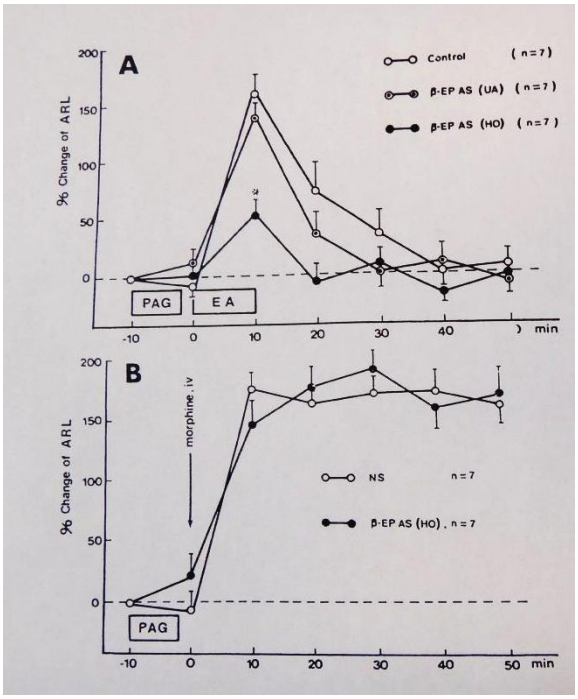


За да се установи кои невропептиди са отговорни за аналгезията се е вървяло по пътя на доказване на разлика с морфинова аналгезия. Поставянето на микроинжекция на налоксон – препарат, който е антагонист на морфина е предизвиквало намаляване на морфиновата и на акупунктурната аналгезия.(Фиг. 3). За да се отдели носителят на акупунктурната аналгезия е направено микроинжектиране на серум в мозъка на плъх и е установено, че антисерум срещу β – endorphin в периакведукталното пространство на сивата част на мозъка предизвиква намаляване на акупунктурната аналгезия, но не на морфиновата аналгезия - Xie at all 1984. (Фиг. 4). Разликата между двете части на тази фигура – А и В се дължат на използването на антисерум срещу β – endorphin. Ако антисерумът разпознава (НО) или не разпознава (UA) плъховия β – endorphin при поставяне в доза 4 μ l в постоянно закрепена канюла в мозъка, след електроакупунктура в Zusanli(ST 36) и Qunlun UB60 за 10 мин (А) и след интравенозното поставяне на морфин (В). Този резултат говори за директно активиране на опиоидните рецептори в сивото пространство на мозъка, докато при акупунктурата ефекта се получава чрез отделянето на β – endorphin.

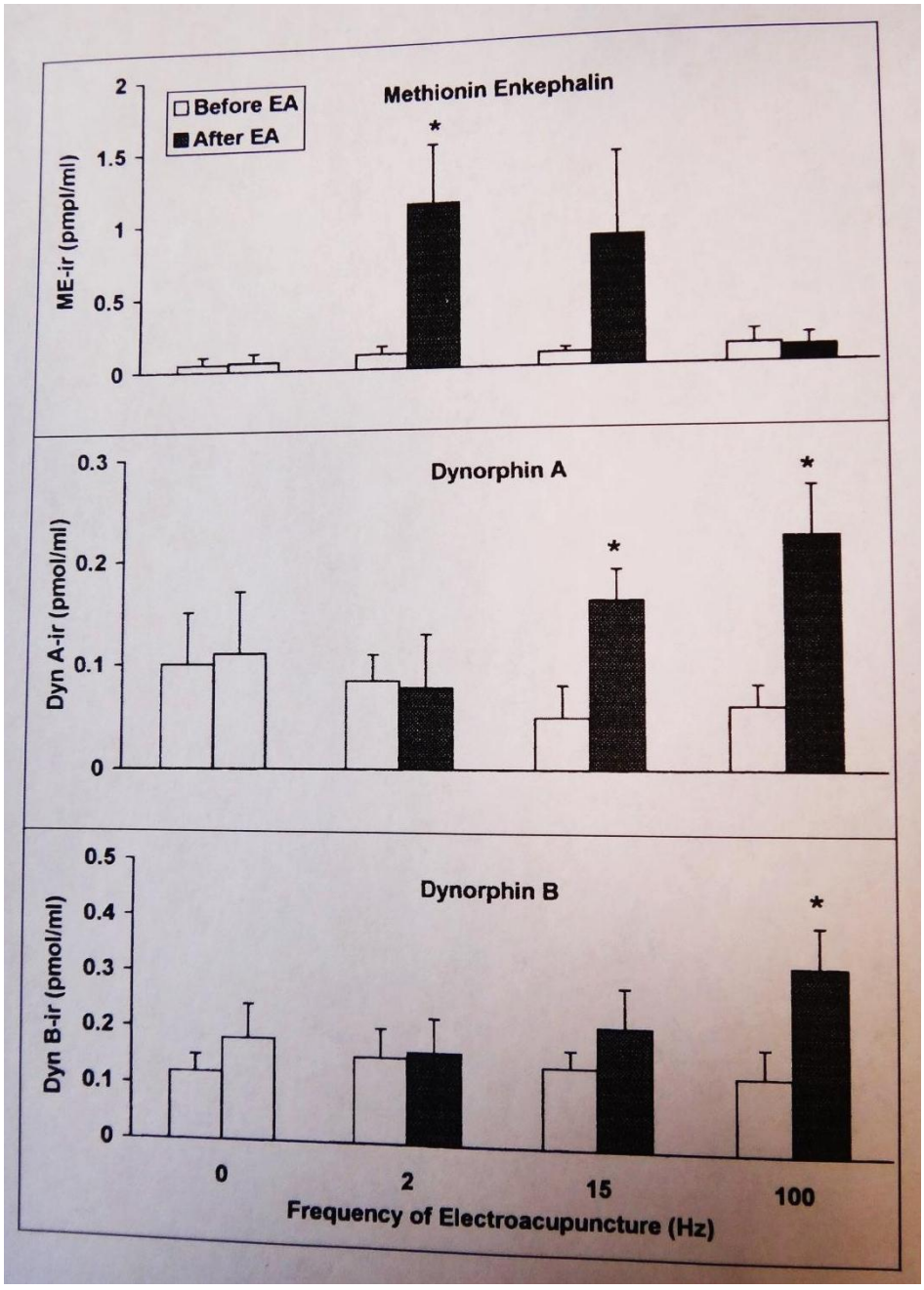
За да се установи по-точно какъв вид пептид са проведени опити с различна честота на електростимулация – (Fei et all 1986) , при които е доказано, че може да се специфицира отделянето на вид ендорфин без да се стимулира отделянето на другите видове. За целта спиналното субарахноидално пространство на плъх е било перфузирано със церебрална



Фиг. 3



флуидна течност преди и след електроакупунктура на Zusanli ST 36 and Sp. 6 Sanyinjiao, при използването на 2,5 и 100 Hz. (Фиг. 5) . установено е, че при честоти до 2 херца се отделя енкефалин, а при честоти от 100 хц се отделя динорфин.



Къде и как се прилагат тези резултати. Традиционно акупунктурата се използва при третиране на остра и хронична болка с успех при около 70 % от болните с болки в кръста и гърба, артрит, мускулни спазми и болки, мигрена и други (Ulett 1989, Ng et al 1992, Thomas and Lundberg 1994, Anderson et al 1976).

Депресия

Това е демонстрирано в Китай при major depression, което е било правено по два пъти седмично за 30 мин с електростимулация (Han 1986, Lou et al 1990).

Насоките на нашата работа са преди всичко установяване на промените в хормоналния баланс на организма чрез взимане на кръв преди и след завършване на курс на лечение и изследването и с HPLC.

EO3. OPTIMIZED PCR METHODS FOR THE DETECTION OF INFECTIOUS AGENTS CAUSING FEVER AND RASH SYNDROME

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Abstract

Introduction: Many infections agents can cause a rash and fever in addition to other symptoms. Diseases that present with fever and rash are usually classified according to the morphology of the primary lesion. Rashes can be categorized as maculopapular (centrally and peripherally distributed), petechial, diffusely erythematous, vesiculosis and nodular. Potential causes include viruses, bacteria, spirochetes, rickettsiae etc.

The **objective** of this study was to optimized protocols of amplification methods for detection of the infectious agents (measles, rubella, parvovirus B19, *Rickettsia conorii*, *Mycoplasma pneumoniae* and *Coxiella burnetii)*.

Materials and Methods: 36 serum samples collected from ill individuals during acute and/or recent infection were tested. The following molecular methods were used: extraction of infectious DNA/RNA from started material serum sample; amplification methods (conventional PCR/RT-PCR techniques) by consensus primers for detection of specific DNA/RNA regions; electrophoretic analysis in 2% agarose gel to visualize the PCR products.

Results: Positive PCR signals were detected in 10/36 (27.8%) serum samples. The frequency of proving infectious nucleic acid in the tested pathogens (measles, rubella, parvovirus B19, *Rickettsia conorii*, *Mycoplasma pneumoniae* and *Coxiella burnetii)* were 1/36, 0/36, 9/36, 0/36, 0/36 and 0/36, respectively. Our protocols were optimized only for detection B19V-NS1 region (103 nt) and measles (454 nt).

Conclusions: The timely and specific diagnosis with molecular methods in children and adult with fever and rash can be extremely important. An accurate characterization of the skin lesions and a thorough history can help narrow the differential diagnosis for a specific patient. The laboratory studies can be useful in confirming the diagnosis.

Key words: measles, rubella, parvovirus B19, *Rickettsia conorii*, *Mycoplasma pneumoniae*, *Coxiella burnetii*, PCR/RT-PCR, electrophoresis

EO4. ВИРУС ЗИКА: МОЛЕКУЛЯРНОБИОЛОГИЧНИ ХАРАКТЕРИСТИКИ, КЛИНИЧНА КАРТИНА, ДИАГНОСТИКА И ЛЕЧЕНИЕ

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EO5. COMPARATIVE ANALYSIS BETWEEN FIELD AND LABORATORY DIAGNOSIS OF NOSEMATOSIS AND VARROOSIS ON HONEYBEE

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In 2015 and 2016 were conducted laboratory tests for parasites of 163 samples of bees of 105 apiaries from different regions of the country. Among 76 beekeepers were carried out survey for observed signs characteristic of these diseases. Results of laboratory tests were compared with the manifested symptoms of the field. The purpose of this study was to compare the clinical signs observed by beekeepers, and the results of laboratory analysis. Laboratory tests on samples of bees show a high degree of infestation with spores of *Nosema spp*. (52.8%) and the mite *Varroa destructor* (12,9%) alone and 18.4% in mixed invasion of both parasite. Negative are 15.9% of the samples. In many cases the anamnestic data is not confirmed by laboratory results. Comparing survey results on the field and laboratory studies conclude that nosemosis prevails while beekeepers indicate varroosis as a major problem. According to data from surveyed beekeepers on signs of parasitic diseases of the apiary at 39.6% it comes to varroosis, but only 2.6% - for nosemosis. Mixed invasion of the two parasites indicate 28.9 percent surveyed beekeepers and the same percentage (28.9%) of them have not observed signs of parasitic diseases. This study confirms the necessity of laboratory testing for accurate diagnosis.
Keywords: honeybees, nosemosis, varroosis, clinical signs, laboratory analyzes

INTRODUCTION

Nosemosis is a disease on bees of global proportions detected in many parts of the world, including Europe, Africa, Asia, America and Oceania [3, 7, 9, 11, 14, 16]. The causative agents of nosemosis are *Nosema apis* Zander and *Nosema ceranae* Fries which are included in genus *Nosema*, belongs to the class *Microsporidia*. Recent studies have shown that *N. ceranae* has changed its feeding habits and is no longer unique to the eastern bee, but has also become a very efficient parasite of European honeybee *Apis millifera* [4, 5, 6, 12]. The Nosema ceranae is perhaps the most recent and virulent [10, 18]. The credibility of this theory is based on the fact that all parasites are dependent on the energy from their hosts to reproduce, causing them significant nutritional stress and eventually, death [19]. According to Paxton [22], N. ceranae is an emerging, potentially virulent pathogen which has spread throughout the world in the past 10 years, which explains why researchers have detected it in both healthy and weak honey bee colonies. Unfortunately, there is no reliable field diagnostic symptom enabling a diseased worker bee to be identified without killing it, nor can the beekeeper recognize an infected queen. The clinical symptoms associated with *Nosema* spp. infection can be seen with other types of colony conditions, so cannot be used to provide reliable diagnosis. Only a method to detect *Nosema* spp. under a light microscope can be used to confirm presence of spores [1, 4, 13, 18].

Today the parasite *Varroa destructor* is found throughout the world, except for Australia [8] causing serious damage in *A. melllfera*. The number of parasites steadily increases with increasing brood activity and the growth of the bee population, especially late in the season when clinical signs of infestation can first be recognized. In addition to its direct action as pathogen, the *Varroa* mite has been proven to be an effective vector in transmitting and activating viruses [20].

The purpose of this study was to compare the clinical signs observed by beekeepers, and the results of laboratory analysis in period of two years (2015-2016).

MATERIAL AND METHODS

The investigation was conducted on 163 samples of bees of 105 apiaries from 19 different regions of the country in period of two years (2015-2016). Among 76 beekeepers were carried out survey for observed signs characteristic of these diseases. Results of laboratory tests were compared with the manifested symptoms of the field.

Sample collection

The bee samples were collected from the hive bottom of died colonies in 19 regions of the country. Every sample contained about 200 bees.

Methods of investigation

The methods were used of *OIE Terrestrial Manual, 2008 - Chapter 2.2.7*. for *Varroa destructor* identification, and *OIE Terrestrial Manual,2013 - Chapter 2.2.4.* for the identification of *Nosema spp*., respectively [17].

Investigation for Varroa destructor

The dead bees from the hive bottom were floated with industrial alcohol, stirred continuously for around 5-10 minutes. The mites that floated to the surface were identified and observed [2]. Than the contents were placed of the sieve on a bright plate, where the mites can be easily identified and counted. The percentage of infestation level was calculated by following formula:

% *V. destructor* = (Number of foretic mites / number of adult bees) x 100

Investigation for spores of Nosema spp.

The abdomens of 20 bees of each sample are obtained in 20 ml of distilled water. Of the suspension are made smears on a glass slide. Smears were air-dried, ethanol-fixed and were stained with Giemsa's stain (10% in 0.02 M phosphate buffer) for 45 minutes. *Nosema spp*. spores had a distinctive appearance, with thick unstained walls and an indistinct blue interior, without visible nuclei. To quantify the average infection level spores were counted and were calculated per bee by method of Oliver [21].

RESULTS

The laboratory results of the percentage of positive samples infected with parasites and negative samples were shown in Figure 1.

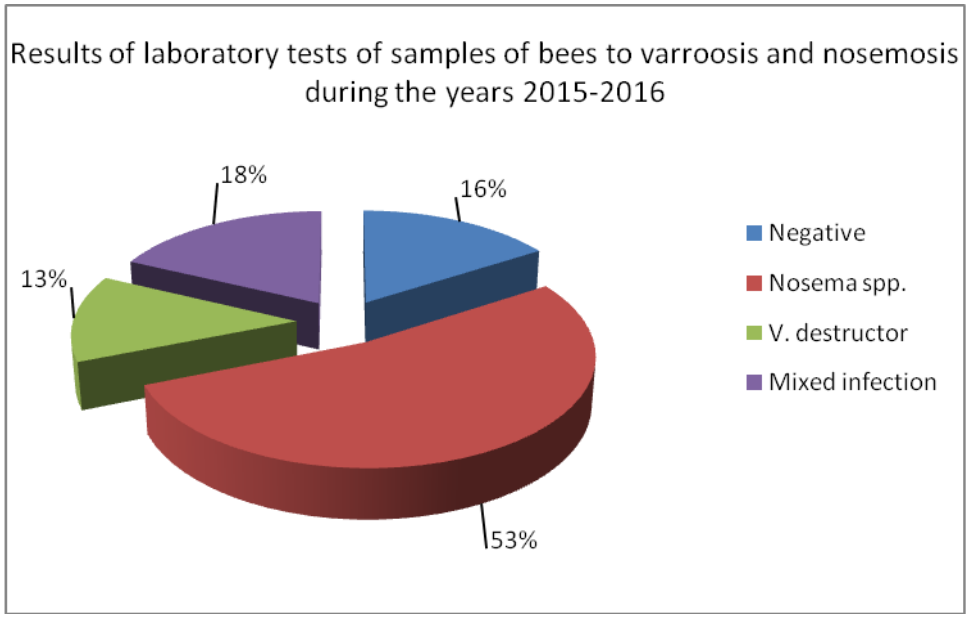

Fig.1. Results of the laboratory tests of samples of bees (2015-2016)

It appears that from 163 investigated samples the higher percentage of positive were for *Nosema spp.* spores – 86 (52,8%). The second place took the samples with mixed invasion – 30 (18,4%) and the lowest percentage of the positive samples were infested with *Varroa* mite – 21 (12,9%). The negative samples were 26 (15,9%).

The results of the percentage of negative and infected with parasites colonies giving by beekeepers were shown in Figure 2.

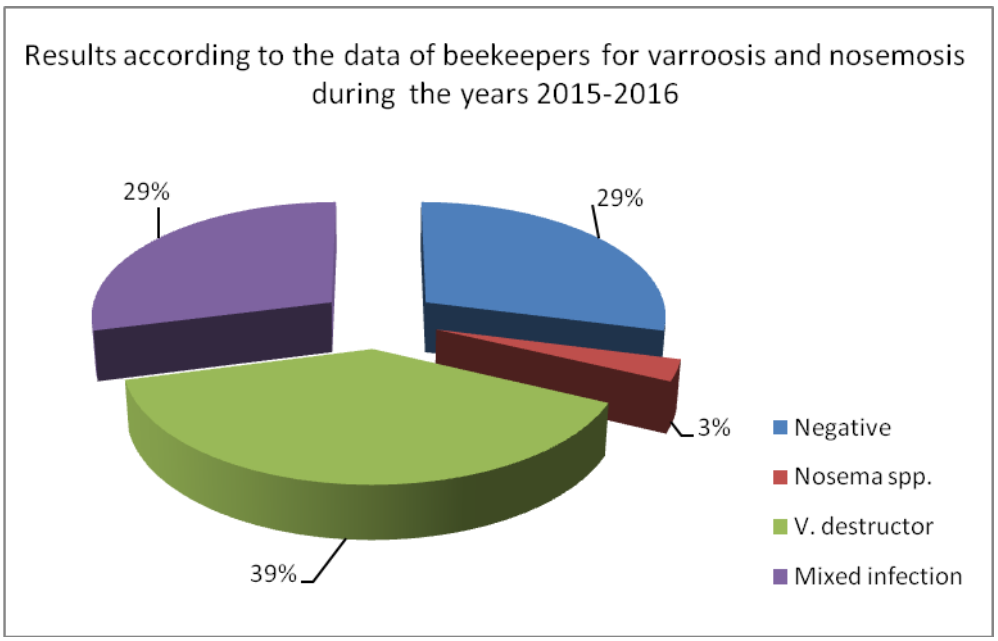

Fig.2. Results from the data of parasitic diseases observed by beekeepers (2015-2016)

Data given by 76 beekeepers showed that the most common disease was varroosis – 30 of the surveyed (39,6%). In second place were reported mixed infections varroosis with nosemosis and negative cases in 22 (28,9%) of the surveyed. The lowest percentage of the reported diseases was nosemosis – 2 (2,6%).

The level of *Nosema spp*. spores invasion in laboratory tested bee samples is given on Fig.3.

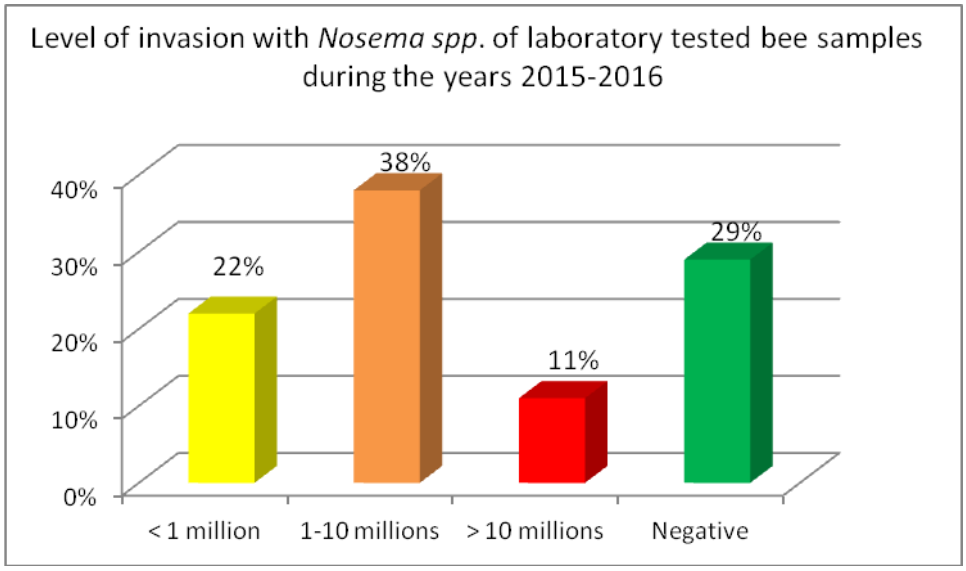

Fig.3. Level of *Nosema spp.* invasion in laboratory tested bee samples (2015-2016)

In our studies we found that the highest percentage (fig.3) of positive samples with spores of *Nosema spp*. had a level of invasion between 1 and 10 million - 62 (38%) of the samples. A degree under 1 million showed 36 (22,1%) of the investigated samples and the highest invasion (>10 million spores per bee) was established in 18 (11%)of the infected samples, respectively (fig.3). Negative samples occupied approximately one-third of all tested – 47 (28,9%).

The level of *Varroa destructor* invasion in laboratory tested bee samples is given on Fig.4.

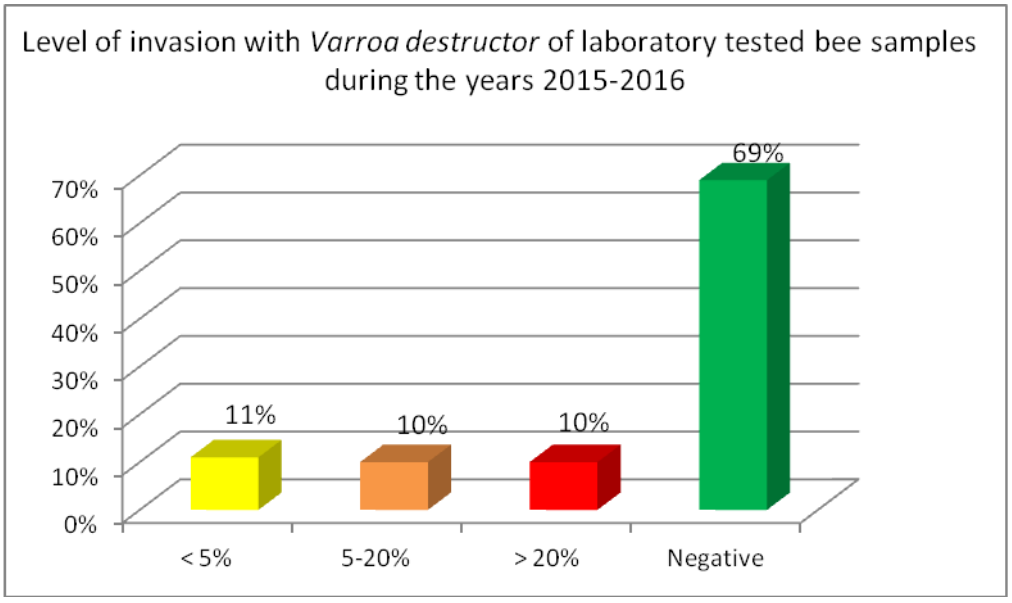

Fig.4. Level of *V. destructor* invasion in laboratory tested bee samples (2015-2016)

The percentage of the infected with *Varroa* mite bees were comparable in all the positive samples with a different levels of invasion. Level of invasion < 5% we found in 18 (11%) of samples, level between 5 and 20% - in 17 (10,5%) and the degree of invasion (> 20%) in 16 (9,8%) of samples, respectively. The highest percentage of the samples were negative – 112 (68,7%).

CONCLUSION

Comparing the results from laboratory studies and data from questionnaires found that they are radically contradictory.

Laboratory studies indicate that the nosemosis is most common in the country, while beekeepers indicate varroosis as a major problem.

The main reason for this contradiction is that beekeepers do not send samples for laboratory examination, which is the most reliable method for diagnosis of bee diseases, especially nosemosis.

Signs of diseases and poisoning of bees have very similar symptoms and it is difficult to recognize only on the apiary.

Our studies on the project will continue in other regions of the country to establish the prevalence of *Nosema spp.* and *Varroa destructor.*

Acknowledgements: This work is related to a project between IEMPAM- BAS and NDRVMI - BFSA (Study the spread of the causes of parasitic diseases in bees Varroa destructor and Nosema spp. in different regions of Bulgaria)

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EO6. ANTIPARASITE REMEDIES APPLIED IN MOUFLONS (*OVIS MUSIMON*) AND OTHER WILD SHEEP

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Summary

The selection and use of antiparasitic remedies in wild sheep present one of the challenges to the wildlife managers and veterinarians. The present study represents a review of the literature on the experience accumulated in this respect. The summarized literature data show the following: The benzimidazoles most often have been applied for treatment of parasites in wild sheep. Their effectiveness varies according to the parasite species. It has been high against gastrointestinal nematodes and relatively lower against lungworms. Some drugs, for example oxfendazole has been 100% effective against *Moniezia* spp. It has been established that albendazole is readily accepted even by fastidious animals. Two-day treatment has been recommended during the treatment with mebendazole. For long-term control of protostrongylids free-choice availability of fenbendazole-medicated salt has been recommended as a potentially effective management tool. Ivermectin is other medicine tested for treatment of wild sheep. Applied parenterally it has been effective in control of the lung nematodes of *Protostrongylus* genus and mites *Psoroptes ovis*. The dosage of 600 μg/kg body

weight is recommended for parenteral use. The results of orally administrated ivermectin are contradictory – in some cases the drug has been ineffective against lungworms, in others it has had good effect. Most probably this depends on the dosage and scheme of treatment. The parasites more difficult for cure, independently of kind of remedies, have been protostrongylids and *Trichuris* spp. Experiments have shown that repeated administration of some anthelminthics may contribute to the development of parasite resistance. Other ones have demonstrated positive effect of biological control on the gastrointestinal nematodes in animals in captivity.
Key words: *mouflon, bighorn sheep, parasite treatment, benzimidazoles, ivermectin*

Introduction

Parasitoses in animals, including in wild ones, display an array of negative effects. Most of them become a pre-disposing factor for secondary deficiencies and infections, others are detrimental to reproduction and some would lead to lethality of the hosts. In this connection, lately it becomes increasingly clear that parasites can have significant impacts on the dynamics of wildlife populations [17]. On the other hand wild animals present an epidemiological niche for parasites common with the ones in the domestic animals and are often an important source of hazardous parasitic zoonoses. That is way taking measures for fighting parasitic diseases amid the wildlife is not only an indication of a good management in game-breeding and hunting industry but is also a healthcare necessity. The application of anti-parasitic remedies in the wild animals has however, its specifics. For example, the individual animal treatment in wild nature is impossible, the used drugs are tested only in domestic animals, there is a risk of swallowing of bigger quantity of the drug substance when the animals are treated in a group with the food, etc. In this connection the goal of the present study was to carry out a review of the literature of the experience with regard to the anti-parasitic treatment of wild sheep and in this way to help experts in the field with information which would make it easier for them to take action in the control and prevention of the parasitic diseases among them.

Results and Discussion

Six desert bighorn ewes naturally infected with *Psoroptes ovis* have been supplied by the New Mexico Department of Game and Fish aiming to evaluate ivermectin for control of the parasite in these animals [6]. Two ewes in one pen have been injected with ivermectin (10 mg/ml, Lot Number L-646, 471-46B OZ) at the rate of 500 μg/kg. Two ewes in a second pen have been injected at 1000 μg/kg. The ewes in the third pen have remained untreated to serve as controls. The effectiveness of the treatments has been established at 7, 14 and 35 day post-treatment intervals by determining the number of living mites in scrapings taken from the animals. All the sheep have been infected with *P. ovis* at the time of the pretreatment evaluation. Mite populations on the two control ewes have increased in both ear and body locations after that time, indicating that the treatments have been administered at a time when mite populations have been naturally increasing on the animals. At 7 days post-treatment no live mites have been found in ear samples taken from either 500 on 1000 μg/kg treated animals. However, live mites have been found on the poll and withers on one sheep examined at this interval, suggesting that mites may not be killed as readily on body locations as in the ears. No live mites have been found on either ear on body location at 14 or 35 days post-treatment on any of the treated animals. According to the authors the failure to achieve complete control until after the 7 day post-treatment interval suggests that ivermectin is rather slow acting against *P. ovis* in desert bighorn sheep. They have concluded that ivermectin injected intramuscularly at either 500 on 1000 μg/kg appeared to be extremely effective and perhaps completely effective in controlling *P. ovis* in desert bighorn sheep.

Kozakiewicz and Maszewska [8] have established that 100% of mouflons in a hunting centre in the Wielkopolska district, maintained under semi-free conditions, have been infected with gastrointestinal and lung nematodes. The efficacy of Systamex (containing oxfendazole) given in a daily dose of 1.7 mg per kg for 3 consecutive days has been 88.9% against gastro-intestinal nematodes, 75.0% against lung nematodes and 100% against *Moniezia* sp.

Regular weekly faecal examinations for various gastrointestinal nematodes of 23 types of ruminants at a Bern zoo, Switzerland, has helped to identify the high risk groups, which have included elk, mouflon and most of the deer and goats [18]. In most groups, increases in egg counts have coincided with parturition. Albendazole at 5 or 7.5 mg/kg body weight in food at 2.5 g/kg for high risk groups has been readily accepted even by fastidious animals and when given at high peak periods, has resulted in drops of infection.

Four faecal examinations annually have been performed on mouflon and deer at the Lešná zoo, Czechoslovakia, between 1979 and 1984 [4]. Some other ruminants kept in adjoining runs have been also examined. *Muellerius capillaris* (in 55.6%) and *Trichostrongylus* spp. (in 51.9%) have been the most frequent of the 12 nematodes present. *Fasciola hepatica* has been found in 3.7% and *Eimeria* spp. in 44.4%. The principal infection peak has been occurred in April-June, with a second in Sept.-Nov. and for this reason the animals have been regularly dewormed in spring and autumn with Mebenvet (mebendazole) and, occasionally, with Nilverm.

Miller and Hobbs [15] have performed experiments with bighorn sheep. They have established that a single s.c. injection of ivermectin (0.2 mg/kg) has eliminated L1 of *Protostrongylus* from faeces of animals 4 weeks after treatment under laboratory conditions. Bighorns treated with a single injection of ivermectin (0.5 mg/kg) have showed similar results that have lasted over 4 months post-treated period, although some larvae have been recovered from 5 of 23 faecal samples. From these observations the authors have inferred that ivermectin has showed high efficacy against adult *Protostrongylus*, an inference based on observation of L1 disappearance in treated animals relative to no change in L1 levels in untreated controls.

The rafoxanide (salicylanilide) and mebendazole preparation Rafendazol premix has been added to feed for mouflons in hunting areas for 2 or 4 consecutive days in 1988-90 [9]. Faecal examinations have shown high efficacies against gastrointestinal nematodes and lungworms. The 2-day treatment has recommended.

Düwel [2] has studied the efficacy of a single dose of luxabendazole (Fluxacur, Luxacur) of 10 mg/kg (5% suspension), and of a split dose of 2 mg/kg daily on 5 consecutive days (pellets in feed) in sheep experimentally infected with *Fasciola hepatica, Haemonchus contortus, Ostertagia circumcincta, Trichostrongylus colubriformis* and *Cooperia oncophora.* Naturally infected game animals (fallow deer, mouflon and wild boar) have been treated with pellets in feed (2 mg/kg/day for 5 days). The drug has been 97 to 100% effective against immature and adult gastro-intestinal nematodes in sheep. The split dose and the single dose have had similar efficacies. The split dose has been more effective (about 70%) against 4-week-old flukes than the single dose (about 20%), but against older flukes (6 to 12 weeks) both dosage regimens have been similarly effective (≥80%). Based on post mortem examinations, the drug has been >90% effective against intestinal nematodes in fallow deer, >95% effective in wild boars, and highly effective in mouflon.

Protostrongylus sp. may lead to the development of pneumonia and increased levels of adult and juvenile mortality. Though proven effective over the short term, injection of ivermectin requires capturing and handling individual animals, which is difficult with wild animals. Furthermore, frequent reappearance of lungworm following treatment with anthelminthic drugs indicates that repeated administration may be necessary for long-term control of *Protostrongylus* sp. in free ranging populations of bighorn sheep [3]. That is way the authors have evaluated influences of oral administration of ivermectin on lungworm infection levels in 3 free-ranging ewe groups of bighorn sheep. Base of these studies they have concluded that broad-scale oral administration of ivermectin has not been an effective tool for managing lungworm infections in a free ranging population of bighorn sheep.

Lamka et al. [10] have studied the efficacy of ivermectin against lung and gastrointestinal nematodes in the mouflons. The pharmaceutical has been administered orally on 6 consecutive days at a dose of 415 μg/kg per day to each of 19 mouflons infected naturally with *Mullerius capillaris, Chabertia ovina* and *Nematodirus* spp. Their fecal samples collected before, during and after ivermectin administration have been examined. On days 14, 21 and 28 after the finish of the treatment, 2 animals

have been shot, respectively. Their lungs, duodena, colon and ceaca have been necropsied for the parasites. Parasitological examination of the organs has proved a good helminthicidal efficacy of the preparation including that against the adult *M. capillaris.* Based on examination of pooled fecal samples it has been shown that ivermectin rapidly has stopped also the elimination of infective forms (eggs and larvae) of the helminths. The drug has been readily consumed and well tolerated. The animals have not shown signs of side effects.

According to Lamka et al. [11] adulticide effect of the drug in verminous lung foci should be the aim of anthelminthic administrations infected with lungworms. This collective has tested anthelmintic efficacy of flubendazole (FBZ) against *M. capiollaris* in mouflon. The study has been conducted in 16 mouflons in a small game preserve. An FBZ dose of 3 x 15 mg/kg live weight has been chosen to be tested. Samples of mixed droppings have been collected before treatment, during it and after its termination. LPG values have been determined by larvoscopic examinations. Four (2 x 2) mouflons have been shot on day 7 and 14 after treatment termination and they have been subjected to detailed helminthologic examinations (macroscopic description of pulmonary verminous lesions, larvoscopy of verminous foci and mucus smears from the respiratory tract, larvoscopy of individual droppings). The animals have been extremely willing to ingest the drug applied with feed, the dosing schedule being confirmed. Pre-treatment LPG values of mixed droppings have fallen rapidly after the treatment started, and the excretion of *M. capillaris* larvae completely has terminated beginning on day 7 after treatment termination. Pulmonary and coprological LPG findings in the shot mouflons have been minimum, in one animal only, in the others the findings have been zero. Macroscopic findings have shown that all shot mouflons have suffered from the infection to a large extent before treatment. The authors have concluded that the therapeutic efficacy of FBZ administered at a dose of 3 x 15 mg/kg of body weight can be in general evaluated highly positively. According them the drug administration quickly has stopped larvae excretion in the mouflon droppings due to adulticide effect in the verminous foci of the lungs.

Gastrointestinal and bronchopulmonary nematode infections and the efficacy of netobimin (Hapasil®) have been analyzed by way of fecal examination in 10 female mouflons in Spain [14]. Before treatment all 10 mouflons have had *Trichostrongylus axei*, *Teladorsagia circumcincta* and *Marshallagia* spp.; six have had *Nematodirus* spp., two - *Trichuris* sp., one - *Capillaria* sp., seven - bronchopulmonary *Dictyocaulus filaria* and 10 mouflons have had protostrongylid lungworms (*M. capillaris*, *Protostrongylus rufescens*, *Cystocaulus ocreatus* or *Neostrongylus linearis*). Netobimin (7.5 mg/kg) has been 100% effective against *T. axei*, *T. circumcincta*, *Marshallagia* spp., and *D. filaria* infections whereas one animal has continued eliminating *Nematodirus* spp. eggs. The drug also has been effective against *Capillaria* spp. but not against *Trichuris* spp. or protostrongylid infections.

Lamka et al. [12] have studied anthelmintic efficacy of ivermectin administered in mouflons in different doses aiming to select the optimum dose. The study has been realized during winter season in game herd in 23 mouflons of 10-11 months old. Before treatment larvae of *M. capillaris* (strong infection) and eggs of *Nematodirus* spp. and *Oesophagostomum* spp. (mild infection) in common faecal samples have been found. The mouflons have been randomly divided in four groups: control group (3 head) and 3 experimental groups (6-8 animals in group). Before experimental treatment each animal in experimental groups has been weighted and marked. The doses of ivermectin 0.20, 0.60, and 100 mg/kg of body weight have been used. The drug has been administered s.c. All mouflons have been placed into enclosured area of 4 hectare. 28-60 days after treatment mouflon have been hunted and detail helminthological examinations (lung macroscopical evaluation, larvoscopy of lung verminous spots, larvoscopy and ovoscopy of individual faeces, necropsy of gastrointestinal organs and quantification of nematode adults) have been carried out, LPG and EPG values have been determined. The study has been realized as a simple blind experiment--all helminthological examinations have been done without knowledge of the used therapy (control x experimental animals) or ivermectin dose (experimental animals). Macroscopical examinations of lungs have shown symptoms of strong *M. capillaris* infection (numerous verminous spots) in all 23 mouflons. Larvoscopical examinations (lung

tissue, individual faeces) after treatment have become negative in 19 treated animals, in the rest of animals the mild larvoscopical finding has been determined. Pretreatment common LPG values (2100-2230) in control animals have been confirmed, findings in verminous spots of these animals have been highly positive (massive presence of live larvae, eggs, and rests of nematode adults). The qualitative findings (quantitatively were findings under the detection limit for the used method) were minimum in 4 mouflons of all treated groups. Pretreatment ovoscopical findings in control animals have been confirmed, too. Necropsy examinations of gastrointestinal organs have been highly positive in all control animals (mean finding of 95 adults of determined nematodes), in treated animals the findings have been positive in 3 animals of different groups (up to 2 adults only). In respect to reached results, pathogenicity of found nematodes, age of experimental mouflons, and economy of treatment, a dose of 0.60 mg/kg of body weight ivermectin dose as optimum for parenteral treatment of mouflon nematodoses has been recommended.

In an effort to control *Protostrongylus* spp. in a Rocky Mountain bighorn sheep herd of approximately 30 animals, fenbendazole-medicated salt has been placed on the Stillwater bighorn winter range in Montana (USA) for four consecutive winters, 1990 to 1993 [5]. Sheep of all age and sex classes have been observed using the medicated salt throughout the study period. Prevalence and average number of lungworm larvae per gram of bighorn feces have declined significantly ($P < 0.05$) from pretreatment levels (1987 to 1989), and have remained low throughout the study period. The authors have concluded that free-choice availability of fenbendazole-medicated salt is a potentially effective management tool for long-term control of protostrongylid lungworms.

Two groups of 6 mouflons infected with *M. capillaris* have been treated daily with mebendazole or flubendazole at 7.5 mg/kg body weight for 3 consecutive days in the period January-March 1998 and 1999 [13]. Faecal excretion of L_1 larvae have decreased in both groups after treatment, but the decrease has been more rapid in flubendazole-treated animals. Prevalence of L_1 larvae and worm eggs in lung lesions have been higher in mebendazole-treated animals.

Velik et al. [19] have treated repeatedly adult mouflon ewes with therapeutic doses of albendazole (ABZ, p.o. 7.5 mg/kg of body weight/day, for five consecutive days). Animals (treated or control) have been sacrificed 24 h after the fifth dose of ABZ and liver and small intestine have been collected to prepare microsomes. The activities of several biotransformation enzymes have been measured in both hepatic and intestinal microsomes. A significant increase in the activity and amount of cytochromes P4501A (CYP1A) has been observed in both tissues of ABZ treated mouflons compared to control animals. No other biotransformation enzymes tested have been affected by five ABZ doses. The in vitro biotransformation of ABZ has been studied in hepatic and intestinal microsomes from ABZ treated and control mouflons. Concentrations of two main ABZ metabolites – pharmacologically active ABZ sulfoxide and pharmacologically inactive ABZ sulfone have been analysed. A significant increase in rate of formation of ABZ sulfone (which is catalysed by CYP1A) has been observed in hepatic as well as in intestinal microsomes from ABZ treated animals. According to the authors the enhancement of ABZ deactivation by its repeated administration may affect the anthelmintic efficacy of this drug and may contribute to the development of parasite resistance.

Konjević et al. [7] have examined two live and one dead mouflon (*Ovis ammon musimon*) with a history of weakness, tremors, and paralysis. After a detailed gross and histologic examination and a bacteriologic, parasitologic, and rabies evaluation, a preliminary diagnosis of tick paralysis has been established. A thorough field search has revealed 13 affected mouflons found in the open hunting ground “Sveti Juraj” near the town of Senj (Croatia), along with an additional 35 mouflon carcasses. All 13 mouflons have been placed in a quiet, semi-dark stable. All detectable ticks have been removed manually, and the animals have been topically treated with 250 ppm of Amitraz (*N*,*N*′-[(Methylimino)dimethylidyne]di-2,4-xylidine) water emulsion (Taktic 12.5% EC, Intervet International, 5830 Boxmeer, Netherlands). The collected ticks have been identified as *Ixodes ricinus*, *Dermacentor marginatus*, and *Haemaphysalis punctata*. In the following 24 hr, all treated animals have been

recovered fully. This report describes a naturally occurring outbreak of tick paralysis in free-ranging mouflons from a karst habitat.

Cazapal-Monteiro et al. [1] have described their experience with control of parasites in Marcelle Natureza, a zoological park located in San Martín de Guillar (Outeiro de Rei, Lugo) where a large number of wild animals living in an area of ca. 20 hectares divided into large parcels. Continuous grazing has enhanced infection of animals by gastrointestinal nematodes, which sometimes has provoked severe diarrhea. Control of parasites has been based on anthelmintic administration 2 times / year. Despite the efficacy of the deworming, animals have become quickly reinfected since contamination of parcels with infective stages (L3 larvae of nematodes). After the observation of high values of eggs per gram of feces of gastrointestinal nematodes (≥ 500 epg) one month following the deworming of mouflon (*Ovis musimon*), one study has been conducted to establish the effect of the incorporation of a mixture of spores of ovicidal and nematophagous fungi (*Mucor circinelloides* + *D. flagrans*, respectively,) to the feed concentrate, on the risk of animal challenge. By means of the addition of 2x106 parasiticides fungi spores per kg of feed concentrate, and providing this mixture every 2 weeks, the animals have been able to maintain a rate of elimination of 50-150 eggs per gram (epg). After one year of using this biological therapy, deworming has not been necessary among wild animals, so this procedure is recommended by the authors in grazing wild herbivores in captivity.

In their study on the diseases in zoo animals from Lahore zoo, Pakistan, Nemat et al. [16] have pointed that Mouflon sheep are the most affected animals among ungulates in the period 2009-2013. The authors show the drugs which animals have been treated. Against the parasitic diseases ivomec injectable has been used.

We have summarized the bigger part of the above given data in the Table 1.

Parasites, Animals	**Medicine**	**Dosage and Scheme of Treatment**	**Results and Conclusions**
Psoroptes ovis **Desert bighorn sheep**	**Ivermectin**	**500 or 1000 µg/kg i.m.**	**Effective in controlling *P. ovis* in desert bighorn sheep.**
GIN and LN ***Moniezia* sp.** **Mouflons**	**Oxfendazole**	**A daily dose of 1.7 mg per kg for 3 consecutive days**	**It has been effective 88.9% against gastro-intestinal nematodes, 75.0% against lung nematodes and 100% against *Moniezia* sp.**
GIN **Mouflons**	**Albendazole**	**5 or 7.5 mg/kg body weight in food at 2.5 g/kg**	**It has been readily accepted even by fastidious animals and when given at high peak periods, has resulted in drops of infection.**
M. capillaris, Trichostrongylu	**Mebendazole and,**	**Animals have been regularly**	

***s* spp.*, Fasciola hepatica, Eimeria* spp. Mouflons	occasionally, Nilverm	dewormed in spring and autumn.	
Protostrongylus **spp.** Bighorn sheep	Ivermectin	Single s.c. injection (0,2 or 0,5 mg/kg)	It has shown high efficacy against adult *Protostrongylus.* It has eliminated L1 of *Protostrongylus* from faeces of animals 4 weeks after treatment. Bighorns treated with a 0,5 mg/kg have showed similar results that have lasted over 4 months post-treated period.
GIN and LN Mouflons	Premix from salicylanilide and mebendazole	Premix has been added to feed for 2 or 4 consecutive days.	High efficacies against these parasites. The 2-day treatment has been recommended.
GIN Mouflons	Luxabendazole	Split dose of 2 mg/kg daily on 5 consecutive days (pellets in feed)	Based on post mortem examinations, the drug has been highly effective against intestinal nematodes in mouflons.

Table 1. Literature data of antiparasite treatment in some wild sheep.
GIN – gastrointestinal nematodes; LN – lung nematodes

Parasites, Animals	Medicine	Dosage and Scheme of Treatment	Results and Conclusions
LN Bighorn sheep	Ivermectin	Oral administration	It has not been an effective tool for managing lungworm infections in a free ranging population of bighorn sheep.
GIN and LN Mouflons	Ivermectin	Orally on 6 consecutive days at a dose of 415 µg/kg per day to	Good efficacy including against the adult *Mullerius capillaris.* It rapidly has stopped also the elimination of infective

		each of mouflons	**forms (eggs and larvae) of the helminths.** **No side effects.**
M. capillaris **Mouflons**	**Flubendazole**	**3 x 15 mg/kg live weight**	**Efficacy can be in general evaluated highly positively. The drug quickly has stopped larvae excretion in the mouflon droppings due to adulticide effect in the verminous foci of the lungs.**
GIN and LN **Mouflons**	**Netobimin**	**7.5 mg/kg**	**100% effective against most of the parasite, without *Trichuris* spp. and protostrongylids**
M. capillaries ***Nematodirus* spp.** ***Oesophagostomum* spp.** **Mouflons**	**Ivermectin**	**0.20, 0.60, and 1.00 mg/kg of body weight, s.c.**	**A dose of 0.60 mg/kg of body weight IVM dose as optimum for parenteral treatment of mouflon nematodoses has been recommended.**
***Protostrongylus* spp. Rocky Mountain bighorn sheep**	**Fenbendazole-medicated salt**	**For four consecutive winters**	**free-choice availability of fenbendazole-medicated salt is a potentially effective management tool for long-term control of protostrongylid lungworm**
M. capillaries **Mouflons**	**Mebendazole or flubendazole**	**7.5 mg/kg body weight for 3 consecutive days**	**Better effectiveness with flubendazole.**
Ixodidae **Mouflons with tick paralysis**	**Amitraz water emulsion**	**Topically treated with 250 ppm**	**In the following 24 hr, all treated animals have been recovered fully.**

Table 1: Continued. Literature data of antiparasite treatment in some wild sheep.

The summing-up and analyses of all of the literature data give us grounds for drawing the following conclusions:

The benzimidazoles most often have been applied for treatment of parasites in wild sheep. Their effectiveness varies according to the parasite species. It has been high against gastrointestinal nematodes and relatively lower against lungworms. Some drugs, for example oxfendazole has been 100% effective against *Moniezia* spp. It has been established that albendazole is readily accepted even by fastidious animals. Two-day treatment has been recommended during the treatment with mebendazole. For long-term control of protostrongylids free-choice availability of fenbendazole-medicated salt has been recommended as a potentially effective management tool.

Ivermectin is other medicine tested for treatment of wild sheep. Applied parenterally it has been effective in control of the lung nematodes of *Protostrongylus* genus and mites *Psoroptes ovis*. The dosage of 600 μg/kg body weight is recommended for parenteral use. The results of orally administrated ivermectin are contradictory – in some cases the drug has been ineffective against lungworms, in others it has had good effect. Most probably this depends on the dosage and scheme of treatment.

The parasites more difficult for cure, independently of kind of remedies, have been protostrongylids and *Trichuris* spp.

Experiments have indicated that repeated administration of some anthelminthics may contribute to the development of parasite resistance. Other ones have demonstrated positive effect of biological control on the gastrointestinal nematodes in animals in captivity.

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EO7. ANTIOXIDANTS IN THE LIVER OF *FASCIOLA HEPATICA* INFECTED RABBITS AFTER MIXED BASIC SALTS APPLICATION

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Abstract

The effect of new synthesized mixed crystals of hydroxy sulfates of Zn, Co, Cu and Mn on the liver antioxidant status in experimentally *Fasciola hepatica* infected rabbits was studied. Two kinds of triple basic salts $(Zn_xCo_yCu_{1-x-y})_4(0H)_6S0_42H_20$ (29,89 mass % Cu^{2+} ; 64 mass % Co^{2+} ; 19,99 mass % Zn^{2+}); $(Zn_xCo_yMn_{1-x-y})_4(OH)_6S0_4.2H_20$ (33,05 mass % Zn^{2+} ; 10,70 mass % Co^{2+} ; 9,02 mass % Mn^{2+}) were synthesized and applied in *F. hepatica* infected rabbits in acute stage of the helminthosis. Antioxidant balance comprising quantity of the vitamins C, A and E, SOD and CAT activities and Zn, Cu, Co and Mn content was investigated in the livers of the infected and treated with the salts rabbits.

Antioxidant imbalance in the livers of *F. hepatica* infected rabbits was developed. It was expressed via reduced vitamines C, A and E content, changed enzyme activities and lower Zn, Co, Cu and Mn contents. Only Zn-Co-Mn salt has restored the developed hypovitaminosis E and C. Both triple salts administration normalized trace element losses and SOD-activity. They did not effect CAT activity and hypovitaminosis A. Zn-Co-Mn compound could be used for an effective control of fasciolosis in rabbits by the enhance of their antioxidant defense system. The results clearly showed the presence of antioxidant imbalance in the rabbits in acute stage of fasciolosis.

Key words: triple basic salts, antioxidants, liver, rabbits, fasciolosis

Introduction

The hosts defensive reaction in many parasitic diseases is manifested by an increased production of reactive oxygen species (ROS). Intensified action of ROS coincides with disturbances of the antioxidant system, responsible for their inactivation.

The susceptibility of rabbits to various parasitic diseases and high mortality of the young animals are hindering the development of rabbit meat industry.

Up to now, only neutral salts of trace elements are generally applied to correct mineral deficiencies in farm animals and rarely they have been applied to restore the normal mineral balance under parasitic infections. During the last years basic (hydroxy) salts and their mixed crystals are applied to restore the elemental deficiencies in experimentally infected with helminthes animals (6, 13).

The aim of our study is estimation of antioxidant abilities (levels of vitamins A, C and E, trace elements zinc (Zn), manganese (Mn), cobalt (Co) and copper (Cu), enzymes superoxide dismutase (SOD) and CAT activity) in the livers of rabbits in acute phase of fasciolosis after treatment with different triple hydroxy salts of Zn, Co, Mn and Cu.

Material and methods

The experiment was carried out on 32-days old male Chinchilla rabbits. Twenty rabbits were divided into six groups: group 1- control (healthy animals), group 2 - healthy and treated with $(Zn_xCo_yMn_{1-x-y})_4(OH)_6S0_4.2H_20$ rabbits, group 3 - healthy and treated with $(Zn_xCo_yCu_{1-x-y})_4(0H)_6S0_42H_20$ animals, group 4 - experimentally infected with *Fasciola hepatica* rabbits, group 5 - experimentally infected with *F. hepatica* and treated with $(Zn_xCo_yMn_{1-x-y})_4(OH)_6S0_4.2H_20$ animals, and group 6 - experimentally infected with *F. hepatica* and treated with $(Zn_xCo_yCu_{1-x-y})_4(0H)_6S0_42H_20$ rabbits. The animals from groups 4, 5 and 6 were orally infected with 60 metacercariae of *F. hepatica* per rabbit. Two weeks later the animals from groups 2, 3, 5 and 6 were treated with the triple basic salts respectively per os. The dose of Zn-Co-Mn salt was 0.029 g/per rabbit and that of Zn-Co-Cu salt was 0.021 g/per rabbit. The animals were kept in individual stainless steel cages. They were fed with a basal semisynthetic diet for young rabbits. The experiment was lasted on the 60" day post infection. The rabbit livers were collected for further investigations.

All of the procedures with the animals were conducted in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Specific Purposes and the current Bulgarian laws and regulations.

Rabbit livers were investigated for vitamins A, C and E levels, trace elements Zn, Mn, Co and Cu content and enzymes CAT and SOD activities. Liver vitamins levels were determined by HPLC methods, using a fluorescence detector (4). Trace element levels were established by Atomic Absorption Spectroscopy using Varian Techtran, Model 220 (2). SOD activity was determined according to the method of Beauchamp and Fridovich (3), and CAT activity - according to Aebi (1) method. Statistical analysis of the results was done by variation analysis and Student's t-test.

Results

Vitamin A, C and E levels in the livers of the infected with *F. hepatica* rabbits were reduced compared with controls. Vitamin A level was decreased with 25%, vitamin C level - with 40% and vitamin E level - with 51% in the infected animals. Liver vitamin A content in the infected and healthy rabbits was not changed after the treatment with the both triple salts (Fig. 1). Reduced vitamin C level in the liver of the infected host was slightly increased as a result of Zn-Co-Mn salt application. The vitamin C level was not affected after treatment with Zn-Co-Cu salt (Fig. l). The level of vitamin E in the infected rabbits was increased after the application of Zn-Co-Mn salt but not to the value of the control ($P<0.01$). Zn-Co-Cu was not had any effect on the reduced vitamin E level in the livers of *F. hepatica* infected rabbits (Fig. 2).

Fig. 1: Vitamin A and C level in rabbits (un)infected with *F. hepatica* and (un)treated with triple basic salts.

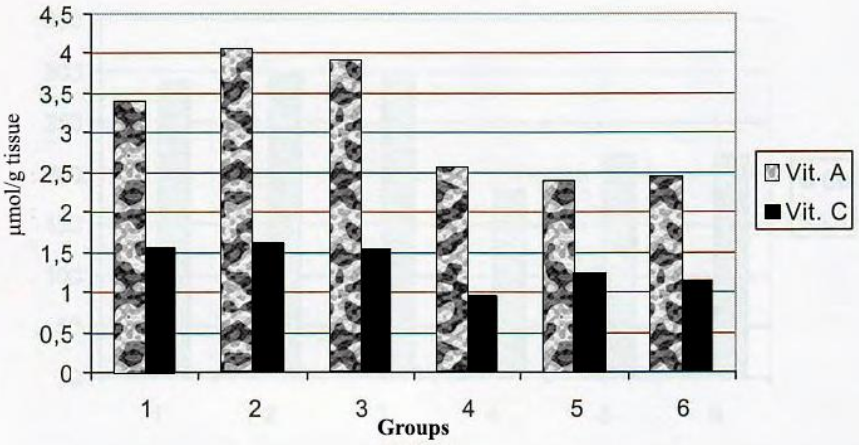
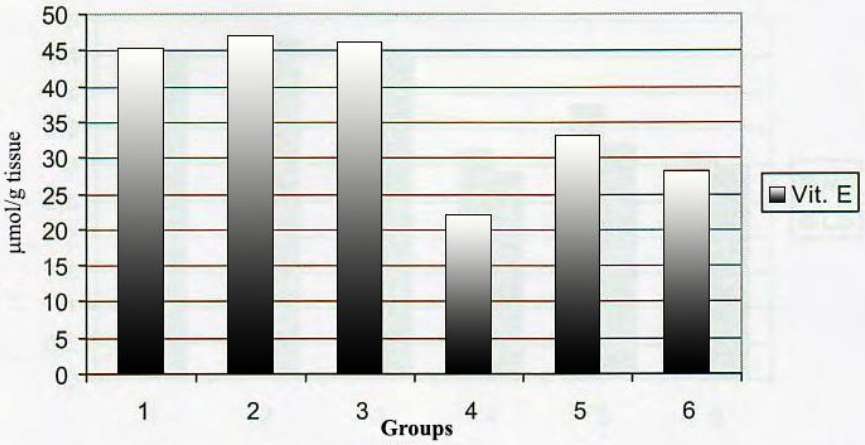


Fig. 2: Vitamin E level in rabbits (un)infected with *F.hepatica* and (un)treated with triple basic salts.



The levels of the investigated trace elements in the livers of *F. hepatica* infected rabbits were reduced compared to the controls. The Cu content was reduced with 19%. The application of Zn-Co-Mn salt was not changed it. The Cu level in livers of the infected rabbits was significantly increased after the application of Zn-Co-Cu salt (Fig. 3). Co and Zn levels were reduced with 36 % in the livers of rabbits with acute fasciolosis. Both applied salts increased the Co and Zn levels in the infected animals ($P<0.05$) (Fig. 4). The reduction of Mn content in the infected livers was with 22 %. The level of this trace element was influenced positively only after the treatment with Zn-Co-Mn salt (Fig. 4).

Fig. 3: Cu and Zn content in rabbits (un)infected with *F.hepatica* and (un)treated with triple basic salts.

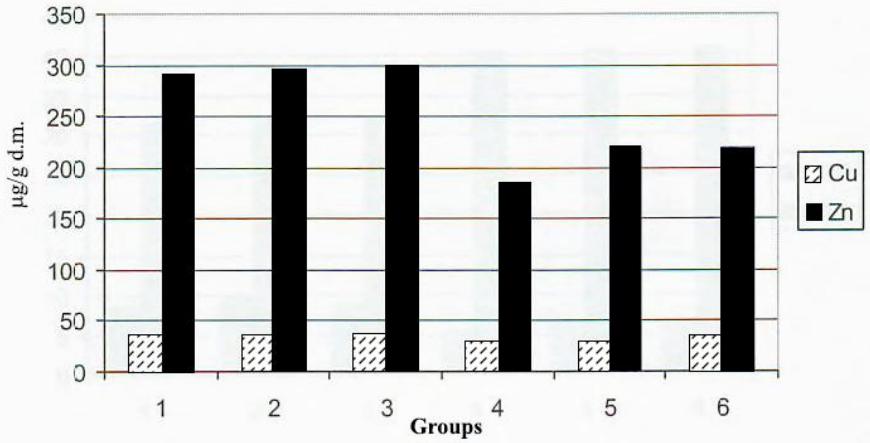
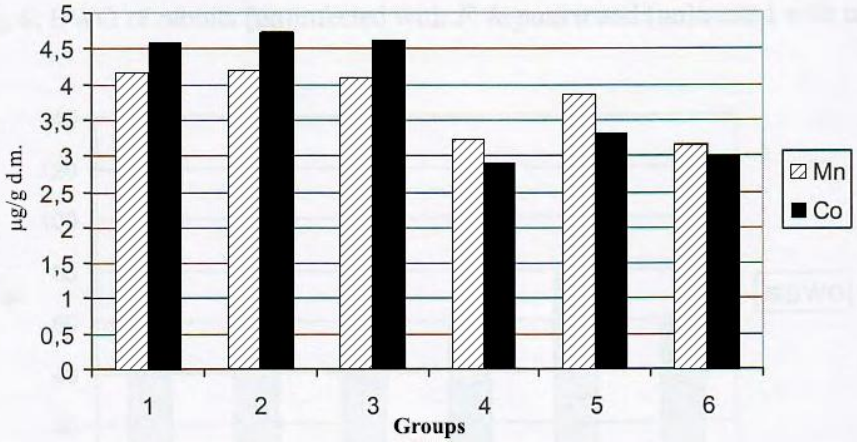


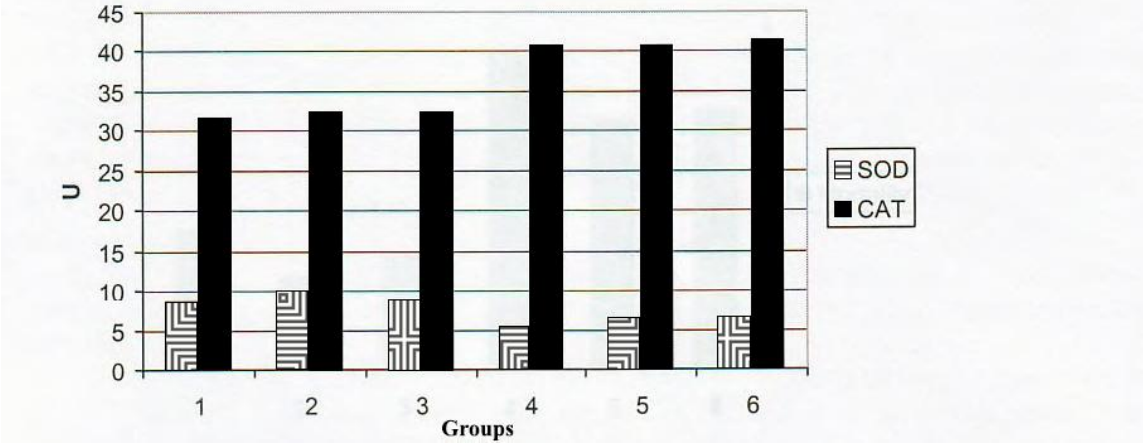
Fig. 4: Mn and Co content in rabbits (un)infected with *F. hepatica* and (un)treated with triple basic salts.



SOD activity was reduced in the livers in the *F. hepatica* infected rabbits compared to the controls ($P<0.01$) (Fig. 5). The application of triple basic salts increased the SOD activity in the infected ($P<0.01$) and healthy animals ($P<0.05$).

CAT activity was increased in the livers of the infected rabbits compared to the control ($P<0.01$). Both salts were not had any effect on the CAT activity ($P<0.01$) (Fig. 5).

Fig. 5: SOD and CAT activity in rabbits (un)infected with *F.hepatica* and (un)treated with triple basic salts.



Discussion

Developed hypovitaminoses A, C and E, trace element imbalance and alterations of enzymes activities are resulted in oxidative stress due to the acute fasciolosis. Vitamin E depletion is much better expressed than that of vitamin C and A. Vitamin E is an excellent biological chain-breaking antioxidant that protects cells and tissues from lipoperoxidative damage induced by free radicals which are increased during parasitoses (7). Vitamin C content is reduced because as a water soluble compound the vitamin is in the front-line of defense against free radicals created by the metabolism of infected host. Vitamin C has been demonstrated to enhance antioxidant ability of vitamin E by reducing the tocopheroxyl radicals back to their active form of vitamin E (9) or sparing available vitamin E (10). Regarding their antioxidant properties there is a synergistic effect of vitamin E and C on the immune response. Vitamin A deficiency in the livers of *F. hepatica* infected rabbits is developed as both the conversion of beta-carotene into vitamin A in the epithelial cells of the intestines and the storing of vitamin A in the livers are disturbed. If vitamin C and E contents are reduced, it means that there is a considerable loss of the vitamin A precursors.

The reduced SOD activity in the livers of *F. hepatica* infected rabbits may be due to the inactivation of the enzyme by interaction with oxygen radicals. Some authors have been reported for the fall of SOD activity by the action of hydroxyl radicals and H_2O_2 (12). It may be a result of injury of the enzyme by the superoxide radical.

The increased CAT activity indicates the highly enhanced capacity to scavenge hydrogen peroxide, produced in the liver cells in response to oxidative stress due to the infection. It may be a compensatory mechanism of the body to get rid of excess peroxides (8).

Antioxidant imbalance due to different parasitic infections is established by some authors (5, 7). Reduced vitamin A and C, carotene and retinal levels are determined in rats or sheep infected with *Fasciola* spp. or *Trychostrongylidae* spp. Significant changes in activity/concentration of antioxidant parameters in acute phase of fasciolosis in rats are observed by Kolodziejczyk et al. (11). Decreased activity of some liver enzymes (SOD, GSH-Px, GSSG-R and CAT) as well as a reduction of vitamins C, E, A and glutathione levels are established.

Well expressed decrease of the levels of the trace elements Zn and Cu and vitamins C, E and A in the livers of rabbits with acute fasciolosis is described in the present study. Our results are in a good agreement with the literature data. The compound containing Zn, Co and Mn showes positive effect on the vitamin E level, SOD activity and Mn, Zn and Co content in the livers of the infected rabbits. The applied biogenic trace elements Zn, Co and Mn almost normalize the antioxidant imbalance due to *F. hepatica* infection.

The presented results clearly show imbalances in levels/concentrations of nonenzymatic and enzymatic antioxidants investigated in the livers of rabbits in acute stage of fasciolosis. Antioxidant-oxidant imbalance is developed in the infected host. Applied in the study Zn-Co-Mn salt can be used to correct the antioxidant imbalance due to fasciolosis.

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EP1. EFFECTS OF NEMATODE INFECTIONS ON TUMORS IN ANIMALS AND ON TUMOR CELL CULTURES

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Abstract

Literature data are summarized in the present review article about the combination of some nematode infections and malignant tumors *in vivo* on animals and *in vitro* on tumor cell cultures. More of the results show a relation between some nematode infections like *Spirocerca lupi* and *Strongyloides stercoralis* and malignant tumors development in pat animals. However, there are investigations revealing tumor growth inhibition in experimental animals under *Trichinella spiralis, Nippostrongylus brasiliensis* and *Ascaris lumbricoides* infections. Data exist about cell growth inhibition of tumor cell cultures under the effect of protein products isolated from the last nematodes and the infected host tissues.

Key words: nematodoses, tumors, animals, cell cultures

Introduction

Literature data are available about the influence of some helminth infections on the host cell proliferation: stimulation of the malignant tumor growth under the effect of schistosomosis, opisthorchosis and clononrchosis [9, 10, 11, 16, 29, 30, 36] and inhibition of the cell proliferation under *in vivo* and *in vitro* combined experiments with trichinellosis, fasciolosis, nippostrongyloidosis, ascaridosis and tumors [12, 15, 21, 22, 33, 34, 35, 36, 37, 39].

Some nematode infections like *Spirocerca lupi* and *Strongyloides stercoralis* have been recognized as a causative factor for the development of cancer. Suggested mechanisms include chronic inflammation elicited by the parasite, changed immune response and a possible tumorigenic effect from certain parasitic secretions. Furthermore, some investigations in experimental animals exist which reveal inhibition of the development of transplanted malignant tumors under infections with the nematodes *Trichinella spiralis, Nippostrongylus brasiliensis* and *Ascaris lumbricoides* and on the growth of tumor cell cultures after treatment with protein products isolated from some nematodes and infected host tissues.

Nematode infected pat and farm animals and tumors

An investigation related to the frequency and pathology of infection with the nematodes *Heterakis gallinarum* and *Heterakis isolonche* in golden pheasants was conducted. Under light microscopy, leiomyomas in the muscular and submucosa of the cecal wall associated with immature *H. gallinarum* worms were observed. The authors considered that the probable causes for the appearance of granulomatous nodules, which could evolve to neoplasias, would be continuous reinfections with *H. gallinarum* provoking a tissue phase of the disease [19].

Spinal B-cell lymphoma and concurrent pulmonary filariasis were diagnosticated in a pet rabbit. The rabbit was presented for investigation for pelvic limb paralysis resulting from extradural spinal lymphoma, presumably rising from the body of the sixth lumbar vertebra. The neoplasm was subsequently immunophenotyped as a B-cell lymphoma. Pulmonary filariasis was an incidental finding at necropsy of the euthanased animal [27].

A rare case of the combination of trichinellosis and oral squamous cell carcinoma in a domestic cat was reported. The animal had been missing from its home for approximately 1 month. After that it

was returned ill and died after few weeks. A small mass was found in the subcutaneous tissue at the rostroventral surface of the left part of mandible. Histopathologic examination revealed mild to moderate chronic–active inflammation associated with helminth fragments and subsequantly larvae of *Trichinella* spp. were detected. In the tissue sections from the oral cavity, there was invasion of the submucosa and connective tissues by a poorly encapsulated and densely cellular squamous cell carcinoma [20].

Spirocerca lupi is a nematode infesting the canine esophagus, where it induces the formation of granulomas and nodules that may transform into sarcomas. Neoplastic transformation of these granulomas to osteosarcoma or fibrosarcoma was described in three dogs infected with *S. lupi*. On the basis of this investigation the authors developed a xenograft murine model of *S. lup*i - associated sarcoma, using tumor material from the infected dogs. Tumor lines of *S. lupi*-associated sarcomas were created in all investigated groups of mice after the tumor inoculation. According to the researchers, this resource would facilitate studies on the malignant transformation of the granulomas, establishment of efficient chemotherapy and radiotherapy, and identification of diagnostic molecular markers for the disease [32].

The objectives of another study were to identify clinical ante-mortem differences between malignant and benign spirocercosis cases in dogs. Medical records of 297 dogs diagnosed with spirocercosis in a Veterinary Academic Hospital were used. Two groups of cases were selected from these records: confirmed benign cases and confirmed malignant esophageal nodule cases. The received results showed that the dogs with spirocercosis-induced sarcoma were significantly older than the benign cases. The difference in age between the two groups of animals may be partially explained by the time taken for the malignant neoplasm to develop and be diagnosed, but may also indicate an increased predisposition to malignant transformation with advanced of the age [8].

Spirocerca lupi infection and tumors of the spinal cord, the esophagus and the skeletal muscle were diagnosed histologically in a pet dog after euthanasia. Caudal esophageal mass was found at post mortem examination. This was diagnosed as a *S. lupi* nodule. *S. lupi* – induced esophageal chondrosarcoma and was believed to be the primary site from which the other, presumably metastatic, lesions originated [17].

Computed tomography imaging, necropsy and histopathological investigations made in a pet dog proved *Spirocerca lupi* induced esophageal neoplastic transformation (extraskeletal osteosarcoma). It was believed to be the primary lesion, and the majority of secondary metastasis to the brain, spine, heart, multiple muscular groups and abdominal organs were observed. Extraskeletal osteosarcomas are generally more aggressive than skeletal osteosarcomas and this may explain the widespread metastasis in the dog body [25].

Data existed that malignant transformation of esophageal lesions to sarcomas, which may metastasize to the lungs, under spirocercosis in dogs, occurred in 8–26% of the cases, and carried a grave prognosis. It was reported about a biochemical study, which was provided to characterize the acute phase response in pat dogs with benign and suspected malignant spirocercosis at the time of diagnosis, and during anti-helminthic treatment. The concentrations of all positive serum acute phase proteins were significantly higher in suspected neoplastic cases compared to benign ones [23].

Clinical, blood, serum biochemistry and parasitological assessments were performed on four hospitalized dogs which were indicated to be infected with *Dirofilaria immitis*. In the histopathological examinations, pulmonary adenocarcinoma deriving from bronchial epithelium was identified in one of the dogs, and nematodes *D. immitis* were encountered in bronchial and bronchiolar lumens, interstitium of the lungs, and bile ducts [31].

A case of esophageal *Gongylonema pulchrum* infection and esophageal squamous cell carcinoma in adult female vari (Lemur macaco variegates) was observed. The lemur had lived in a zoo and had a clinical history of dyspnea, vomiting, and anorexia. At necropsy, a whitish, soft, nodular, centrally necrotic mass was found in the caudal third of the esophagus. In addition, numerous intraepithelial nematodes *G. pulchrum* were observed in the entire esophagus. A relation between the infection with *G. pulchrum* in the host esophagus and the development of esophageal squamous cell carcinoma was suggested [4].

Nematode infected experimental animals, tumors and tumor cell cultures

It was observed that the growth of Walker sarcoma was entirely suppressed or greatly enhanced in *Nippostrongylus brasiliensis* infected rats depending on the timing of the tumor-cell inoculum in relation to the parasitic infection. Early, before arising of the immune response to the parasite, the tumor did not grow, but, once the immune response had developed, tumor growth was enhanced. The growth of a syngeneic adenocarcinoma in mice was suppressed in *N. brasiliensis* infected mice whether the infection was given before, with, or after the tumor inoculum. The authors suggested that this effect could be prevented by antilymphocyte serum [15].

Stimulation of the tumor growth was found out in rats, under combined effect of *Trichinella spiralis* infection and nickel sulfide (Ni_3S_3) induced sarcoma. Rats first were infected with 3000 *T. spiralis* larvae and 5 days later they were injected with nickel sulfide into the gastrocnemius muscles [14].

More data were published about observed antineoplastic effect under experimental trichinellosis.

It was established that *Trichinella spiralis* infection in mice carried out 28 days prior to sarcoma-180 intraperitoneal transplantation, produced small but statistically significant increases in the length of both the incubation period and survival time, but did not affect the clinical phase of the tumor development. Administration of tumor cells 56 days following the helminthic infection had no detectable effect on any parameter of tumor development studied. The results were consistent with the hypothesis that *T. spiralis* infection temporarily altered the host reaction to sarcoma-180, possibly at the level of non-specific macrophage activity [18].

The antineoplastic effect of long-term *Trichinella spiralis* infection was assessed by daily observation of the development and progression of neoplastic nodules in mice. For this purpose the experimental animals were orally infected with 200 *T. spiralis* larvae 176 days preceding subcutaneous administration of solid B-16 melanoma cells. Control mice developed tumors by day 28 following tumor challenge, while none of the corresponding *T. spiralis*-infected animals demonstrated any signs of neoplasia. All control mice died within 60 days, while none of the nematode-infected animals developed detectable neoplasms. This phenomenon suggested that the presence of well-established larval cysts was capable of stimulating host antineoplastic activity [21].

It was reported for protection from intraperitoneally applied ascites neoplasia sarcoma-180, which was found to be statistically significant, under different larval doses of *Trichinella spiralis* infection and challenge intervals in mice. Mice infected with 100 or 200 larvae were more resistant to ascites sarcoma-180 progression than uninfected controls or infected with 300 or 400 *T. spiralis* larvae animals. Protection was observed in the mice challenged with the tumor 2 weeks after nematode infection but not at 6, 8 or 34 weeks [22].

It was established that solid tumor B-16 melanoma development was inhibited in mice with chronic trichinellosis. In the infected tumor-bearing animals, the tumor induction intervals were longer and the tumor size was subsequently smaller than in the control mice. When the number of tumor cells in the inoculum was less than that required to produce 100% tumor incidence in the uninfected mice, significantly more of the animals with *Trichinella spiralis* infection of 2 months duration remained tumor-free [26].

The possibility of the involvement of histamine in the anti-neoplastic effect of *Trichinella spiralis* on the growth of a murine fibrosarcoma was tested using an H_2-receptor antagonist (cimetidine) and an H_2-receptor agonist (tolazoline). The tumor fibrosarcoma was originally induced by 3-methylcholanthrene in mice. Mice were orally infected with 200 *T. spiralis* larvae, 8 days before subcutaneous inoculation of the tumor cells. Cimetidine or tolazoline were administered intraperitoneally. The antitumor effect of *T. spiralis* was detected to be the strongest during intestinal stage of the disease when higher production of mastocytes and T-suppressor lymphocytes was established. The authors assumed that H_2-receptor-bearing cells (e.g. suppressor T-cells) partially

suppressed the anti-neoplastic effect of *T. spiralis*. The effect of enhanced from the helminth infection suppressor T-cells, known to possess H_2-receptors, was blocked by the H_2-receptor antagonist cimetidine, which increased the parasite induced anti-neoplastic effect [28].

The effect of *Trichinella spiralis* infection was studied as a modulator of intradermally applied oncogen Shopes's fibroma virus, causing benign adenoma in rabbits. Thirty-five days after the nematode infection and 9 days after the virus injections tumor lesions were noticed. The rabbits pretreated with *T. spiralis* exhibited much lower virus titres than the untreated controls, which was evidently related to a certain degree of aspecific immunity conferred by the parasite. The results indicated that *T. spiralis* produces, in rabbits, resistance to Shope's fibroma virus and its neoplastic effect [3].

Cross antitumor resistance was detected under experimental trichinellosis and chemically induced carcinogenesis in rats. An insignificant decrease in the numbers of 3,4-benzpyrene induced malignant fibrous hystiocytoma and inhibition of the development of 7,12-methylbenzoanthracene induced breast fibroadenoma were established after prophylactic infection with *Trichinella spiralis* in rats [2].

Murine forestomach carcinoma (cell line MFC), ascitic hepatoma (cell line H22) and sarcoma (cell line S180) tumor models were used to test the anti-tumor effect of *Trichinella spiralis in vivo* in mice. Groups of tumor-bearing mice were given caudal vein injection of crude extracts of adult worms and newborn larvae of *T. spiralis*. These treatments inhibited tumor growth and were dose-dependent. Furthermore, the anti-proliferative activity of crude *T. spiralis* extract was examined *in vitro* using tumor lines MFC, H22, S180, human chronic myeloid leukemia cell line (K562) and hepatoma cell line (H7402). Tumor cell proliferation *in vitro* was measured by methyl thiazolium stain and was inhibited in dose-dependent manner. Cell cycle analysis indicated that the application of crude *T. spiralis* extracts arrested the cell cycle of lines K562 and H7402 in G1 or S phase. The authors concluded that the nematodes *T. spiralis* contain anti-tumor active agent [38].

The ability of *Trichinella spiralis* and *Trichinella britovi* infections to develop a protection against Walker 256 carcinosarcoma in host body was studied in rats by macro- and microscopical methods. The rats were initially infected with different doses of *T. spiralis* and *T. britovi* larvae. Three weeks p.i. the animals were subcutaneous grafted with cells of Walker 256 solid tumor. Three months later, after clinical examination, the rats were sacrificed and investigated. According to the received results, the authors concluded that both *T. spiralis* and *T. britovi* infections protected the host against the tumor invasion, but *T. spiralis* developed more powerful effect [24].

A study was provided for investigating the effect of *Trichinella spiralis* infection on tumor growth and metastasis of B16-F10 melanoma in mice. After oral infection with *T. spiralis* larvae, B16-F10 cells were injected subcutaneously and intravenously into experimental mice to evaluate tumor growth and metastatic potential, respectively. It was established that tumor growth and lung metastases in *T. spiralis* infected mice were significantly reduced compared with control mice. In order to elucidate the mechanism of this action, cytokine arrays were conducted using mouse serum. On the basis of these results the authors suggested that *T. spiralis* infection reduced tumor growth and metastasis through a complex transition in cytokine regulation profiles in the host [13].

It was described that *Trichinella spiralis* infection conferred effective resistance to tumor cell expansion. Latter, a T7 phage cDNA display library was constructed to express genes encoded by *T. spiralis*. Organic phase multi-cell screening was used to sort through candidate anti-tumor proteins from *T. spiralis* in a transfected human chronic myeloid leukemia cell line (K562) and a human hepatoma cell line (H7402) using the display library. The protein encoded by the A200711 gene of *T. spiralis* was identified and analyzed using protein analysis software. To test the antitumor effects of A200711, variations in cell proliferation and apoptosis were monitored after recombinant pEGFP-N1-A200711 was transfected into H7402 tumor cells. The results showed that the expressed target gene successfully induced apoptosis in H7402 hepatoma cells [39].

A study was provided for investigating the effect of excretory/secretory proteins from *Trichinella spiralis* on apoptosis of NCI-H446 small-cell lung cancer cells *in vitro*. The expression of Bcl-2, Fas and

FasI mRNA was detected by RT-PCR. C-myc protein expression level was examined by Western blotting and immunofluorescence assay. It was proved that *T. spiralis* excretory/secretory proteins may inhibit apoptosis of NCI-H446 small-cell lung cancer cells by reducing the apoptosis protein C-myc and Bcl-2 mRNA levels, and causing the increase of Fas/Fasl mRNA ratio [5].

Thermostable biologically active substances (BASes), inhibitors of cell proliferation, were isolated from the livers of healthy and *Trichinella spiralis* infected rats. Their effects were investigated *in vitro* on the cell viability of primary Graffi myeloid tumor cell cultures and human permanent tumor cell lines HeLa and T-24. The inhibiting effect of BAS isolated from the livers of *T. spiralis* infected rats on Graffi myeloid tumor cells viability was the strongest compared to the effects of BASes on the other cell cultures and to the effect of BAS isolated from livers of healthy rats [37].

In different articles it was supposed that the infection with nematodes *Trichinella spiralis* may provoke anti-tumor effect by activating macrophages or stimulating cell-mediated immune response in the host, and/or possessing tumor-associated antigens and anti-tumor active substances of the parasite [7, 14, 18, 40].

The antitumor effects of helminthes *Toxoplasma gondii* and *Toxocara canis* egg antigens were investigated in comparison with Bacillus Calmette Guerin (BCG) (known to have anticancer distinctive) *in vivo* on solid WEHI-164 fibosarcoma transplanted to mice. Tumor size was measured. It was established that *T. gondii* parasites and *T. canis* egg antigens induced inhibition of the tumor growth like BCG in the tumor fibrosarcoma mouse model [6].

The effect of whole worm extract of *Ascaris lumbricoides* was investigated on solid Lewis lung carcinoma, transplanted to mice. The animals were intraperitoneally injected with worm extract of *A. lumbricoides*, and subcutaneously injected with Lewis lung carcinoma cells at right axillary region, in different successions. The results indicated that the tumor formation was affected by the whole worm extract of *A. lumbricoides* which had an inhibitory effect on tumor growth [41].

A study was carried out to investigate the effect of different doses of crude antigens of *Ascaris lumbricoides* on the secretion of IL-6 and TGF-β of human lung adenocarcinoma cell cultures (A549 cells), and the apoptosis of A549 cells. It was established that the cell cycle of A549 cells was blocked in G0/G1 phase induced by crude antigens of *A. lumbricoides*. The increased apoptosis rate of A549 cells induced by crude antigens might be related to the reported in the investigation changes in the levels of TGF-β and IL-6 [12].

The effects of the antifilarial drug diethylcarbamazine citrate (DEC), excretory-secretory (ES) material from the filarial parasite *Setaria equina* or a combination of both were examined on the status of oxidative stress and pathogenesis of hepatocellular carcinoma (HCC) induced by diethylnitrosamine and 2-acetylaminofluorene in rats. Pathogenesis of liver cancer and ES treatment were evaluated using histological investigation, level of antioxidant and liver function enzymes. Repeated ES doses increased the activity of antioxidant enzymes and showed a protective effect on liver architecture. DEC could modulate the later effects when combined with ES. This study could indicate the effect of *S. equina* ES as antioxidant against rat HCC, while DEC could modulate such effect when combined with it [1].

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EP2. THE ROLE OF THE PARASITE VARROA DESTRUCTOR AS VECTOR OF VIRUSES ON HONEY BEE APIS MELLIFERA

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ABSTRACT:

Varroa destructor (Anderson and Truman, 2000) previously described as *Varroa jacobsoni* (Oudemans, 1904) (a closely related mite) is a parasitic mite of honey bees. According to results of scientific research projects, the main cause of honey bee colony loss is this mite, which can be found in almost every apiary in Europe. This mite is an external parasite that attaches to the body of *Apis* species, and breeds within the colony by laying its eggs within capped brood and feeding on *Apis* larvae. Varroa is present on all continents with the exception of Australia. Depending on climatic conditions, the damage caused by *V. destructor* appears from autumn to early spring during the overwintering phase, leading to general weakening and often complete losses of colonies. High level infestations can be a direct cause of colony loss, but the mite is also a vector of a number of viruses. The ectoparasitic mite *Varroa destructor* and honey bee pathogenic viruses have been implicated in the recent demise of honey bee colonies. Several studies have shown that the combination of *V. destructor* and deformed wing virus (DWV) poses an especially serious threat to honey bee health. Nowadays the bees viruses like as Sacbrood Virus (SBV), Acute Bee Paralysis Virus (ABPV), Chronic Paralysis Virus (CPV), Izraeli Acute Paralysis Virus (IAPV), Kashmir bee virus – (KBV), Deformed Wing Virus (DWV), Cloudy wing virus (CWV), Varroa destructor virus-1 (VDV-1) are associated with mite. Although bee viruses usually persist as unapparent infections and cause no overt signs of disease, they can dramatically affect honey bee health and shorten the lives of infected bees under certain conditions. Even with proper management it is impossible to keep apiaries 100% free from *V. destructor* mites. This pathology causes commonly called varroasis (also known as varroatosis or varrosis).

Key words: varroa destructor, bee viruses, vector

INTRODUCTION:

Honeybees form an integral part of the human food chain. In addition to producing both honey and beeswax, honeybees provide essential pollination services for hundreds of crops, including apples, almonds, avocados, beets, berries, cabbage, canola seeds, carrots, cherries, cotton, cucumbers, kiwi, melons, peaches, pears, plums, soybeans, squash, and tomatoes. It is estimated that 1/3 of the human diet comes from insect-pollinated plants, and honeybees provide 80% of that pollination. Various studies have suggested that bees pollinate over $15 billion in crops in the United States every year.

Unfortunately, honeybee colonies have also suffered much devastation in recent years. Repeated parasitic infestations, bacterial, viral, and fungal infections, and increased environmental stresses on the honeybee population have contributed to huge losses. Mite infestations in the late 1980s and 1990s practically have eliminated wild honeybee colonies and have caused the loss of 50-70% of managed bee colonies (Peterson, 2009). More recently, in late 2006 and early 2007, large numbers of bee colonies have been lost for no apparent reason. This phenomenon is known as Colony Collapse Disorder and represents a great threat to the honeybee population and their essential agricultural functions in the USA.

VARROA DESTRUCTOR

The mite *Varroa destructor* is an obligatory ectoparasite of the honey bee (*Apis mellifera*) and is one of the major threats to apiculture worldwide. For thousands of years, Varroa mites have been parasites on the Asian honey bees *Apis cerana*, giving this species the opportunity to learn to live with the mites. The first honey bee colony losses attributed to the Varroa mite have been reported in the Far East during the 1960's; the mites have since spread to most areas of the world where *Apis mellifera* are kept. Unfortunately, the European honey bee *(Apis mellifera)* did not have any time to adapt to this pest, which was accidentally introduced in Europe by international trade in 1977 and then later in North Africa, America and New Zealand as well. Scientists have intensively studied this parasite. They have researched the biology of the mites, its potential as a vector for disease and specific approaches to control. This, in turn, has led to the development of effective, safe medicines the strategic use of which has helped to prevent even greater damage to honey bee populations. In the beekeeping industry, at least 18 (Sammataro and Yoder, 2012) virus types and strains have been recorded as disease pathogens of adult bees and brood. Almost all are RNA viruses.

Bees are well protected against viral infection with their chitin body shell and gut coating; therefore, a virus particle existing on the outside body or cuticle is unlikely to infect the cells of the bee. However, parasitic mites can penetrate the exoskeleton and leave the bee vulnerable to infection from bacteria and viruses.

Varroa is especially harmful because it is an important vector in the spread of bee viruses (Martin et al., 2012). When the varroa mite pierces both the body of pupae and adult bees with its mouthparts, in addition to creating a site for the entry of infections, the mite can also introduce viral infection directly into the bloodstream. If viruses can enter the bee's cell structure via the bloodstream (haemolymph) or broken internal tissue (in the mid-gut), major damage can be done. The damage caused to colonies by viral infection varies according to a number of factors including the type and strain of virus involved, the strength and health of the colony (including the infestation levels of both varroa mites and Nosema sp.), weather conditions, the season and food availability.

Di Prisco et al., 2011 have provided the first evidence that Varroa destructor is IAPV replication-competent and capable of vectoring IAPV in honeybees. The honeybees became infected with IAPV after exposure to Varroa mites that carried the virus. The Israeli acute paralysis virus (IAPV) is a significant marker of honeybee colony collapse disorder (CCD). The copy number of IAPV in bees has positively correlated with the density of Varroa mites and time period of exposure to Varroa mites. This

study has showed an active role of Varroa mites in IAPV transmission and has sheds light on the epidemiology of IAPV infection in honeybees.

According to (Genersch, 2006) honey bees (*Apis mellifera*) productively infected with d*eformed wing virus* (DWV) through *Varroa destructor* (*V. destructor*) during pupal stages develop into adults showing wing and other morphological deformities. Deformed wing virus (DWV) is a honey bee pathogen which, like other honey bee viruses, generally persists as an inapparent infection. Outbreaks of clinical DWV infections have been characterized by the occurrence of morphologically abnormal bees and have been invariably associated with infestation by the parasitic mite *Varroa destructor* (Ball and Allen, 1988; Martin, 2001; Nordström, 2003). In accordance with (Gisder et al., 2009) deformed wing virus (DWV) normally causes covert infections but can have devastating effects on bees by inducing morphological deformity or even death when transmitted by the ectoparasitic mite *Varroa destructor.* In order to determine the role of *V. destructor* in the development of crippled wings, they have analyzed individual mites for the presence and replication of DWV. Their results have supported the correlation between viral replication in mites and morphologically deformed bees. Locke et al. (2012) have also confirmed in their experiments that Varroa mite infestation may be important to the development and maintenance of damaging DWV titers in colonies.

Nordström (2003) has investigated the distribution of deformed wing virus (DWV) in adult females *Varroa destructor* and in their progeny in relation to the pupal host. Bees have been investigated to evaluate acquisition and transfer of DWV by the mites. The results clearly have shown that adult female mites regularly act as competent vectors of DWV, however, they do not acquire or transfer virus on all possible occasions. Mother mites may contain DWV while the pupal host remains free from overt infection and both mother mites and mite progeny may not acquire detectable amounts of DWV from an infected host bee. However, a majority of mites feeding on pupae that emerge with deformed wings have to contain DWV. Their data have also demonstrated that both adult and immature mite progeny most likely acquire DWV from DWV-infected host bees and not from their mother mites. Several studies have shown that the combination of *V. destructor* and deformed wing virus (DWV) poses an especially serious threat to honey bee health. Mites transmitting virulent forms of DWV may cause fatal DWV infections in the developing bee. Pupae parasitised by mites not inducing or activating overt DWV infections and may develop normally (Schöning, 2012).

According to Martin et al. (2012) the mite increases the prevalence of a single viral species, deformed wing virus (DWV), from ~10 to 100%) within honey bee populations, which accompanied by a millionfold increase in viral titer and a massive reduction in DWV diversity and predominance of a single DWV strain. Therefore, the global spread of Varroa has selected DWV variants that have emerged to allow it to become one of the most widely distributed and contagious insect viruses on the planet.

Francis et al. (2013) have been investigated the viral titres (Acute-Kashmir-Israeli complex and deformed wing virus) in honey bees and varroa mites from 23 colonies (15 apiaries) under three treatment conditions. The experiment has been provided in the next variants: 1. Organic acids (11 colonies); 2. pyrethroid (9 colonies) and 3. untreated (3 colonies). They have found that in colonies where varroa treatment reduced the mite load, colonies overwintered successfully, allowing the mites and viruses to be carried over with the bees into the next season. In general, AKI and DWV titres have not shown any notable reaction to the treatment and steadily have increased over the season from April to October. In the untreated (control group), titres have increased dramatically. Viral copies have correlated to number of varroa mites. Most colonies that collapsed over the winter have had significantly higher AKI and DWV titres in October compared to survivors. Only treated colonies have been survived the winter.

The ability of the parasitic mite *Varroa destructor* to transmit Kashmir bee virus (KBV) to the Western honey bee (*Apis mellifera*) has been investigated by exposing pupae from a KBV-negative colony to varying numbers of adult female mites from KBV-positive colonies (Chen et al., 2004). The virus status of pupae and the mites have been determined after five days by RT-PCR. They have found a significant relationship between KBV-positive pupae and exposure to KBV-positive mites. No pupae have been virus-positive when all the mites introduced into a given cell subsequently have been tested negative. Mites testing positive for KBV have transmitted virus about 70% of the time. The percentage of KBV-positive *V. destructor* in a given cell also has increased significantly.Virus-free mites have become virus-positive by cohabiting in the same cell with virus-positive mites, and they have calculated the mite-to-mite transmission rate as 51%.

In accordance with (Sumpter and Martin, 2004) the feeding activities of *Varroa* mite provides a new route of transmission for some bee viruses, earlier found only in inapparent form. It has been shown that APV, DWV, slow paralysis virus (SPV) and Kashmir bee virus (KBV) can all be successfully transmitted between honey bees during mite feeding activities. These four viruses are also known to multiply rapidly when artificially injected into bee pupae. Mites only carry viruses so long as they are attached to an overtly infected bee. According to the investigations (Nordstrom 2000) when a virus-free phoretic mite moves from an uninfected to an infected adult bee, it will begin carrying the virus.

Tentcheva et al. (2004) have investigated in *Varroa* samples, the following four viruses: DWV (100% of the apiaries), SBV (45% of the apiaries), ABPV (36% of the apiaries), and KBV (5% of the apiaries). Their latter findings have supported the putative role of mites in transmitting these viruses. Taken together, these data indicate that bee virus infections occur persistently in bee populations despite the lack of clinical signs, suggesting that colony disease outbreaks might result from environmental factors that lead to activation of viral replication in bees.

Shen et al. (2005) have studied the role of varroa mites in infections of Kashmir bee virus (KBV) and deformed wing virus (DWV) in honey bees. They have performed quantitative comparison of viral infections between bees with and without mites by dot blot analysis and enzyme-linked immunosorbent assay (ELISA). Under natural and artificial mite infestations, bee pupae contained significantly higher levels of Kashmir bee virus (KBV) and deformed wing virus (DWV) RNAs and KBV structural proteins than mite-free pupae. Moreover, in mite-infested bee pupae, DWV had amplified to extremely high titers with viral genomic RNA being clearly visible after separation of total bee RNA in agarose gels. Linear regression analysis has shown a positive correlation between the number of mites introduced and the levels of viral RNAs. The detection of viral RNAs in the nymph and adult mites underline the possible role of varroa in virus transmission. However, most groups of virus-free adult mites (9/12) were associated with bee pupae heavily infected by viruses, suggesting that the elevated viral titers in mite-infested pupae more likely resulted from activated viral replication. Based on these observations and their concurrent research demonstrating suppressed immune responses in bees infested with mites. They propose that parasitization by varroa suppresses the immunity of honey bees, leading to activation of persistent, latent viral infection.

CONCLUSION

Varroa mites not only cause significant damage to the bees by feeding on their hemolymph, but also act as a vector for viral diseases. The wounds inflicted by mites may also be contaminated with bacterial or fungal organisms. These secondary infections can be recognized based on typical symptoms associated with them and often lead to severe damage to the colony. In order to prevent this, it is crucial that colonies have to be monitored for Varroa infestation regularly. Control the infestation has been taken when necessary.

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Session F

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FO1. CLINICAL CASE OF TOXIC EPIDERMAL NECROLYSIS

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Toxic epidermal necrolysis or Lyell's syndrome is severe *life-threatening* adverse drug reactions, with a high mortality rate. The drugs most commonly involved are: antibiotics; anticonvulsants; antiretroviral drugs; nonsteroidal anti-inflammatory drugs, allopurinol. **Case report:** A 68-year-old female, presented with complaints of fever and extensive rashes on the skin of the face, the neck and the trunk, severe itching of the skin, ulcerations and erythema of the conjunctiva and the oral cavity and difficulty in swallowing. She has a period of two or three days of general discomfort and fatigue, rash, fever up to 38°C, sore throat, arthralgia, myalgia, loss of appetite and have been treated with antipyretics, antibiotic - ampicillin, antihistamines, vitamins. Her state worsened during the next 3 days, so she was hospitalized in the Toxicology clinic. UMHATEM "N.I.Pirogov". Multiple maculopapular and bullous eruptions, plaques were present all over the body, blisters that cover a substantial portion of the body. The entire skin covering the body surface was denuded and peeled off with minor manipulation - Nikolsky's sign. Intraorally, multiple oral ulcers of the buccal mucosa, tongue, palate, labial mucosa, soft palate were seen. Hemorrhagic erosions were also seen on both the upper and lower lips. Conjunctivitis and ulceration of genitalia was also noted. The lesions got slowly better with serum therapy, fluid and electrolyte replacement, *systemic* corticosteroids, antihistamines, antibiotic, vitamins, H2 blockers, topical care of mucosal changes. Evolution was satisfactory with epidermization and she was discharged from the hospital after 1 months.

Key words: Toxic epidermal necrolysis, Lyell's syndrome

Introduction

Toxic epidermal necrolysis (TEN) or Lyell's syndrome is severe *life-threatening* adverse drug reactions, characterized by *bullous and erosive lesions involve oral, ocular, and genital mucosa and vast areas of the skin with extensive dermo-epidermal detachments.*

Various etiologies have been proposed, but drugs have been chiefly characterized as the offending agents. The drugs most commonly involved are: antibiotics; anticonvulsants; antiretroviral drugs; nonsteroidal anti-inflammatory drugs, allopurinol. *An immunologic response to immunocomplexes formed by metabolites of the causal drug and the common tissue antigens is thought to be responsible for this disorder.* Recovery is usually slow and may take 3–6 weeks. As a rule, skin lesions heal without scarring, whereas mucosal scarring and strictures are frequent late complications (1, 2, 11).

Worldwide incidence is 1-2 cases per million population per year (4). It can affect all age groups but is more common in elderly people, perhaps due to the increased numbers of drugs that they are prescribed. Mortality due to TEN is most often cited as 30–50% (1, 3–5). Sepsis and multi-organ failure are the main causes of death.

A case of TEN, treated in the Toxicology clinic and Burn Centre, Emergency University Hospital "Pirogov" was presented.

Case report: A 68-year-old female, presented with complaints of fever and extensive rashes on the skin of the face, the neck and the trunk, severe itching of the skin, ulcerations and erythema of the conjunctiva and the oral cavity and difficulty in swallowing. She has a period of two or three days of general discomfort and fatigue, rash, fever up to 38°C, sore throat, arthralgia, myalgia, loss of appetite and have been treated with antipyretics, antibiotic - ampicillin, antihistamines, vitamins.

There was no history of previous hypersensitivity reaction to drugs.

Her state worsened during the next 3 days, so she was hospitalized in the Toxicology clinic. On general examination, she was well-oriented and conscious. Initial vital signs were: heart rate 88 beats per minute, blood pressure 145/89 mmHg, respiratory rate 26 breaths per minute Multiple maculopapular and bullous eruptions, plaques were present all over the body, blisters that cover a substantial portion of the body. The entire skin covering the body surface was denuded and peeled off with minor manipulation - Nikolsky's sign. Intraorally, multiple oral ulcers of the buccal mucosa, tongue, palate, labial mucosa, soft palate were seen. Hemorrhagic erosions were also seen on both the upper and lower lips. Conjunctivitis and ulceration of genitalia was also noted.

Based on the history and clinical presentation, a diagnosis of TEN was given.

The routine laboratory investigations were within normal limits.

A chest radiograph of the patient did not show any active lesion.

Immunoassays revealed an expressed lymphopenia affecting all lymphocyte subpopulations, with the exception of NK cells, high levels of IgG. The patient was positioned in air-fluidised beds in burn unit and managed symptomatically by administering intravenous fluid and electrolyte replacement, *systemic* cortico-steroids, antihistamines, antibiotic - klacid, human plasma, vitamins, H2 blockers, topical wound care with chlorhexidine acetate (Bactigras), topical treatment of mucosal changes - vaginal globules, ophthalmic preparations. Evolution was satisfactory with epidermization and she was discharged from the hospital after 1 months (Fig. 1, 2, 3).

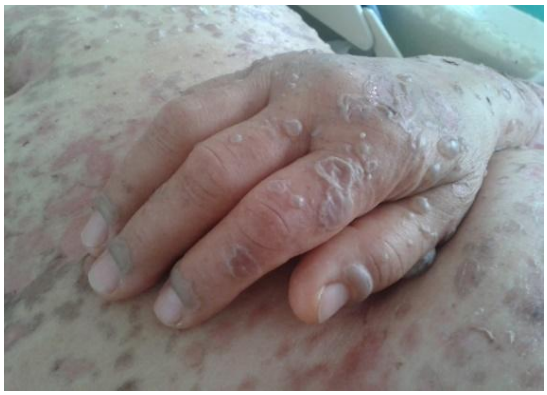

Fig. 1

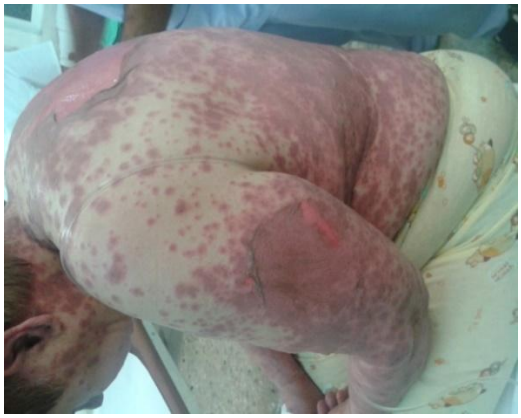

Fig. 2

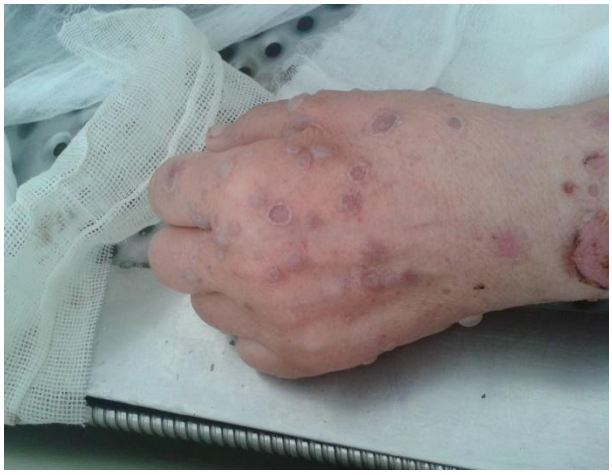

Fig. 3

Discussion

Toxic epidermal necrolysis is a severe acute skin disorder, described for the first time in 1956 by Alan Lyell. TEN is thought to be an immunological disorder and to be one of a family of three skin disorders. TEN is considered to be the more serious, followed by Stevens-Johnson syndrome and erythema multiforme, in order of severity of disease.

Definitions vary and another classification system works on the fact that SJS and TEN are related conditions which can be differentiated by the degree of skin involvement. Less of the epidermis sloughs off in SJS, whereas TEN may be defined as involving >30% of the total body surface area. (4, 5)

An allergic reaction to a drug is most often the cause among adults. The exact cause of the violent skin reaction is unknown. There appears to be an immune response leading to the rejection of the skin and mucous membrane. There is thought to be an immune complex-mediated hypersensitivity reaction to the presence of toxic drug metabolites which accumulate in the skin. This reaction results in the destruction of keratinocytes. Specifically, cytotoxic T lymphocytes cause keratinocyte damage and subsequent necrosis, mediated by granzyme B. Cytotoxic molecules including FasL and granulysin have been implicated as causing the widespread keratinocyte apoptosis (8, 11).

Most cases involve the use of medications such as antibacterial sulfonamides, non-steroidal, anti-inflammatory drugs, anticonvulsants, some antibiotics. However, other etiologies, including infection, malignancy, and vaccinations, may exist (11).

Onset can occur at any age. TEN affects many parts of the body, but it most severely affects the mucous membranes, such as the mouth, eyes, and vagina. The severe findings of TEN are often preceded by 1 to 2 weeks of flu-like symptoms.

These symptoms may mimic those of a common upper respiratory tract infection.

The disease consists of a prodrome of malaise, lethargy, and fever, followed by erythema and massive bullae formation.

Pathologically, there is epidermal necrosis and vesication at the dermal-epidermal junction, but the dermis is relatively normal. The mucous membranes are usually included in the destructive process. Mucous membrane involvement occurs early in 90% of cases and commonly precedes other symptoms. (8). The conjunctivae, buccal, nasal, pharyngeal, tracheobronchial, perineal, vaginal, urethral and anal mucosae may all be involved.

When the rash appears it may be over large and varied parts of the body, and it is usually warm and appears red. In hours, the skin becomes painful and the epidermis can be easily peeled away from the underlying dermis. Bullous and erosive lesions involve oral, ocular, and genital mucosae; and vast areas of the skin with extensive dermoepidermal detachments (3, 6, 9).

The patients have to be seen by a multidisciplinary team and must to received intensive supportive care. This included early fluid and electrolyte replacement, aggressive nutritional supplementation, *systemic* corticosteroids, antihistamines, intravenous antibiotherapy, oxygen therapy through a canula, or assisted ventilation according to the patient's clinical condition and blood gas analysis administration of inactivated human plasma, intravenous immunoglobulin, prevention of stress ulcers with H2 blockers, hydrotherapy under intravenous sedation and with the application of a chlorhexidine solution, local treatment of mucosal changes, prevention of ocular problems through daily observation by an ophthalmologist, who will prescribe appropriate ophthalmic medication (antiseptic solutions, topical antibiotics) and monitoring of vital functions (4, 7, 12).

As a rule, skin lesions heal without scarring, whereas mucosal scarring and strictures are frequent late complications. Late eye complications, potentially leading to blindness, occur in up to 50% of cases. Sepsis and multi-organ failure are the main causes of death. Hospital stay was between 13 and 30 days without mortality. Recovery is usually slow and may take 3–6 weeks (10).

In our patient the cutaneous tissue and the oral mucosa showed a sustained and relatively quick re-epithelialization, which allowed the patient to be discharged home after 30 days of hospitalization.

Conclusion: Severe adverse drug reactions as toxic epidermal necrolysis, is a rare, but very serious dermatological lesions, characterized by the sudden onset of high fever, with signs of systemic toxicity and intense muco-cutaneous *lesions.*

The main treatment is the immediate suspension of the culprit drug and the patient's hospitalization in a service that can provide intensive care and minimize the risk of infection.

Key words: Toxic epidermal necrolysis, Lyell's syndrome

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FO2. STUDY ON ACUTE POISONING WITH ANTIHYPERTENSIVE AND ANTIARRHYTHMIC MEDICINES

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Objective: To study the characteristics of acute poisoning with antihypertensive and antiarrhythmic medicines. Methods: In a retrospective epidemiological study, all cases of antihypertensive and antiarrhythmic medicines (AAM) poisoning were analyzed for 3 years period. Demographic data, previous illness were obtained retrospectively. The cases of poisonings were evaluated with respect to clinical course, therapy and outcome. Results: In the period from January 2011 to December 2013, a total 896 patients with acute exogenous intoxications were treated in the Toxicology Clinic, Department for adults, UMHATEM "N.I.Pirogov", Sofia, Bulgaria. 136 cases (15.2%)of them were poisoning with AAM - beta-blockers, calcium channel blockers, angiotensin converting enzyme inhibitors, etc. 95 (69.9%) were female and 41 (30.1%) male, between the ages of 18 and 98. 61 individuals (44.9%) were poisoned by only one drug. In 75 (55.1%) remaining cases, intoxications were mixed, including other different medications or psychoactive substances. 112 cases (86,8 %) were intentional – a result of a suicide attempt. The severity of poisonings varied from moderate to extremely severe. In 17 patients poisoning occurs with the signs of exotoxic shock, 3 of them – with fatal outcome. In the examined group three patients died of cardiogenic shock and secondary acute respiratory failure resistant to therapy. Conclusion: Patients with acute poisoning from AAM were a large proportion of all patients, received acute poisoning and represent a serious challenge for the physician - toxicologist.

Key words: acute poisoning, antihypertensive, antiarrhythmics medicines.

Introduction

Modernizing of contemporary style of living – sedentary way of life, unhealthy nutrition, low health culture of the population and some other factors have led to rise of rhythm disturbances and progression of arterial hypertension (AH) more often at young age as well. Such are the results of Framingham study, which included 3900 healthy people, observed for a 10-year period (3, 10). At the recommendations of the European hypertension association, respectively the European cardiology society, there has been emphasized that monotherapy lowers effectively arterial pressure with a small number of patients. There must be a combination of at least two types of medicines to be achieved control under heightened pressure. For more comfort at the market there are fixed in one tablet antihypertensive combinations (FC) of two, (more rarely of three) medicines, most often one of them is a diuretic (1, 2, 4, 6, 8).

Modern antihypertensive FC contain different but mutually complementing one another in the mechanism of action of the medicaments which leads to a bigger effectiveness and lower prices of them. They are recommended as very good for treatment of AH with certain proofs from several large studies for the biggest benefit in reduction of undesirable extreme cardiovascular events. Nowadays medical practice has at its disposal unimaginable great number of antihypertensive and diuretic means, allowing refinement at combining of single representatives. In this way is reached not only maximum antihypertensive effect but also favourable outcome of all complications, connected with the disease (5, 7, 9).

Antihypertensive medicines are a mixed group, including diuretics, blockers and beta-adrenergic receptors /beta-blockers/, blockers of alpha-adrenergic receptors, calcium antagonists, inhibitors of angiotensin converting enzyme (ACE inhibitors) sartans, centrally effecting sympatholytics, vasodilatating medicines, ganglioblocking means.

At the market there appeared a lot of various commercial names of most of the generic antihypertensive medicaments. Most of them are at acceptable prices, widely used in medical practice and same are well known to patients. They often become means for self-poisoning, used by both healing patients as well as their relatives. The various commercial names of one and the same generic medicament lead to unintentional mistakes. Prolongation of patients' lives and progress of atherosclerotic processes are also prerequisites for overdosage and intoxications.

Objective: To study the characteristics of acute poisoning with antihypertensive and antiarrhythmic medicines.

Methods: In a retrospective epidemiological study, all cases of antihypertensive and antiarrhythmic medicines (AAM) poisoning were analyzed for 3 years period. Demographic data, previous illness were obtained retrospectively. The cases of poisonings were evaluated with respect to clinical course, therapy and outcome.

Criteria for inclusion of the cases

Patients over 18 years old with confirmed diagnosis, treated in the Toxicology Clinic of UMHATEM "N.I.Pirogov" in the period from 2011 to 2013. Monitoring of blood pressure and heart rate were performed. There has to be at least one ECG record.

Results and discussion

In the period from January 1st , 2011 to December 31st 2013, totally 896 patients with acute exogenous intoxications were treated in the Toxicology Clinic, Department for adults, UMHATEM "N.I.Pirogov", Sofia, Bulgaria. 136 cases (15.2%) of them were poisoning with AAM – beta-blockers, calcium channel blockers, angiotensin converting enzyme inhibitors, etc. (Fig. 1).

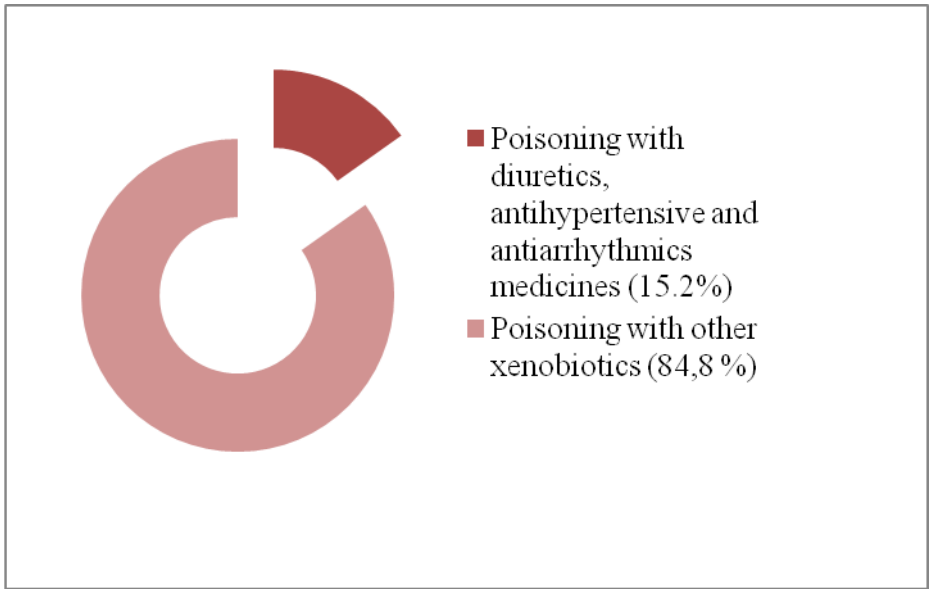

Fig. 1 Distribution of poisoning according to type of xenobiotics (relative share)

95 people (69.9%) were female and 41 (30.1%) male, between the ages of 18 and 98 (Fig. 2, Fig. 3).

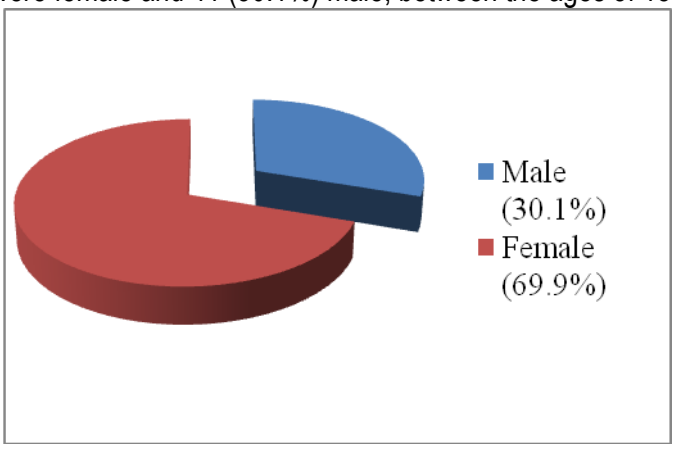

Fig. 2 Distribution by gender

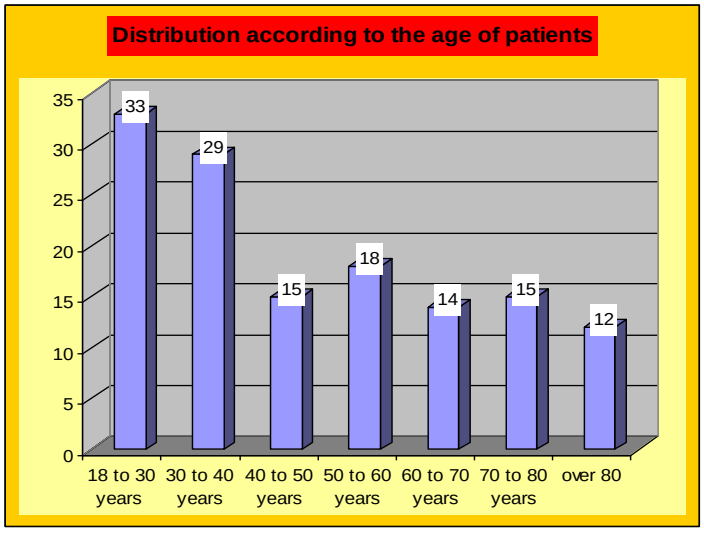

Fig. 3 Distribution according to the age (N)

61 individuals (44.9) were poisoned by only one drug. In 75 (55.1%) cases, intoxications were mixed, including other different medications or psychoactive substances. 18 patients had taken also antidepressants, 10 – bensodiasepins, 12 – alcohol, 7 – antibiotics, 8 – antipyretics, 7 – nonsteroid resolvents, 5 – antidiabetes medicaments, 4 – cardiac glycosides, 7 – nitrates, 6 – statins, ahistamins, protectors of the gastric mucosa, mucolytics, immunomodulators and other medicaments (Fig. 4).

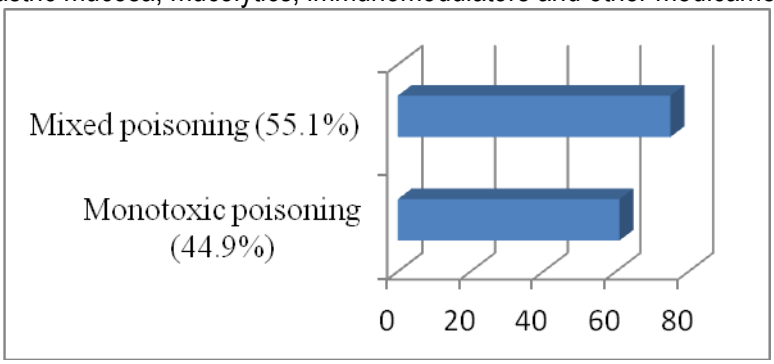

Fig. 4 Distribution according to type of intoxication

23 patients had taken four and more than four medicaments. In 112 cases (86.8%) poisonings were intentional – as a result of a suicide attempt. 11 patients had taken a higher dose of drugs on alcohol background. 5 patients with atherosclerotic changes had used repeatedly the daily dose of the prescribed medicaments. 4 patients had taken antihypertensive medicaments from one and the same group but with different commercial names. Four patients had been taking medication by mistake (Fig. 5).

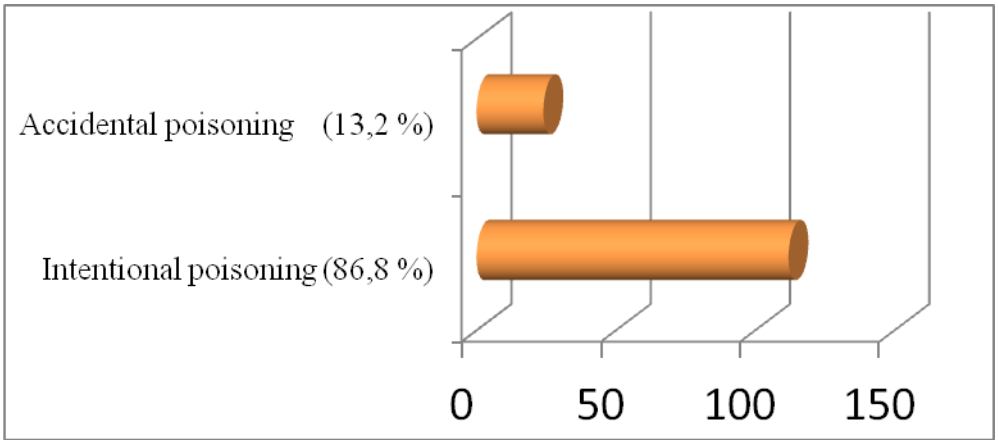

Fig. 5 Distribution according to the reason for poisoning

The severity of poisonings varied from moderate to extremely severe.

In 17 patients (12.6%), poisoning occurred with signs of exotoxic shock, three of them with fatal outcome. Two of the dead patients until the 12th hour after their receipt in hospital, and the third one (a female) – on the sixth day of entrance. Mortality rate was 2.2%.

The patients, admitted to the hospital a short time after the incident, are most often with not completely manifested clinical picture. In patients, entering at hospital later after the incident, the clinic picture is manifested to the fullest extent.

66 people were admitted in the hospital with normal indices of arterial pressure. ECG data show the following: 52 patients were with bradycardia, 18 – with tachycardia, 4 – with AV block I degree. There

were 12 patients with paroxysmal atrial fibrillation and 7 people were with extrasystoles. 43 patients were with normal ECG records (Fig. 6).

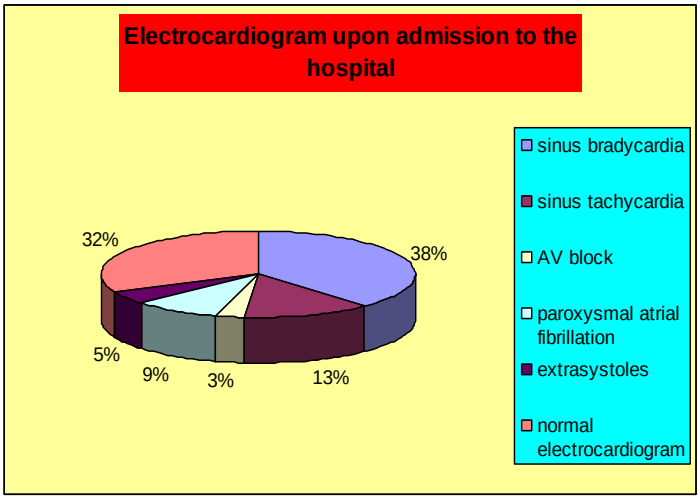

Fig. 6 Changes in electrocardiograms

The patients were treated in the hospital from several hours to 14 days. Prolonged hospitalization is often determined by complications. Seven patients were with imflammatory pulmonary changes, two others were with pulmonary edema.

The treatment of AAM patients starts with depuration of the gastrointestinal tract. Replacing corrective therapy include electrolyte and glucose solutions, HAES steril 10% or 15%, according to the values of blood pressure and central venous pressure (CVP).

In patients with tachycardia, non-selective beta blocker is administered (most often propranolol hidrochlorid with the lowest possible dose, achieving therapeutical effect).

When it is impossible to normalize the arterial pressure applying infusion solutions, there must be used catecholamine support. In patients with bradycardia parasympaticolytics (atropine sulphate) is administered.

If necessary, it can be administered oxygen ventilation, antibiotics, anticoagulants, gastric mucosa protectors and other symptomatic medication. In cases of extreme bradycardia should be placed a temporary cardiostimulator.

Conclusion

Patients with acute poisoning from AAM were a large proportion of all patients, received acute poisoning and represent a serious challenge for the physician - toxicologist.

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FO3. ATOMIC ABSORPTION SPECTROMETRY – METHOD FOR ANALYSIS OF CD-INDUCED TOXICITY

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According to the World Health Organization cadmium (Cd) is one of the most dangerous environmental pollutants. Sources of Cd pollution are cadmium-nickel batteries and accumulators, PVC products, pigments for ink and paints (cadmium yellow) and alloys. The International Agency for Research on Cancer has classified Cd as a proven human carcinogen. Chronic intoxications with Cd in humans occur as a consequence of consumption of contaminated food and water, and as a result of smoking. It is assumed that one of the mechanisms by which Cd induces toxic effect is a disruption of biometal ions homeostasis [1]. Cd^{2+} is a steric mimic of calcium (Ca). Both divalent cations have the same overall charge and very close ionic radii. Studies on experimental animals exposed to chronic Cd intoxication demonstrate that the toxic metal ion causes a decrease in the Ca concentration in kidneys. The observed effect is due to altered function of protein kinase C, which is important in the activation of metal-regulatory transcription factor (MTF-1). Lower Ca concentrations are determined in the liver of Cd-intoxicated animals compared to the control group. Most likely, Cd inhibits the absorption of Ca from the duodenum. The toxic metal ion also decreases iron (Fe) concentration in the liver of Cd-treated mice. Cd displaces Fe from ferritin and increases the content of free Fe ions in the kidneys. In addition, the

toxic metal ion greatly reduces the absorption of Fe from the gastrointestinal tract. Significantly higher levels of zinc (Zn) are measured in the livers of animals exposed to the toxic metal ion to the control group. It has been shown that Cd intoxication stimulates the synthesis of metallothionein, which may be the cause of Cd- induced increase of Zn in the liver. To the best of our knowledge there is a lack of systematic information about the effect of the toxic metal ion on biometal homeostasis in other organs.

Herein we present detailed novel information about the effects of Cd on biodistribution of Ca, Fe, copper (Cu) and Zn. The accumulation of the toxic metal ions in the organs of Cd-intoxicated mice and its effects on homeostasis of the biometal ions were studied by atomic absorption spectrometry. The results revealed that Cd is accumulated in all organs of Cd-intoxicated animals. No significant effect on the Zn homeostasis was observed. Exposure of the animals to Cd induced a significant alteration of Ca, Cu and Fe biodistribution. The results demonstrated that Cd exerts toxic effects by disturbance of biometal ions homeostasis. The advantages of the atomic absorption spectrometry as a method for analysis of Cd-induced toxicity are discussed.

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FO4. HAZARD AND IMMEDIATE MEASURES AGAINS THE PRODUCTION AND APPLICATION OF THE HERBICIDE GLYPHOSATE (ROUNDUP)

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Today, the Earth's population increases and natural resources diminish. The global food crisis is escalating poverty, hunger and social tensions have growing GM crops last more than 30 years. Over 80% of the land dedicated to GM crops has at least one genetic element of tolerance to herbicides. Corporate "Monsanto" create a group of GM crops "as a solution to the crisis" and benefit, misinformed government agencies, farmers and the general public about the "safety" of the herbicide "Roundup". Glyphosate - the active ingredient, however it is classified as "probably carcinogenic to humans", according to the WHO, leading to disturbances in ecosystems. The primary breakdown product of glyphosate in plants, soil and water aminomethylphosphonic acid (AMPA) is toxic to humans and animals by the herbicide. Glyphosate and AMPA have been shown to have "genotoxic" - they interfere with the ability of cells to replicate DNA and reproduce, leading to potential genetic mutations, increased risk of cancer, many chronic diseases related to reproduction and aging. So it needed immediate action by governments of all countries to restrict or prohibit GMO foods.

Key words: glyphosate herbicides, Roundup, Monsanto, health, reproduction, GMO

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РЕАЛНА ОПАСНОСТ ЗА ЗДРАВЕТО И НЕЗБАВНИ МЕРКИ СРЕЩУ ПРОИЗВОДСТВОТО И ПРИЛОЖЕНИЕТО НА ХЕРБИЦИДА ГЛИФОЗАТ(ROUNDUP)

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Днес населението на планетата Земя стремглаво нараства, а природните ресурси драстично намаляват. Глобалната хранителна криза, която изостря крайните бедност и глад и нарастващото социално напрежение, наложи отглеждането на ГМО културите през последните повече от 30 години. Сега повече от 80% от земята отдадена за ГМ култури притежава поне един генетичен елемент на толерантност към хербициди. Корпоративният гигант „Монсанто” създаде група генетично модифицирани култури, като решение на кризата и се облагодетелства, водейки продължителна кампания на дезинформация, за да убеди правителствените агенции, земеделските производители и населението като цяло, че хербицидът „Раундъп” е ”безопасен”. Насърчава фермерите да пръскат колкото Roundup е необходим, за да се преборят с плевелите. Почти всички тези култури са посадени с Roundup-ready семена и храните от тези култури попадат в организма ни ежедневно. Това крие огромен риск за здравето! Глифозатът – активната съставка на популярния хербицид „Раундъп“ и много други хербициди – е класифициран като „възможен канцероген за човека“, според СЗО. Глифозатът е отрова, разстройва способността на тялото да се прочиства, води до дисбаланс и е в основата на много хронични заболявания, свързвани с репродукцията и със стареенето. Затова са необходими незабавни мерки за забраната му.

Ключови думи: глифозат, хербициди, пестициди, Roundup, Монсанто, здраве, репродукция, генно модифицирани култури

Въведение

Хербицидите са една трета от световния пазар за пестициди.

Глифозатът е най-продаваният хербицид в света и един от най-широко използваните препарати за убиване на плевели в Европа. Той се използва широко в земеделието, в парковете и обществените пространства, около железопътните линии и в градините. Също така е от решаващо значение за отглеждане на генетично модифицирани (ГМ) култури, много от които са модифицирани, за да са резистентни на глифозат.

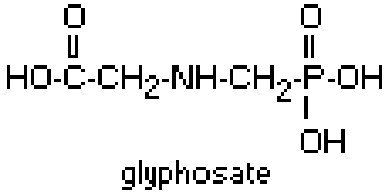
Изложение

Какво представлява глифозатът?

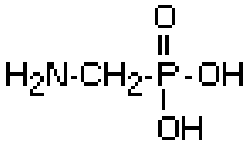
Глифозатът (N-(phosphonomethyl)glycine) е системен, широкоспектърен хербицид, който действа, като блокира ензим, отговорен за създаването на протеини в растенията. Това означава, че той е токсичен за всяко растение, което не е генетично модифицирано, за да издържи на веществото. Глифозатните хербициди съдържат и други съставки, включително повърхностно активни вещества за подобряване на усвояването им от растенията.

Първичен продукт от разграждането на глифозат в растенията, почвата и водата е aminomethylphosphonic acid (AMPA), киселина чиято химична структура е много близка до тази на глифозат (Фигура 1). Самата AMPA няма търговско използване. [28]

Figure 1. Structural formulae of glyphosate and AMPA



glyphosate



AMPA

Част от глифозата, попаднал в човешкия организъм, може да се разгради до киселина – aminomethyphosphonic acid (AMPA). Установено е, че AMPA е дори по-токсична за хората и животните от опасния хербицид. Един процент от глифозата остава в тялото една седмица след експозиция. Глифозат и AMPA са показали, че са "генотоксични" - те пречат на способността на клетките да копират точно ДНК и да се възпроизвеждат, което води до потенциални генетични мутации и повишен риск от рак .

Приложение

Къде се използва?

Употребата на глифозат за убиване на плевели е била патентована от Монсанто през 1970 г. и търговската марка Roundup на компанията става най-продаваната, въпреки, че първите биотехнологични продукти на Монсанто не са генномодифицирани култури, а противоречиви генномодифицирани лекарства за крави, хормон за растеж за едрия рогат добитък, наречен rBGH или rBST. Семената на Монсанто, резистентни към глифозат (Раундъп Реди) са на върха на биотехнологичната сцена от повече от десетилетие – създавайки почти монопол за Раундъп хербицида на компанията. Раундъп е най-продаваният пестицид в света и е спомогнал за превръщането на Монсанто в петата в света агрохимическа компания. Монсанто в момента е най-голямата компания за семена в света, отговаряща за почти една четвърт от световния частен пазар. Значителен дял от пазара на Монсанто идва от земеделските производители, които са задължени да използват Roundup Ready хербицида върху Roundup Ready генетично модифицирани (ГМ) култури.

Глифозатът е широко използван от земеделските производители, за да изчистят плевелите от полетата преди засаждане или преди семената на засадената култура да са покълнали. Също така, понякога глифозат се напръсква върху зърнени култури, маслодайна рапица, царевица и слънчоглед преди прибиране на реколтата, за да изсъхне тя. Тази употреба е известна като desiccation - десикация (изсушаване). Глифозатът се използва за борба с плевелите при отглеждането на лозя, маслинови дървета и други овощни градини и често се използва, в паркове, обществени места и покрай железопътни релси и магистрали. В световен мащаб през 2011 г. са били използвани около 650 000 тона от продукти, съдържащи глифозат, а прогнозите сочат, че употребата ще се удвои до 2017 година. Ако повече ГМ култури се одобрят в ЕС, нивото на използването им може да се увеличи с до 800%[11]. В момента в ЕС има подадени 14 заявления за отглеждането на ГМ култури, устойчиви към глифозат които очакват одобрение. Подадени са заявления за още 58 ГМО, за 17 от които ЕОБХ е издал положително становище.

Глифозатът е най-често използваният хербицид от земеделците във Великобритания, използва се и върху 39% от земеделската земя в Германия. Доколкото ни е известно, няма налична информация за количеството и площите, върху които се използва глифозат в България. През 2012г. в Съединените щати почти половината от цялата земеделска земя е засадена с Roundup Ready култури на Монсанто. По-голямата част от соята, внасяна в Европа от Латинска Америка за фураж е Roundup устойчива. Данните показват, че

използването на глифозат, както и на други хербициди се увеличава драстично, когато се отглеждат устойчиви на Roundup ГМ култури.

Методи

За наличието на глифозат е необходимо тестване в урина, кръв, мляко.

В момента се правят много малко изследвания за наличие на глифозат от публичните власти, въпреки широкото му използване. Властите в Европа не провеждат тестове за наличие на глифозат при хората, а тестове за храна не са чести. Доколкото ни е известно в България не се провежда никакъв мониторинг в тази посока.

Поради липсата на публични данни Приятели на Земята Европа извърши тестове на проби от урина на доброволци от 18 страни в Европа. Резултатите показват следи от глифозат в пробите от всички страни. Оказва се, че в България всяка десета проба е отчела наличието на глифозат.

Това повдигна сериозни опасения относно наличието на глифозат в човешкото тяло, както и въпроси, свързани с излагане на въздействието на други химикали, използвани с глифозата.

Приятели на Земята Европа алармират да знаем от къде е произхода на този глифозат и какво причинява той на телата ни, както и на околната среда като цяло. Необходимо е да знаем, защо правителствата не извършват мониторинг за експозиция на глифозат. Защо храната ни не е тествана рутинно, за да се гарантира, че тя не съдържа глифозат? Какви стъпки, ако има такива, се предприемат за намаляване на излагането ни на въздействието на глифозат?

Здравни аспекти

Данни от опити с животни показват, че когато се консумира глифозат, около 23% от него се абсорбира в тялото [17]. Той може да бъде намерен в кръвта и телесните тъкани [1] и е показано, че може да премине през плацентата по време на бременност [18]. Една малка част може да се разгради до aminomethylphosphonic acid (AMPA). Активният химикал глифозат в Roundup, е неприемливо съединение, защото уврежда ДНК-то, причинява рак и действа като ендокринен (хормонален) дисруптор (вещество, разстройващо функцията) на телесните клетки. Тъй като глифозат е толкова широко използван, повечето хора са изложени на това редовно.

Глифозат-базираните хербициди имат различни нива на токсичност, но могат да бъдат фатални при хората [11]. При по-ниски дози са показали, че са токсични за човешките клетки, включително за плацентните и ембрионалните клетки [3]. Установено е, че AMPA е дори по-токсична за хората от глифозата [4].

Съществуват и доказателства, че глифозатът може да наруши функциите на човешката ендокринна система, което може да предизвика необратими ефекти на определени етапи от живота, например по време на бременност. Проучвания върху плъхове показват увреждане на нивата на тестостерон в мъжките екземпляри [14], а изследвания на клетъчни култури, показват, че глифозатът блокира рецептори за мъжките полови хормони [14] и че инхибира производството на естроген [19]. Ако глифозатът оказва влияние върху човешката хормонална система, излагане на каквото и да е ниво може да представляват потенциална заплаха за здравето.

В районите на Южна Америка, в които се отглежда соя има съобщения за повишение на вродени дефекти. Едно проучване в Парагвай установи, че бебетата на жени, които живеят в рамките на един километър от полета, пръскани с глифозат, е два пъти по-вероятно да имат вродени дефекти [7]. Лабораторни изследвания са показали малформации в жабешки и пилешки ембриони, изложени на хербициди, базирани на глифозат [20].

Глифозат и AMPA са показали, че са "генотоксични" - те пречат на способността на клетките да копират точно ДНК и да се възпроизвеждат, което води до потенциални генетични мутации

и повишен риск от рак [21]. В Еквадор и Колумбия, където глифозатни хербициди са били използвани за контрол на производството на кокаин, проучвания са открили генетични увреждания и увеличение в количествата на спонтанни аборти по време на периода на пръсканията [22] [9]. В района Чако в Аржентина, където се отглежда соя, честотата на ракови заболявания се е увеличила четири пъти през последното десетилетие [23]. Проучване на американският Институт за отговорна технология показва, че новите болести, една от които е свързана с непоносимост към глутена са предизвикани от ГМ храните и хербицида Раунъп, с който се пръскат те.
Съединението глифозат действа също така като силно хелиращо вещество. Това означава, че то се свързва с основните минерали, от които се нуждае тялото ни като желязо, магнезий, цинк и кобалт – минерали, важни за функцията на мускулите, нервната трансмисия и имунното здраве.
Глифозатът засяга и бионаличността на В витамините и на аминокиселината триптофан. Това може да предизвика всякакъв вид неврологични заболявания.
Глифозатът – активната съставка на популярния хербицид „Раундъп“ и много други хербициди – е класифициран като „възможен канцероген за човека“.
Оказва се, че ГМ и храните с високо съдържание на глифозат са много силно свързани с: високото кръвно налягане, инсултите, диабета и метаболитните разстройства, затлъстяването, Алцхаймер, деменция и Паркинсон, Множествена склероза, възпалителното заболяване на червата и чревните инфекции, няколко различни вида рак..

Екологични аспекти
Понеже глифозатът е предназначен да убива растенията, той може да има пагубен ефект върху дивата природа, да доведе до намаляване на биоразнообразието на обработваемите земи и да унищожи източниците на храни за птици и насекоми. Проучвания във Великобритания, разглеждащи въздействието на резистентни на хербициди ГМ култури върху биоразнообразието установяват, че култури, третирани с хербицида глифозат могат да окажат неблагоприятно въздействие върху птиците, живеещи на земеделските земи [23].
Наред с прякото въздействие върху растенията е установено, че глифозатът се отмива от почвата в реки, потоци и подпочвени води [24]. Водното замърсяване представлява заплаха за водните организми и има данни от проучвания в Северна Америка, че хербициди, съдържащи глифозат могат да бъдат токсични за жаби [25]. Това е от особено значение, т.к един от трите вида земноводни са застрашени от изчезване. Установено е, че чернодробните клетки на шаран са повредени от излагане на хербицида глифозат [26]. Популациите на полярните мечки и делфините също са засегнати. Констатира се наличието му и при пчелите.
Глифозатът също оказва влияние върху почвеният химичен състав. Докато в някои почви, глифозатът се свързва с почвените частици, което го прави инертен, в други видове почви той остава активен и се разгражда от почвените микроорганизми, засягащи биологични и химични процеси около корените на растенията, включително способността на растенията да фиксират азот [27], което води до необходимостта от повишаване на нивата на азотни торове.
Екологичният ефект от употребата на конвенционални хигиенни средства също не е за прнебергване!

Относно процеса на одобрение
Глифозатът е одобрен за използване в целия ЕС през 2002 г., но европейските регулаторни агенции не извършват свои собствени изпитвания за безопасност, а вместо това, разчитат на данните, предоставени от производителите. Повечето от тези данни идват от изследвания на индустрията, а не от рецензирани научни изследвания, а първоначалните данни не са на разположение за независим контрол и оценка. Процесът на одобрение на глифозата през

2002 г. не прецени, дали той може да наруши човешките хормони и репродуктивна система. Процесът определя нивото за "допустима дневна доза" - “acceptable daily intake” (ADI), която за глифозат в момента е 0,3 mg на килограм телесно тегло. Това е по-високо от нивата, препоръчани от някои производители между 0.05 mg и 0.15mg/kg. Независима научна оценка през 2012 г. предложи ниво на 0.025mg/kg [2].
През 2010 г., германската федерална служба за защита на потребителите и безопасност на храните (BVL) допусна подновяване на разрешението на Монсанто за употребата на глифозата.
Европейската комисия се съгласи да удължи съществуващото одобрение с три години до 2015 г., за да даде на компанията повече време за подготовка на заявлението. На 28 октомври 2015 г. Европейският парламент отхвърли с огромно мнозинство предложението държавите членки да могат самостоятелно да забраняват вноса и продажбата на разрешените в ЕС генно модифицирани храни и фуражи на своя територия. Мотивите –че е отстъпление от единния пазар и митническия съюз и противоречи на принципите за свободното движение на стоки.
С изумление разбирам, че ПРЗ съдържащи глифозат на Монсанто Юръп С.А. – Белгия отново са разрешени за паралелна търговия в България с решение влизащо в сила от **26.01.2016г. до 18.02.2023 г.**
Процесът по одобрение на ЕС разглежда само проучвания на глифозат, а не на действителната формула на хербицида, т.е. това, което се използва от фермери и градинари. Някои изследвания показват, че други компоненти могат да се комбинират с глифозата и да се повиши неговата токсичност [13]. Изследователите предупреждават, че фокусът само върху глифозата подценява потенциалните опасности [5]. Това трябва да попадне в обхвата на изследванията в светлината на новите правила. Ендокринните експерти казват, че е необходим по-предпазлив подход. Няма друг подход освен пълна забрана на ГМО храните. “Не трябва да се заблуждаваме, че с някакви закони и граници можем да контролираме нещо, което в същността си е непредсказуемо и автоматично неудържимо.”- казва Д-р Майкъл Антониу, Главен лектор по молекулярна патология.
Налице са достатъчно доказателства за екологичните и здравните последици от глифозата, които предизвикват безпокойство. „Като се има предвид, че резултатите от нашите тестове показват присъствие на глифозат в телата на хората, ние искаме да знаем как глифозатът, намерен в пробите човешка урина е влязъл в тялото и какви могат да бъдат последиците от постоянната експозиция на ниски нива на глифозат. Какво се случва с глифозата, който остава в тялото?”- Приятели на Земята Европа.

Незабавни мерки:
ЕС и националните правителства да започнат програма за мониторинг на глифозат в храните и фуражите, включително вносни суровини за изхранване на животни, като ГМ соя. Нивата на глифозат (както и на разградения продукт AMPA) в околната среда трябва да бъдат наблюдавани, като се обхванат водните системи и почвата. Тези програми за мониторинг трябва да бъдат всеобхватни и резултатите трябва да бъдат направени публично достояние незабавно.
Националните правителства трябва да въведат програма за намаляване на употребата на глифозат, а изсушаването чрез глифозат (пръскане на културите малко преди прибирането на реколтата) трябва да бъде забранено незабавно. Всички други начини за използване на глифозат трябваше да бъдат оценени до 2015 г., като съществуващите максимално допустими граници на остатъчни вещества (MRLs) следва да се преразгледат.
Не трябва да бъдат разрешавани в ЕС (и не само там) устойчиви към глифозат генетично модифицирани култури.

Всички преработватели на храни и търговци на дребно трябва да минимизират излагането на своите клиенти на остатъци от глифозат, чрез определяне на продукти „без глифозат" от техните доставчици. Те трябва да разширят своята вътрешна програма за мониторинг на пестициди и да включват глифозата в редовното тестване.
В състава на продукти за растителна защита да се включват само вещества, за които е показано, че определено са от полза за растениевъдството и които не се очаква да окажат вредно въздействие върху здравето на хората или на животните, нито пък неприемливо въздействие върху околната среда. За да се постигне еднаква степен на защита във всички държави, решението за допустимост или недопустимост на такива вещества следва да се взема на общностно равнище въз основа на хармонизирани критерии. Тези критерии следва да се прилагат при първото одобрение на активно вещество. За вече одобрени активни вещества, критериите следва да се прилагат при подновяването или преразглеждането на одобрението им. [32]
Следва да бъде насърчавано разработването на методи, при които не се извършва изпитване върху животни, с цел да се получат данни, които са от значение за човека, и да се заменят използваните понастоящем изследвания на животни. [32]
От етични съображения оценката на активно вещество или на продукт за растителна защита не следва да се основава на изпитвания или изследвания, при които активното вещество или продуктът за растителна защита умишлено се дава на хора с цел да се определи за активното вещество „нивото", при което не се наблюдава въздействие върху човека. [32]
По сходен начин токсикологичните изследвания, проведени върху хора, не следва да се употребяват за понижаване на границите на безопасност за активни вещества или продукти за растителна защита. [32]

Заключение

Глифозатът – е опасен за човешкото здраве. Според СЗО „са налице достатъчно доказателства, за да класифицира глифозатът, като вероятно канцерогенен за човека". Генетично модифицираните култури и пръсканите с Roundup плевели, както и животните хранени със зърнени храни, отгледани като Roundup-ready култури и храните от тези култури, попадат в чинията ни всеки ден. Това означава, че в тялото ни се натрупва токсичен товар във времето. Затова са необходими незабавни мерки от правителствата на всички държави, токсичните ГМО храни да не попадат в хранителния ни режим, за да живеем с по-малък риск от заболявания и по-дълго.

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FO5. VITAMIN D AS A PREVENTION FOR DIABETES

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FO6. GHRELIN AND ITS IMPLICATION ON DIABETES

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FO7. EFFECT OF MELATONIN ON TYPE 2 DIABETES

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FO8. TREATMENT OF DIABETES USING MORINGA OLEIFERA (LEAF)

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FO9. MAXIMAL AEROBIC TEST AS A TOOL FOR ASSESSMENT OF FUNCTIONAL CAPACITY OF ATHLETES

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Drawing up of a suitable training program for athletes is essential for reaching high sport achievements. There are various tests to assess the functional status of athletes. One of the most common tests used to define an appropriate training program is the maximum aerobic test (VO_2max test). It provides objective data on the functional capabilities of the athletes as the parameter *maximum* rate of *oxygen* consumption (VO_2max) gives a reliable information about the state of the cardiovascular system, lung capacity and condition of skeletal muscle. This test has good reproducibility and is considered the "gold standard" in the coaching practice. In this test the oxygen consumption is increased many times when compared to the consumption at rest, which could induce oxidative stress (OS). In this study the effect of VO_2max test on oxidative status of athletes was assessed. *Our results indicated that the test* performance leads to OS development, as the degree of oxidative changes depends on the training level of the respondents. In well trained athletes the changes in OS biomarkers are hardly to be established because of regular training that improves the functioning of endogenous antioxidant systems and in this way prevent the induction and development of OS. So change or lack of change in pro/antioxidant status of athletes can serve as an indicator of adaptation processes occurring in the body as a result of the training process.

FO10. HOME DIAGNOSTIC AND SCREENING BY MEANS OF SMARTPHONES AND MEDICAL DEVICES - CLINICAL APPLICATIONS

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Abstract

Nowadays, electronic devices are entering boldly even the lives of ordinary people. Along with other amenities, the applications related to health are also extremely important. Fortunately, the life-threatening indicators may be tracked online and transmitted to physician in real time. Parameters are tracked during rest, work or sport. Under control are also devices in the body - pacemakers, insulin pumps and others. Companies producing electronic devices compete in the creation of new applications related to health. Physicians will be able to prescribe these devices to track diabetes and wellness, or optimize a patient's diet. Some of these are available as consumer versions today, but we'll eventually use them to diagnose psychiatric or neurologic conditions early. Health monitoring system aims to treat symptoms before they could even come to the surface and hence prevent illness. The idea is to provide monitoring system in case the patient is not in clinical environment. The analyses showed that the proposed system is convenient and reliable and ensures data security at low cost. In addition, the

developed system is equipped to generate warning messages to the doctor and patient under critical circumstances.

Key words: delivery of health care, health monitoring system, smartphone apps, mobile health, self-diagnostic, telemedicine, electronic health files, Apple, Android

Advances in technology have contributed to many changes in human daily life, for example mobile phone has become more than a tool for communication. The new generation of mobile phones known as smartphones, support many functions such as Internet Browser, Java application, GPS, Bluetooth/infrared and other future functions. These powerful functions make the smartphones suitable for use in the health monitoring field. Portable health systems can comprise various types of small physiological sensors, which enable continuous monitoring of a variety of human vital signs and other physiological parameters such as heart rate, respiration rate, body temperature, blood pressure, perspiration, oxygen saturation, electrocardiogram (ECG), body posture and activity etc. Furthermore, due to embedded transmission modules and processing capabilities portable health monitoring systems can facilitate portable wearable unobtrusive solutions for continuous all-day and any-place health, mental and activity status monitoring [6].

Smartphone based activity recognition has recently received remarkable attention in various applications of mobile health such as safety monitoring, fitness tracking, and disease prediction. To achieve more accurate and simplified medical monitoring, the authors propose a self-learning scheme for patients' activity recognition, in which a patient only needs to carry an ordinary smartphone that contains common motion sensors. After the real-time data collection though this smartphone, we preprocess the data using coordinate system transformation to eliminate phone orientation influence [4].

The idea is to provide the monitoring system even if the patient is not in clinical environment. In general practice, apps could play an important future role in supporting medical education and practice. Another objective is to explore medical students' perceptions regarding the potential of these apps for training and subsequent work as a physician. [9].

Basically, the existing system is used for health monitoring only available in hospital and outside. Monitoring can be done when the patient is on the bed. From that, monitoring and recording of physiological parameters of patients outside the clinical environment is becoming increasingly important in research as well as in applied physiology and medicine in general. As a result, patient still can be continuously monitored even if he had been discharged or can be used as personal health monitoring. In order to provide the health monitoring system when the patients are out of clinical environment many things needs to be considered. The first thing is mobility. The system need to be unobtrusive for the daily life of users, easy to use (user- friendly) and easy to set up. Secondly, the low cost system is more prefered. Last but not least, reliable data transmissions everywhere and at anytime should be provided [3].

The ubiquitous use and advancement in built-in smartphone sensors and the development in big data processing have been beneficial in several fields including healthcare. Among the basic vitals monitoring, pulse rate monitoring is the most important healthcare necessity. A multimedia video stream data acquired by built-in smartphone camera can be used to estimate it. In this paper, an algorithm that uses only smartphone camera as a sensor to estimate pulse rate using Photoplethysmograph (PPG) signals is proposed. The results obtained by the proposed algorithm are compared with the actual pulse rate and the maximum error found is 3 beats per minute. The standard deviation in percentage error and percentage accuracy is found to be 0.68 % whereas the average percentage error and percentage accuracy is found to be 1.98 % and 98.02 % respectively [10].

The delivery of psychiatric care is changing with a new emphasis on integrated care, preventative measures, population health, and the biological basis of disease. Fundamental to this transformation are big data and advances in the ability to analyze these data. The impact of big data on the routine treatment of bipolar disorder today and in the near future is discussed, with examples that

relate to health policy, the discovery of new associations, and the study of rare events. The primary sources of big data today are electronic medical records (EMR), claims, and registry data from providers and payers. In the near future, data created by patients from active monitoring, passive monitoring of Internet and smartphone activities, and from sensors may be integrated with the EMR [7].

It has been developed a patient-centered, smartphone-based, diabetes care system (PSDCS). This study aims to test the feasibility of glycosylated hemoglobin (HbA1c) reduction with the PSDCS. That study was a single-arm pilot study. The participants with type 2 diabetes mellitus were instructed to use the PSDCS, which integrates a Bluetooth-connected glucometer, digital food diary, and wearable physical activity monitoring device. The primary end point was the change in HbA1c from baseline after a 12-week intervention [6].

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the US. Emerging employer-sponsored work health programs (WHP) and Digital health intervention (DHI) provide monitoring and guidance based on participants' health risk assessments, but with uncertain success. DHI-mobile technology including online and smartphone interventions-has previously been found to be beneficial in reducing CVD outcomes and risk factors, however its use and efficacy in a large, multisite, primary prevention cohort has not been described to date. We analyzed usage of DHI and change in intermediate markers of CVD over the course of one year in 30,974 participants of a WHP across 81 organizations in 42 states between 2011 and 2014, stratified by participation log-ins categorized as no (n = 14,173), very low (<12/yr, n = 12,260), monthly (n = 3,360), weekly (n = 651), or semi-weekly (at least twice per week). We assessed changes in weight, waist circumference, body mass index (BMI), blood pressure, lipids, and glucose at one year, as a function of participation level. We utilized a Poisson regression model to analyze variables associated with increased participation. Furthermore, participants previously underrepresented in WHPs (females and Hispanics) and those with an increased number of CVD risk factors including age and elevated BMI show increased adherence to DHI, supporting the use of this low-cost intervention to improve CVD health [11].

The telemedical system focuses on the measurement and evaluation of vital parameters, e.g. ECG, heart rate, heart rate variability, Body Temperature, plethysmography. Based on design of a (Wireless) Body Area Network connected to an Android smartphone the Real-Time system features several capabilities: Data acquisition in the (W)BAN plus the use of the smartphone sensors, patient localization, data storage, analysis and visualization on the smartphone, data transmission and emergency communication with first responders and a clinical server. Smart and energy efficient sensor nodes acquire physiological parameters, perform signal processing and data analysis and transmit measurement values to a coordinator node. In the second design sensors are connected via cable to an embedded system. Data are transferred via Bluetooth to an Android based smartphone.

Health monitoring system (HMS) has gained attention of many researchers & has created new health and wellness dimensions with a holistic approach to life. Modern medicine, being the most prevalent and widely practiced and are limited only to primary, secondary and tertiary preventions only. However, HMS aims to treat symptoms before they could even surface and hence prevent illness. For an illness to develop the first thing which occurs is a change in cell energy levels. If this change is ignored, a change in bio-chemicals occur, if this too is ignored the blood test would show an abnormality and if this is also ignored, structural changes occur and the person falls ill [4].

Online telemedicine systems are useful due to the possibility of timely and efficient healthcare services. The system is conceptualized to provide an interface between the doctor and the patients for two-way communication. The main purpose is to facilitate the remote cardiac patients in getting latest healthcare services which might not be possible otherwise due to low doctor-to-patient ratio. The developed monitoring system is then evaluated for 40 individuals (aged between 18 and 66 years) using wearable sensors while holding an Android device (i.e., smartphone under supervision of the experts). The performance analysis shows that the proposed system is reliable and helpful due to high speed. The analyses showed that the proposed system is convenient and reliable and ensures data security at low cost. In addition, the developed system is equipped to generate warning messages to the doctor

and patient under critical circumstances [5]. For example: cardiovascular diseases are the most common cause of death worldwide and are characterized by arrhythmia (i.e. irregular rhythm of heartbeat). Arrhythmia occasionally happens under certain conditions, such as stress. Therefore, it is difficult to be diagnosed using electrocardiogram (ECG) devices available in hospitals for just a few minutes. Constant diagnosis and monitoring of heartbeat is required to reduce death caused by cardiovascular diseases.

Mobile healthcare system has emerged as a potential solution to assist patients in monitoring their own heart condition, especially those who are isolated from the reference hospital. This paper proposes a self-diagnostic electrocardiogram system for mobile healthcare that has the capability to perform a real-time ECG diagnostic. The self-diagnostic capability of a real-time ECG signal is achieved by implementing a detrended fluctuation analysis (DFA) method. The result obtained from DFA is used to display the patient's health condition on a smartphoneanytime and anywhere. If the health condition is critical, the system will alert the patient and his medical practitioner for further diagnosis. Experimental results verified the validity of the developed ECG diagnostic application on a smartphone. The proposed system can potentially reduce death caused by cardiovascular diseases by alerting the patient possibly undergoing a heart attack [2].

In a project [8], the authors sought to design, develop, and evaluate a prototype mobile cloud-based mHealth app, "PD Dr", which collects quantitative and objective information about Parkinson's disease (PD) and would enable home-based assessment and monitoring of major PD symptoms. The authors designed and developed a mobile app on the Android platform to collect PD-related motion data using the smartphone 3D accelerometer and to send the data to a cloud service for storage, data processing, and PD symptoms severity estimation. To evaluate this system, data from the system were collected from 40 patients with PD and compared with experts' rating on standardized rating scales.

The evaluation showed that PD Dr could effectively capture important motion features that differentiate PD severity and identify critical symptoms. For hand resting tremor detection, the sensitivity was 0.77 and accuracy was 0.82. For gait difficulty detection, the sensitivity was 0.89 and accuracy was 0.81. In PD severity estimation, the captured motion features also demonstrated strong correlation with PD severity stage, hand resting tremor severity, and gait difficulty. The system is simple to use, user friendly, and economically affordable. The key contribution of this study was building a mobile PD assessment and monitoring system to extend current PD assessment based in the clinic setting to the home-based environment. The results of this study proved feasibility and a promising future for utilizing mobile technology in PD management [8].

Interesting application is the so-called stroke riskmeter (displayed as a blue icon) - making survey of patient’s indicators and calculating the risk of stroke.

These devices will send real-time ECG information, temperature, position, heart rate, stress level, or caloric burn through the Web to any clinician or pediatrician in the world. That's a lot of data. We're going to be able to use those in the inpatient and outpatient.

Another example is dermatology. The patient can take a standard camera or a dermatoscope and take a picture of a skin lesion. Instead of making a dermatology consultation and possibly wait for months, you can send the image directly to dermatologists, and they can tell whether it is a melanoma or a mole. Soon, new applications with machine learning will be able to tell whether the lesion is dangerous and track the lesion over time. Such fields as dermatology, pathology, and radiology, which are all based on pattern recognition, are going to be shaped, and in some cases disrupted, by technology. Parents of children with ear infections can use a smartphone case with an otoscope [1].

The app can even tell the difference between arterial fibrillation (AF) and a normal rhythm, which is useful for triage or screening, or for a patient with a particular condition. This can be used for gastroenteritis, tracking peak flow in asthma, picking up diseases - even lung cancer can be detected through breath assessment. We're in an era of quantified self-help, using patches and other devices that can track your breath to determine your blood alcohol level, or the quality of your breath. These also can

track your hydration level, and we will use these to pick up disease early. Devices can track your voice. Smartphones can be used as a sensor for detecting mental health [1].

Some of the tools and technologies we have on the table are already here and will reshape health and medicine. One of them is a medical "tricorder." Almost every consumer and parent will have this kind of technology in their home in a couple of years. You hold it to your forehead, and it tracks your temperature, heart rate, and oxygen saturation, and calculates your blood pressure. These are currently in clinical trials, but will eventually replace the digital thermometer. We're going to see the integration of these technologies at home, which provide data that will upload through your smartphone to your clinician [1]. This will be tele-doctor. Nurse going home visits and send images and data in the doctor's office and he would not waste time.

We will have smart point-of-care lab tests, which require you to spit in a tube or dip a strip in your urine. An app on your phone will analyze the data and send the influenza screen or urine analysis results to your clinician or pediatrician. There will be many ways for consumers to be empowered to use these at home.

A simple skype conversation may diagnose autism**.** For example: children with autism change eye contact throughout conversation; now researchers can track and measure eye movements [1]. So in the next 5 or 10 years, the patients and the doctors are going to get more reliable information from these devices and apps, which can make our lives better and increase the convenience of consulting with patients. Moreover, patients' lives will be improved.

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FO11. ИНДИВИДУАЛИЗИРАНАТА ТАРГЕТНА ТЕРАПИЯ – НОВИ ИЗИСКВАНИЯ КЪМ ТЪКАННАТА ОБРАБОТКА НА БИОПСИЧНИТЕ МАТЕРИАЛИ

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Въведение – Индивидуализирана таргетна терапия е съвременен подход в лечението на онкологичните заболявания, основаващ се на избора на медикамент, въз основа на доказването в туморната тъкан на биомаркери /ДНК, РНК, протеини/ с установена предиктивна /предсказваща резултата от лечението/ стойност. Източник на информация за наличието или отсъствието на предиктивните фактори /EGFR, KRAS, BRCA1, BRCA2, HER2/ може да бъде само фиксираният във формалин и включен в парафин /FFPE/ биопсичен тъканен материал от тумора на конкретния пациент.

Цел - Да се оцени значението на качествената тъканна обработка на биопсичните материали като ключово условие за пригодността им при изследване на предиктивни биомаркери и определянето на индивидуализарана таргетна терапия.

Материал и методи – Сравняват се резултатите от генетични изследвания на предиктивни биомаркери във формалин-фиксирани, включени в парафин тъканни проби /FFPE/, подложени на различни условия на фиксация и тъканна обработка.

Резултати – Решаващи фактори за запазване на диагностичната пригодност на тъканните проби при изследване на предиктивните биомаркери имат минималното време между прекратяването на тъканното кръвоснабдяване до поставянето във фиксатор, използването изключително на 10% неутрален буфериран формалин в съотношение 10:1 спрямо тъканта, строго лимитирано време за фиксация – от 6 до 72 часа. Надвишаването на температурата над 58°C в процеса на тъканна обработка води до необратимо дефрагментиране на ДНК, хидролиза на РНК и прекомерно омрежване на протеините със създаване на кръстосани връзки. Възникването на тези изменения е причина за получаване на неинтерпретируеми резултати от генетичните изследвания и невъзможност за избор на таргетни терапевтични медикаменти.

Изводи – Своевременната и адекватна фиксация и щадящата тъканна обработка на биопсичните материали са решаващо условие за успешното прилагане на диагностичните методи за избор на индивидуално специфична стратегия на противотуморната терапия.

Ключови думи – Индивидуализирана таргетна терапия, предиктивен фактор, адекватна фиксация, тъканна проба, HER2, KRAS, BRCA1/2, FFPE.

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INDIVIDUALIZED TARGETED THERAPY - NEW REQUIREMENTS FOR PROCESSING TISSUE BIOPSY

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Introduction - Individually targeted therapy is a modern approach in the treatment of oncological diseases, based on the choice of drug, depending on evidence in tumor tissue biomarkers /DNA, RNA, proteins/ identified predictive /predictive of treatment outcome/ value. Source of information on the presence or absence of predictive factors /EGFR,KRAS, BRCA1, BRCA2, HER2/ can only be fixed in formalin and embedded in paraffin / FFPE/ biopsy tissue from the tumor material of the patient. **Objective** - To assess the importance of quality tissue processing biopsy as a key condition for their suitability in the study of predictive biomarkers and defining individualized targeted therapy. **Material and methods** - Compare the results of genetic studies of predictive biomarkers in formalin-fixed, paraffin-embedded tissue samples / FFPE /, subject to various conditions of fixation and tissue processing.
Results – A determinant factors for maintaining the diagnostic capability of tissue samples in the study of predictive biomarkers have the minimum time between the termination of tissue blood supply to putting in fixative used exclusively in 10% neutral buffered formalin at a ratio of 10:1 relative to tissue strictly limited time fixation - from 6 to 72 hours. Exceeding the temperature above 58°C in the course of tissue treatment leads to irreversible defragmentation of DNA, RNA, and hydrolysis of excessive cross linking of proteins by creating crossed links. The occurrence of these changes is the reason for getting subject of interpretation results of genetic research and inability to choice of target therapeutic drugs.
Conclusions - Timely and adequate fixation and gentle tissue handling biopsy materials are determinant for the successful implementation of diagnostic methods for the selection of individual specific strategy of anti-tumor therapy.
Keywords - Individually targeted therapy, predictive factor, adequate fixation, tissue sample, EGFR, HER2, KRAS, BRCA1 / 2, FFPE.

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FP1. ВИТАМИН Е – СТИМУЛАТОР И ИНХИБИТОР

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Абстракт: Витамин Е се намира в големи количества в растителни масла, яйчен жълтък, черен дроб, зелени и листни растения. Въпреки че витамин Е има множество функции при хора и животни, ключовата му роля е на антиоксидант, като защитава клетките от оксидативно увреждане. От откриването му, няколко проучвания са показали, че недостиг на витамин Е причинява увреждане на възпроизводителната функция при хора и лабораторни животни. Въпреки това, ефектите на витамин Е дефицит или неговото допълване към фертилитета са разнопосочни, тъй като при женските има стимулиращ ефект над репродуктивния тракт и

фоликулогенезата; докато при мъжките, последните данни го препоръчват като инхибитор, стопираш туморно тъканни разраствания. Ето защо, цялостен преглед относно ефекта на витамин Е е необходим, за да се разбере правилно ролята му с цел поддържане и запазване на качествата на гаметите.
ключови думи: *витамин Е, фертилни функции*

Abstract: Vitamin E is found in vegetable oils, egg yolks, liver, green and leafy plants. Although vitamin E has many functions in humans and animals, its key role as an antioxidant, protecting cells from oxidative damage. Several studies have shown that vitamin E deficiency causes impaired fertility in humans and animals. However, the effects of vitamin E deficiency or supplementing to fertility are different, since the female has a stimulating effect on the reproductive tract and folliculogenesis; while in males, the latest data recommend it as an inhibitor, stopping tumor tissue growths. Therefore, a comprehensive review on the effect of vitamin E is the need to properly understand its role in order to maintain and preserve the quality of gametes.
Key words: *vitamin E, fertility functions*

Витамин Е е един от четирите мастно разтворими витамини, необходими за всички бозайници. Природните му форми се синтезират в растенията и се състоят от група свързани съединения, токофероли и токотриеноли, които показват различна степен на биологична активност. В структурно отношение, съединенията съдържат хидрохиноново ядро и изопреноидна верига, но се различават по отношение на разположението на метиловата група на страничната верига на ароматния пръстен. Структурните разлики контролират активността на витамин Е в различните съединения. α-токоферол е най-разпространен и показва най-висока степен на биологична активност от естествено срещащи се форми на витамина [23].

Витамин Е се намира в изобилие в растителни масла, яйчен жълтък, черен дроб, зелени и листни растения [30]. Предлаганите на пазара източници са синтезирани от дестилати, вторични продукти от растителното производство, които съдържат смес от токофероли, по-устойчиви са на окисление и следователно по-стабилни по време на обработка и съхранение. Окислително разрушаване на витамин Е се повишава от излагане на кислород, топлина, влага, UV светлина [9].

Историческа информация

Витамин Е бил открит през 1922 г. от Evans and Bishop и наречен токоферол. Те идентифицирали неизвестни досега съединения в пшеничен зародиш, маруля и люцерна. Тези органични съединения са определени от съществено значение за нормалното възпроизводство на женски плъхове [14]. "Токоферол" произлиза от гръцката дума "*tokos*" (раждане на дете или потомство), гръцката дума "*pherein*" (да произвеждам), и окончанието "ол", което структурно го определя като alcohol [11]. Най-ранните проучвания с витамин Е са фокусирани върху различни аспекти на репродуктивните способности на селскостопански животни, като се предполагат някои биохимични функции на витамина в резултат на антиоксидантната му активност [22]. През 1957г. е доказано, че некроза на черния дроб при плъхове и ексудативна диатеза при пилета може да бъде предотвратено, чрез включване на компоненти, съдържащи витамин Е (напр. бирена мая) [35].

Абсорбция и транспорт

Приетият витамин Е асоциира с липидите в стомашно-чревния тракт, поради своят хидрофобен характер. Първоначално навлиза в чревния лумен във връзка с триглицериди, но неговата абсорбция не се случва, докато тези съединения се разградят, чрез липаза-медиирана хидролиза в не естерифицирани мастни киселини и β-моноглицериди - структури достатъчно малки за да преминат през мембраната на тънките черва. Не естерифицираните мастни киселини и β-моноглицеридите взаимодействат с жлъчни соли и образуват смесени мицели. Поради изключително малкия си размер, смесените мицели са равномерно диспергирани във

водната фаза на червния лумен [12]. По-специално, витамин Е се абсорбира, посредством образуване на алкохол при обикновена дифузия [3]. Само 20-30% от приетия витамин Е се абсорбира, обаче, ефективността на усвояването е обратно пропорционална на наличното количество в храната [27]. След това, витамин Е навлиза в клетъчните органели или техните мембрани, или остава в цитозола. Тези фракции, преминават през ендоплазмения ретикулум, след това в апарата на Голджи, където им се добавя протеинова част и се пакетират в секреторните везикули като хиломикрони. Поради своя липопротеинов компонент става сравнително разтворим и се освобождава, чрез екзоцитоза в лимфната циркулация. Като хиломикронен компонент навлиза в кръвта с лимфно вливане през дясното предсърдие.

Подобно на други мастноразтворими витамини, витамин Е се транспортира до кръвта, свързан с молекула носител. Въпреки това, за разлика от витамин А, който използва специфичен носител - протеин, витамин Е се транспортира чрез циркулация като компонент на серумен липопротеин [2]. Интересно е, че липопротеините са видово специфични. Така например, говедата, поддържат по-голямата част от техния витамин Е в липопротеините с висока плътност (HDL) [20]. Обратно, при човека по-голямата част от циркулиращите липиди и витамин Е са с ниска плътност (LDL) [8].

Обща функционалност на витамин Е

В биологичните системи витамин Е един от най-силните естествени антиоксиданти. Той предотвратява образуването на пероксиди и свободни радикали от клетъчни липиди, като по този начин се запазва целостта на клетъчната мембрана или понататъшна клетъчна деструкция. Витамин Е функционира в синхрон със Se /селен/, първичен антиоксидант. Se е значим компонент на глутатион пероксидаза и фосфолипид хидропероксид глутатион пероксидаза, ензими, които функционират в хидрофилни среди (напр. клетъчния цитозол, интерстициалната течност) и клетъчни мембрани [16], (Фигура). Ин витро изследвания доказват, че всяка α-токоферол молекула има поведенчески свойства за неутрализиране на два пероксидни радикала, като така предотвратява по-нататъшно оксидативно увреждане [5]. Добавянето на витамина, подобрява клетъчния и хуморален имунитет [29], неутрофилната активност, функцията на Т-клетките, активността на макрофагите, продуцирането на антитела [32; 38], инхибира агрегацията на тромбоцитите, чрез потискане пероксидацията на арахидонова киселина [28]. Той може също да функционира като кофактор в клетъчното дишане, тъй като ограничава активността на цитохром С редуктазата [12].

Фигура. Антиоксидантна система локализирана в клетката

Недостиг на витамин Е

Мускулно заболяване е най-често съобщаваната проява на недостиг на витамин Е и /или недостиг на Se в животновъдството [34]. Симптомите и разстройства на недостиг на витамин Е са различни, в зависимост от засегнатия вид. Те включват разстройства на нервната система,

кръвоносната система, мускулната система, сърдечно-съдовата система, имунната система, и репродуктивната система [39] Освен това, дефицит на витамин Е може да доведе до редица заболявания на черния дроб, бъбрек и бял дроб, и в мастната тъкан [21]. Обхватът на участието на витамин Е в различни системи на организма е може би най-добре описан от Blaxter and Brown, който заявяват, че дефицита на никои друг витамин не води до такова голямо разнообразие от клинични признаци и патологични промени [4]. От друга страна, хранителни изисквания за оптимална доза от витамин Е липсват при редица видове, тъй като множество променливи повлияват необходимите диетични концентрации. Метаболитното и енергийно търсене, наличието на други хранителни компоненти, такива като Fe, Cu [17] витамин А, каротеноиди, нитрати, може да увеличат хранителните нужди за витамин Е. Докато присъствието на Se, сяра-съдържащи аминокиселини, витамин С, и / или някои мастно разтворими антиоксиданти ще намалят изискването му [9].

Витамин Е като потенциален стимулатор

Витамин Е поддържа женския фертилитет при домашни птици, включително производството на яйца, фертилността и люпимостта им [33]. Хранене на свине майки с 50 IU допълнително витамин E/kg дажба сухо вещество увеличава млякото и коластровото съдържание, евентуално засилва имунитета при новородени прасенца. Подобно на свине и говеда, при бременни овце минимални количества витамин Е преминават в плацентата за развитието на плода, а коластрата е основен източник на витамина за новородените. По време на последните 28 дни от бременността токоферола води до зависимо от дозата повишение на концентрациите му в коластрата и серумните нива на тридневни агнета [31]. Овце, допълнени с 330 IU витамин Е/ден, в последните три седмици от бременността им, не показали разлика в теглото, физическото състояние, плодовитостта в сравнение с контролна група. Въпреки това, нивата на смъртност в опитните агнета е по-ниска [26].

Обратно, Segerson и Ganapathy намират тенденция към подобряване фертилитета на яйцеклетките при овце, третирани с мускулни инжекции от 136 IU витамин Е и 10 мг Se [36]. Приложението на витамина може да подобри ендометриума при жени с неизяснен стерилитет, вероятно с антиоксидантния и антикоагулантния ефект [6]. Прилагане на витамин Е, компенсира намаляване теглото и обема на яйчник, което може да се дължи на стимулиране секрецията на гонадотропини [25]. Витамин Е като силен антиоксидант може да предотврати фоликулярна дегенерация и атрезия, следствие от оксидативен стрес и така компенсира намаляването на броя на фоликулите. Например, витамин Е и витамин С приложени като антиоксиданти след лечение с вещество, което води до липидна пероксидация в яйчника и увеличаване на атрезивни фоликули имали положителен ефект [19].

Витамин Е като потенциален инхибитор

Редица литературни източници показват, че добавката витамин Е значително поддържа мъжките функции, включително обема на сперма, концентрация, жизнеспособност и подвижност на сперматозоидите. Съвременни проучвания обаче, насочват вниманието в инхибиращата роля на витамин Е над растежа на простатния тумор, проучено ин витро [24] и ин виво [15]. Изследване на 2974 пациенти с 17-годишно проследяване посочва, че ниските нива α-токоферол се свързват с повишен риск от рак на простатата [13]. Ароматазата превръща андрогените в естрогени и се смята за локален източник на естрогени, които улесняват растежа на хормон-чувствителни туморни клетки. Следователно, инхибирането на ароматаза е важен химио-профилактичен метод. Съобщено е, че витамин Е потиска експресията на ароматаза в две човешки карциномни клетъчни линии ин витро, по зависим от концентрацията начин [7]. Предполага се, че ароматазната активност и експресия са молекулна мишена на витамин Е, което може да е основано отчасти на инхибиторния ефект върху хормонално зависим рак. Третиране с витамина не е повлияла стероидната редуктаза в тумори на простатата, но,

значително намалява ароматазната експресия. Това предполага, намаляване на андрогенното активиране и по този начин и на стероидната сигнализация в простата на плъхове [37]. Освен това, промените в генната експресия в третирана с витамин Е група показват намаляване на оксидативния стрес и може да се обясни с по-слаб темп на некроза в тъканта. В здравата тъкан, намаляване на оксидативен стрес се желае по отношение на превенцията [18]. Достатъчно доказателства, от предклинични проучвания [24], епидемиологични наблюдения [13], и контролирани проучвания [1] показват, че витамин Е предотвратява развитието или прогресирането на рак на простатата, като действа чрез потискане на метаболизма на естрогените и андрогенната сигнализизация.

Заключение

В заключение, настоящият обзор показва важността на витамин Е за биологичните системи и въпреки това разнопосочното му действие, проявено в дозо-зависим начин или тъканно специфично. Витамин Е от една страна действа локално, като инхибитор на туморната тъкан, а от друга протектира и благоприятства женската репродуктивна система. Въпреки наличието на изследвания в областта, то те дават информация за ефекта от добавката. Необходимостта от задълбочаването действието на витамин Е е неизбежна с цел изясняване на конкретни механизми, генна зависимост, локализация на протеиново ниво.

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FP2. TARGETED MIGRATION OF STEM CELLS IN THE MODEL OF BRAIN TRAUMA

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Abstract

Objective: to develop technique of stem cells migration to the brain along trunks of cranial nerves during brain injury modeling.
Methods: 40 anesthetized white rats were fixed in stereotaxis and subjected to craniotomy (20 rats at anterior cranial fossa (group 1) and 20 rats at posterior cranial fossa (group 2). 100 μl of brain tissue were bilaterally aspirated from somatosensory zone and cerebellar cortex in 1st and 2nd groups, respectively. Mesenchymal stem cells (labeled with PKH67 green fluorescent linker) were injected in 10 minutes: intranasally (10 animals) and to Meckel cavity (10 animals) in both groups. Animals were observed and sacrificed at certain periods of time after the operation during three weeks.
Results: Appearance of small groups of mesenchymal stem cells in cranial cavity was established in 30 minutes after the operation. The highest fluorescence was observed at the damaged zone from 14 till 21 day after labeled stem cells implantation. Mesenchymal stem cells were predominantly distributed at the damaged zone in anterior cranial fossa after intranasal injection, and injection of stem cells to Meckel cavity resulted in their pronounced accumulation in the zone of cerebellar cortex injury (posterior cranial fossa).
Conclusion: The fact of somatotopic arrangement of mesenchymal stem cells in cranial cavity according to application zone near cranial nerve endings was established. Intranasal injection of mesenchymal stem cells is followed by their distribution in damaged zone in anterior cranial fossa. Injection of mesenchymal stem cells to Meckel cavity leads to accumulation of labeled cells in damaged zone in posterior cranial fossa.
Key words: brain damage, stem cells, somatotopic migration, intranasal, Meckel cavity

Introduction

Brain tissue regeneration with stem cells is a viable new strategy attracting close attention. This is evident from the articles in highly ranked scientific journals [1, 2]. However, major obstacles remain for systemic and intracerebral routes of stem cells delivery into the brain [3]. Intranasal delivery represents an alternative, allowing to bypass the blood-brain barrier, and to avoid trepanation procedures [3]. We compared the migration routes of the mesenchymal stem cells after the microinjections into the intranasal submucosa, and into the Meckel's cave in the rat brain trauma model.

Methods

In rats under ketamine-xylazine-acepromazine anesthesia (55.6 6.6 and 1.1 mg/kg, respectively, intraperitoneally) 100 µl of brain tissue was removed by aspiration with micropipette. Two groups of rats received bilateral destructions in the somatosensory (n = 20) and in the cerebellar (n = 20) cortices. Mesenchylmal cell suspension (30000 cells labelled by PKH67 green fluorescent linker, in 50 µl of phosphate-buffered saline) was microinjected 10 min after the surgery. In each group 10 rats received microinjection into submucosa of the nasal cavity, and 10 rats received microinjection into Meckel's space. The details of the methods are described elsewhere [3, 4, 5]. Animals were sacrificed in 0.5, 1.0, 4.0, 8.0, 24.0, 72.0 hours and 7, 14, 21 days after procedures. Brains were extracted, frozen and sliced for the confocal laser microscopy.

Results and Discussion

After the intranasal application in the rats with somatosensory cortex lesion, PKH67 signal was observed in the olfactory bulb at 0.5 hour, and in the somatosensory zone at 24.0 hours, where it peaked at the 14th-21st day (Figure 1A). In the rats with cerebellar cortex lesion the signal was vague (Figure 1B).

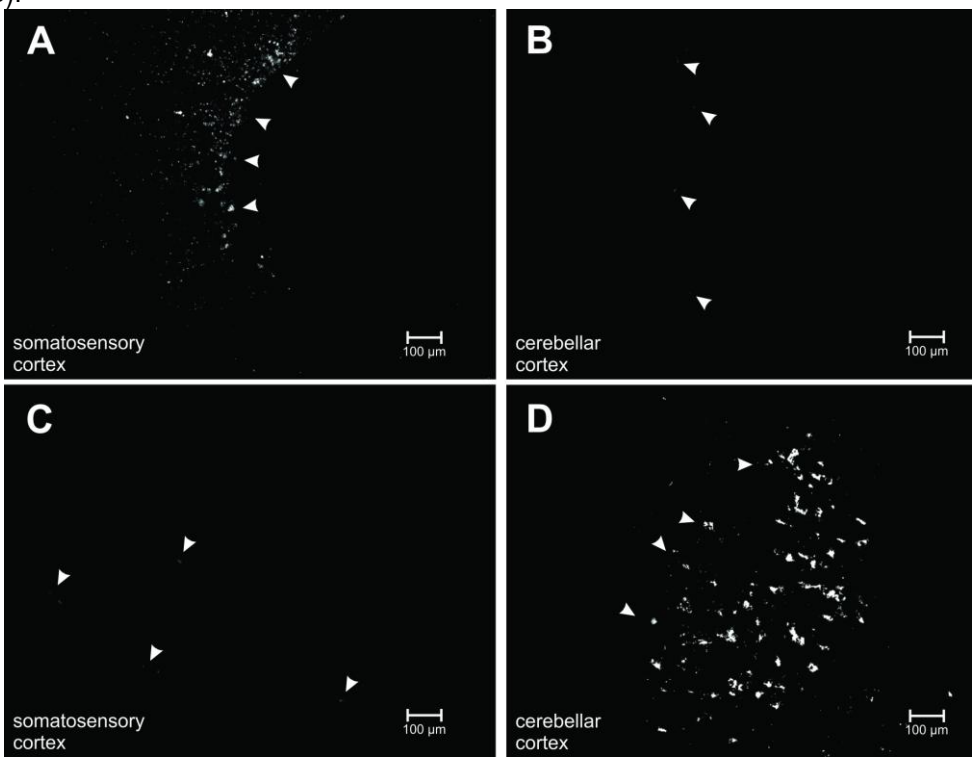

Figure 1. Fluorescent photographs showing the PKH67 labelled cells at 21st day after the surgery in the lesioned brain areas contralateral to the microinjection site. A, B following the intranasal microinjection. C, D following the microinjection into the Meckel's cavity.

Conversely, after the microinjection into the Meckel's cave, the signal was detected in the rats with the cerebellar cortex lesion – in the caudal brainstem at 4.0 and 8.0 hours, and in the cerebellar cortex at 24.0 hours. In the damaged area the signal peaked at 21st day (Figure 1D). In the animals with somatosensory cortex lesion, Meckel's cavity microinjection was followed by a weak signal in the damaged area (Figure 1C).

Conclusion

Our data demonstrate the targeted migration of the mesenchymal stem cells towards the brain tissue of anterior or posterior cranial fossa depending on their delivery into the olfactory or trigeminal routes, respectively.

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FP3. THE MODEL OF ENDOTOXEMIA IN RATS AFTER SUBDIAPHRAGMATIC VAGOTOMY

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Abstract

Escherichia coli lipopolysaccharide (3 μg/kg) injection intravenously to rats (n=23) is accompanied with development of polyphasic fever and shift of nociceptive reactions threshold to hypo- or hyperalgesia according to fever phase. The blockade of afferent and efferent signals after vagotomy disturbs formation of fever and nociceptive reactions pattern. Therefore, obtained data allow explaining the phenomenon of nociceptive reactions and fever threshold shift after imperative change of signals flow through vagus nerve (for example, during qigong practicing).

Key words: fever model, brainstem, vagotomy, control, nociceptive reactions

Introduction

Some years ago a hypothesis has been actively developed on the role of vagal fibres in the initiation of a complex of systemic responses ("non-specific symptoms of sickness" or "acute phase reaction") [1, 2] upon invasion of microorganisms or administration of lipopolysaccharide (LPS) and cytokines to experimental animals [2, 3]. According to the hypothesis, the acute phase reaction is triggered by information coming through afferent fibres of the vagus [3, 4, 5], although there are different views on the involvement of efferent fibres of the nerve in these processes [4]. It is well-known, however, that after subdiaphragmatic truncal or selective vagotomy (this being the main technique at the preparatory stage, used by the supporters of the "vagus" hypothesis) the bulbar nucleus of the vagus showed degenerative alterations [6], which hypothetically may be one of the causes of changed neuroimmune relations during endotoxemia. We focused our attention just on this aspect of the problem. In the present work we attempted to compare the development of the acute phase reaction after systemic application of *Escherichia coli* LPS in rats with preliminary subdiaphragmatic truncal vagotomy and in those with pre-destroyed, by neurotoxin (kainic acid), neurons of the primary projection area of abdominal vagal fibres in the medulla (caudal area of the nucleus of the solitary tract, NST [6, 7]). In this work, in particular, accent is given to the characters of the nociceptive responses during the action of the endotoxin in the organism. The adequacy of the purpose is grounded on the well-known data on the modulatory effect of the vagus nerve and some bulbar nuclei on pain perception and nociceptive transmission [6]. We also collated the physiological findings with the results of an electron microscopic visualization of the caudal NST in rats after subdiaphragmatic truncal vagotomy.

Material and Methods

Experiments were performed in male Wistar rats weighing 230-280 g. The animals were housed four per box. The room was on a 12/12 h light/dark cycle; ambient temperature was maintained at 22 °C. Food and water were available *ad libitum*. The animals were daily handled and habituated to the experimental boxes to minimize stress effects during experimentation for seven days prior to the beginning. A special care was given to post-operative animals (for details see [5]).

One group of animals were subjected to subdiaphragmatic truncal vagotomy (n=7) or sham surgery (n=4) four weeks prior to the experiment. The other group received 0.5 μg kainic acid in 100 nl saline (n=7) or 100 nl of the pyrogen-free saline (PFS; n=5) to the caudal part of the commissural NST (4.5 mm posterior to the interaural line; 0 mm lateral to the midline; and 8 mm down from the skull surface) 29-32 days before the experiment.

Three days before the experiment, a silicon catheter was implanted into the jugular vein to each rat. All operations were conducted under ketamine-xylazine-acepromazine (55.6, 5.5, and 1.1 mg/kg, respectively, i.p.) anesthesia. Intrabulbar injections of the solutions were made with a nanolitre pump (WPI, Inc.-1400, Sarasota, FL, U.S.A.) in accordance with the stereotaxic atlas coordinates (http://labs.gaidi.ca/rat-brain-atlas/).

The nociceptive and febrile responsiveness to intravenous LPS or PFS were tested 29-32 days after the subdiaphragmatic truncal vagotomy or intrabulbar injection of kainic acid. On the day of the experiment the rats were placed in restraining boxes put into a thermostat at 29 °C and 50% humidity. These temperature and humidity levels are optimal for rats in terms of realization of thermoeffector mechanisms [6]. After a 1 h adaptation to the experimental conditions, measurements were started. All parameters (pain sensitivity threshold, deep and surface body temperature) were monitored from 0.5 h before until 5 h after the i.v. injection of LPS or PFS (control). The experiments were started around 9:00 A.M. Each rat was tested twice. One-half of the animals received LPS during the first test and 3 days later and were used in control experiments; the other half was studied in reverse order, i.e., control followed by the LPS test 3 days later.

The pain sensitivity threshold was evaluated during measurements of the tail-flick latency (TFL) to an infrared stimulus of standard intensity. The stimulus intensity was adjusted so that under normal

conditions TFL was ~7 s. The value of TFL corresponded to ~50 °C of the skin temperature in the focus of the light beam (measured in separate experiments). To prevent burns, cut-off time was set at 20 s; if the tail flick response did not occur within this time, measuring was terminated, and the TFL was assigned a value of 20 s (for details of the method see [6]).

Each animal was instrumented with rectal and tail-skin copper-constantan thermocouples (Physitemp, Clifton, NJ, U.S.A.) for deep body (T_c) and skin (T_{sk}) temperature measurements. A colonic thermocouple was inserted 8 cm deep to the anus. A skin thermocouple was attached to the ventral surface of the tail, on the border of its proximal and middle thirds. The T_{sk} was recorded in order to exclude its potential side effect on the results of the tail-flick test [5, 7, 8]. The reference junction of each thermocouple was kept at 0 °C.

Endotoxin 3 μg/kg (LPS, *Escherichia coli,* 0111:B4, List Biological Laboratories, Campbell, CA, U.S.A., lot No LPS-25E) or pyrogen-free saline (PFS, Abbott Laboratories, North Chicago, IL, U.S.A., lot No 18-379-DK) were applied intravenously through a silicon catheter in a volume not exceeding 0.5 ml.

Two and four weeks after subdiaphragmatic truncal vagotomy two and three rats, respectively, (and two sham operated animals, by one for each time of observation) were deeply anesthetized with a lethal dose of pentobarbital, given 0.2 ml of heparin (1000 Units/ml) into the left ventricle, and perfused with 150 ml of 0.9% saline at 40 °C, followed by 0.5 liter of 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4 at 4 °C. The brain was then removed and fixed first in 2.5% glutaraldehyde and then in 1% osmium tetroxide. After fixation the material was washed with buffer, dehydrated in alcohol's of graded strength, and embedded in Araldite. Sections were obtained by the use of a LKB-III Ultratome and scanned with a JEM-IOOB electron microscope (Japan).

Thirty minutes and four weeks after microinjections into the caudal part of the commissural nucleus of the solitary tract the same procedures were made with two and three rats, respectively received kainic acid, and two rats (one for each time of observation), received saline.

The data were statistically treated using the Student's *t*-test. In this study, we also applied one-way and two-way ANOVA test. All data are presented as means ± SE.

Results and Discussion

Intravenous injection of 3 μg/kg lipopolisaccharide induced a significant decrease in TFL (P<0.05) in sham-operated animals (Fig. 1), and in rats with intrabulbar vehicle (Fig. 2). Simultaneously, at 45-55 min after the injection T_c increased from 37.9±0.1 °C by 0.7±0.1 °C (P<0.01) in sham-vagotomized rats and from 37.8±0.1 °C by 0.8±0.1 °C (P<0.01) in rats with the vehicle injection to the NST, while T_{sk} decreased from 35.6±0.2 °C and 35.8±0.2 °C, respectively, by 3-4 °C in both groups. Administration of PFS did not induce significant changes in TFL, T_c and T_{sk}.

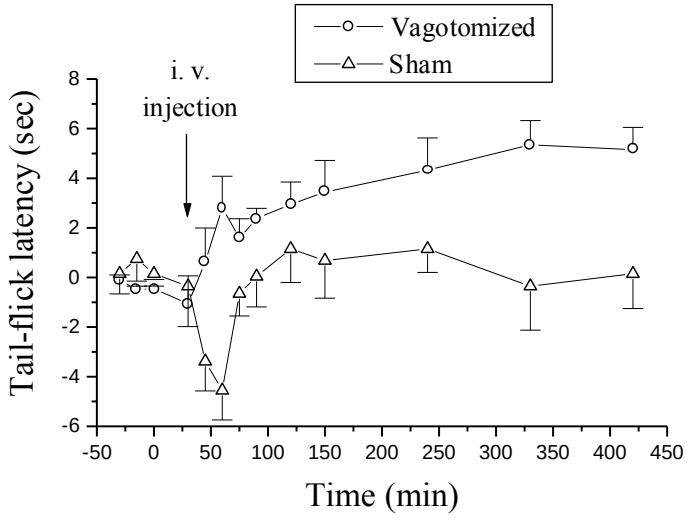

Fig. 1. Nociceptive effect of i.v. injection (arrow) of lipopolysaccharide (3 μg/kg, LPS) in vagotomized (n=7) and sham-operated (n=4) rats. Tail flick latency is shown as mean ± SE.

Injection of LPS to rats whose caudal part of the commissural NST was pretreated with neurotoxin led to a wave-like increase in TFL (Fig. 2), whereas T_c and T_{sk} remained unchanged.

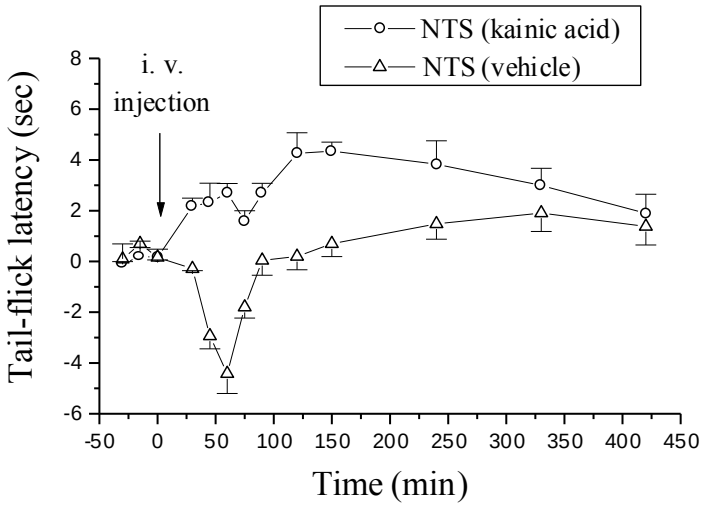

Fig. 2. Nociceptive effect of i.v. injection (arrow) of lipopolysaccharide (3 μg/kg, LPS) in rats preinjected with kainic acid (n=7) or vehicle (n=5) to the caudal part of the commissural nucleus of the solitary tract. Tail flick latency is shown as mean ± SE.

In vagotomized animals the endotoxin also induced a wave-like increase in TFL (Fig. 1) and failed to elicit significant changes in T_c. At the same time, there was some increase in T_{sk} at 45-60 min after the LPS injection.

Two and four weeks after subdiaphragmatic truncal vagotomy the caudal NST exhibited signs of structural alterations both in neuronal processes and cell bodies, visualized by electron microscopy (Fig. 3 a, b). Two weeks after, accumulation of lysosomes and lipofuscin granules in the perikaryon, were observed (Fig. 3a). The outlines of the postsynaptic formations and adjacent glia became less distinct by the second week after vagotomy.

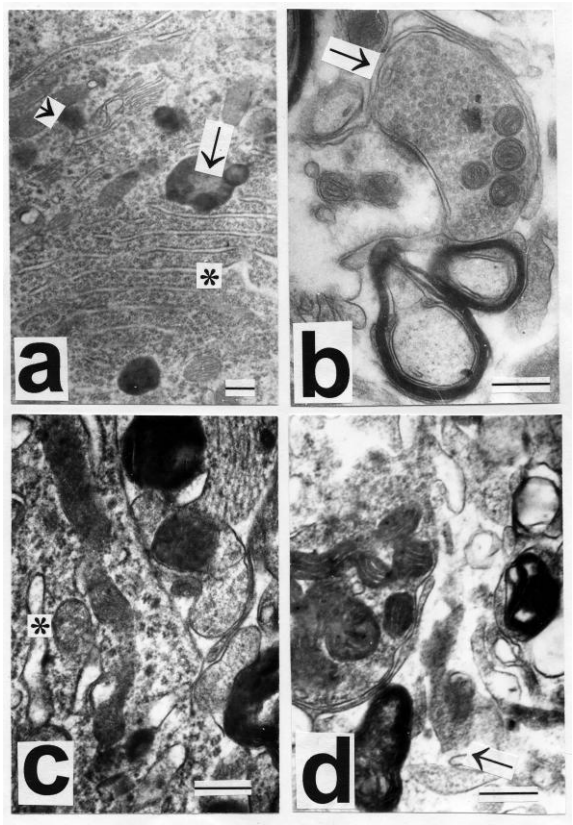

Fig. 3. Electron micrograph of the caudal nucleus of the solitary tract of rats two (a; ×26000) or four (b; ×25000) weeks after subdiaphragmatic vagotomy, and two (c; ×75000) or four weeks (d; ×16000) after microinjection of 100 nl kainic acid (0.5 μg) into the commissural part of the solitary tract.
a – endoplasmic reticulum canals (asterisks), lysosome and lipofuscin granules (arrows); b – axodendritic synapse (arrow); c – cytoplasmic vacuolization, vacuole (asterisk); d – degenerated texture of nervous tissue (arrow).

Four weeks after the surgery, the endoplasmic reticulum canals appeared deformed and lipofuscin granules were present beyond neuronal soma. There were some synaptic contacts of an axon with a dendrite invaginated to its cytoplasm, accumulation of synaptic vesicles (Fig. 3b). We observed widened endoplasmic reticulum canals from 0.05-0.07 μm (the second week of observation after vagotomy) to 0.18-0.19 μm (the fourth week of observation after vagotomy), as compared to 0.02-0.03 μm in sham operated animals.
The axonal hillock in small neurons was found to be lysed. “Dark”-type degenerating thin myelinated neuronal processes were detected both beyond and among the fragments of destroyed cells within the glial cytoplasm.
In animals, which received a microinjection of neurotoxin to the caudal part of the commissural NST, the region showed destruction of the brain tissue. In adjacent tissues, the naked nuclei, swelled mitochondria and disturbance of cellular metabolism were found. A degenerating terminal, consolidation of pre- and postsynaptic membranes, a synaptic contact is discernible (Fig. 3 c, d). No such-like gross structural alterations were observed in saline-treated animals.
In subdiaphragmatically vagotomized rats and in animals who received neurotoxin to the caudal part of the commissural NST, intravenous LPS failed to induce hyperalgesia (Fig. 1, 2), a rise in T_c, and a fall in T_{sk}. It should be noted that TFL depends on T_{sk}: an increase in T_{sk} *per se* results in a decrease in TFL, and *vice versa* [6]. Therefore, the changes in TFL observed in the present study following LPS or PFS administration did not correlate with the changes in T_{sk}. So, the observed changes in TFL were not due to the changes in T_{sk}, but rather developed in spite of them.
The two groups of animals exhibited not only a single-type pattern of TFL changes but also destructive events (caused by neurotoxic kainic acid or vagotomy) in the same portion of the central nerve system, namely in the primary projection zone of abdominal vagal fibres (in the caudal NST). Thus, the structural alterations, following vagotomy, both in the motor vagal nuclei and in the NST are an established fact. In the text, we give the results of our own electron microscopic investigations, which as a matter of fact, are not inconsistent with the available literature data pertinent to vagotomy [2, 3, 5, 9].
In one more series of experiments we simulated structural changes in the NST with local intrabulbar injections of neurotoxin. Four weeks after subdiaphragmatic vagotomy or after kainic acid destruction of neurons in the commissural nucleus of the NST the nociceptive (and temperature) responses to intravenous endotoxin were attenuated, distorted, or blocked. These facts are not surprising and, in principle, do not contradict the accepted view of the NST role in modulation of nociception [6]. Importantly, the modulating role of NST in nociceptive processes is found in endotoxemic rats, too. Since both groups of animals have in common the structural alterations in the NST (after vagotomy or neurotoxin) and the same hyporeactivity to lipopolysaccharide, why isn’t it possible to suggest an important role of the central nerve system post-vagotomy structural changes in the acute phase reaction to the endotoxin? Hypothetically, after endotoxin invasion and production of endogenous pyrogens, the latter may interact with neuroglial elements of the NST not via the abdominal vagal fibres but, e.g., circumventricular organs or various messengers (nitric oxide) released from the vascular endothelium and penetrating to the brain, e.g., in the *area postrema.*
It follows that disturbances of the brain-immune interaction and nociception during experimentally simulated acute phase reaction are underlain by a disordered functioning of neuronal populations in the

NST, the primary projection area of the vagus, rather than by changed conditions for vagally transmitted signals after vagotomy, as it has been thought [2, 5, 7, 10]. Developing the inferences drawn from the functional changes and structural alterations in the medulla after vagotomy, observed in the present work, it may be suggested that such destructive processes in the nuclei of other cranial nerves after their trauma or injury might negatively affect the various body functions (including nociception).

Conclusion\

Escherichia coli lipopolysaccharide injection into internal milieu is accompanied with development of polyphasic fever and shift of nociceptive reactions threshold to hypo- or hyperalgesia according to fever phase. The blockade of afferent and efferent signals after vagotomy disturbs formation of fever and nociceptive reactions pattern. Therefore, obtained data allow explaining the phenomenon of nociceptive reactions and fever threshold shift after imperative change of signals flow through vagus nerve (for example, during qigong practicing).

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FP4. ON THE MANAGEMENT OF NEUROTRANSMITTER IMBALANCE AFTER THE USE OF NEUROTRANSMITTER LIGANDS OR DIELECTRICS IN HYPOXIA

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Abstract

In the brain, contains a variety of signaling molecules (neurotransmitters, neuromodulators, growth factors, cytokines, free radicals, and fast genes). Functional level shift some signaling molecules associated with activation of protective systems to restore homeostasis signaling molecules in the brain. With the development of pathological processes in the brain (e.g. hypoxia) homeostatic processes are being violated. Prescribing dysfunction of the nervous system may be accompanied by increased imbalance signalling molecules in the brain. In this connection it is expedient to carefully control the neurotrophic effects of drugs for the leveling of side effects in the brain and body.

Key words: brain, signaling molecules, drugs, side effects, dielectrics

Introduction

Regulatory systems were formed in living organisms during the evolution to control various functions. Molecules of different origin interact with receptors and act as signal transducers in the systems of functions control. Derivate of lipids, proteins, carbohydrates and nucleic acids and their complexes are the most common signal molecules. Proteins and their complexes are the most common signal acceptors.

Endogenous ligands interact with receptors for split ms or few ms [1, 2]. Approximately the same time is needed to generate electrical signal in neuron' soma and expand it by axon to effectors (other nerve cells, nerve, muscle, gland and other cells). Neural networks were formed in nerve tissue to "discuss" the importance of input signals and make a decision that will be transferred to effectors (output) in the form of chemical and electrical signals. The use of synthetic pharmacological substances for impaired functions correction is accompanied with imbalance in the interaction between synthetic and endogenous regulatory molecules in the brain. So far, little is known about molecular background of the pharmacoresistance [3]. Adverse effects occur, for example after synthetic cannabinoids intake [4], and the real problem of information processing in the brain is formed.

There is another fundamental and applied problem. The question of regulatory processes in hypoxia still remains unsolved. There are lots of antihypoxants, but their effectiveness is illusive. Special attention in the paper is paid on the solution containing amber based dielectric nanoparticles (ABDN). Hypothetically a number of nanoparticles are able to accept hydrogen ions and free radicals excess. Nanoparticles are polarized and obtain ability to attract charged particles from polar solvent. If that is so, then side effects of oxygen lack will be leveled by ABDN in hypoxia experimental modeling. Therefore the effectiveness

of regulatory processes should increase in hypoxia. The answer to these questions was the main purpose of the study.

Methods

The hypothesis was verified in vitro on hippocampal slices. Electrophysiological experiments were performed using transverse sections (slices) of the hippocampus of 450 microns thickness from 4-week-old male rats (*n*=11) as described earlier [5]. This study was approved by the Animal Care and Use Review Board at the Institute of Physiology of National Academy of Sciences of Belarus. Before test, the slices were preincubated for 1 hour in preincubator (BSC-PT, Harvard Apparatus, USA) in carbogen-saturated artificial cerebrospinal fluid (ACSF) at 20°C. The composition of ACSF comprised (in mmol/l): 124.0 NaCl; 3.0 KCl; 1.25 KH_2PO_4; 1.2 $MgCl_2$; 2.0 $CaCl_2$; 26.0 $NaHCO_3$; 10.0 Glucose, pH 7.3-7.4. During tests, the slices were placed in a temperature-controlled chamber (BSC-ZT, Harward Apparatus, USA) at 29 °C and perfused with hypoxic gas mixture (21% O_2) or carbogen-saturated ACSF at a flow rate 4 mL/min. Recording tungsten microelectrodes (WPI Inc., USA) were placed at the stratum radiatum and stratum pyramidale of hippocampal CA1 region to monitor excitatory postsynaptic potentials (EPSP) and population spikes respectively. Neuronal response was evoked by electrical stimulation of presynaptic Schaffer collaterals by stimulating microelectrode.

Results and Discussion

After preincubation period the subsequent infusion of ACSF saturated with carbogen (first minutes of observation are shown in Figure 1A) revealed a gradual stabilization of EPSP. Neuron populations stayed responsive to liminal stimuli during bolus injection of 30 µl of ABDN alcohol solution into incubation camera and subsequent 5-minute hypoxia. Amplitude of EPSP increased a little for the first two minutes (Figure 1B). Then rapid downfall of evoked responses amplitude was observed. Preliminary bolus injection of 30 µl of 95% alcohol into perfusion solution with hippocampal slice and following hypoxia resulted in complete blockade of evoked responses (Figure 1C). EPSP blockade (as in the Figure 1C) was also observed during hippocampal slices perfusion with hypoxic solution of artificial cerebrospinal fluid. EPSP amplitude was restored after hypoxic test (Figure 1D).

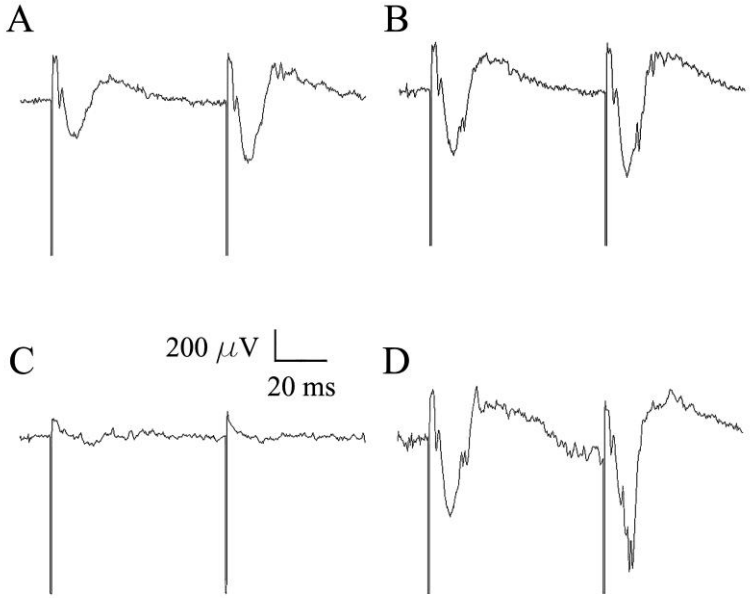

Figure 1. Excitatory postsynaptic potentials evoked by the first and second paired pulses during perfusion of slices of rat hippocampus with artificial cerebrospinal fluid containing 30 µl of alcohol solution of amber based dielectric nanoparticles (ABDN).

A – before perfusion of ABDN ; B – in 60 sec after the start perfusion of ABDN and hypoxia; C - in 90 sec after the start perfusion of 30 mcl spirits and hypoxia; D – in 2 min after subsequent perfusion with artificial cerebrospinal fluid without any substances.

Another problem is determined by the wrong interpretation of non-clinical and clinical studies of psycho- and neurotropic drugs. Adverse effects of synthetic substances were mentioned above. This chapter concentrates on the impairment of natural neurochemical balance in the brain after exogenous ligands of neurotransmitter receptors getting into internal milieu. Millenary evolution stages were accompanied with the development of complicated balanced system of regulatory molecules interaction, for example of nerve cells inhibitors and activators. It is impossible to imagine the harmony of information processing in the brain, comparison of information with previously accumulated experience and decision making without interaction between chemical signal transducers in time and space [1, 2, 6]. Naturally, a variety of regulatory systems and signal molecules takes part in the control of any function. This provides, on the one hand, reliability of any function control and, on the other hand, complicates experimental analysis of concrete role of each regulatory system and corresponding signal molecules. It can be illustrated with several examples. The effectiveness of erect posture maintaining (*Homo erectus*) is firstly determined by intensity and speed of skeletal muscles contraction. This important factor is usually taken into account alone, without brain and skeletal muscles blood supply. The importance of brain blood supply is well demonstrated in case of orthostatic collapse, when contractile and properly developed skeletal muscles become unable to maintain erect posture due to blood flow redistribution and insufficient blood supply of brain. The functioning of neurons, responsible for postural and stretch reflexes, is impaired due to attenuation of brain blood supply.

There is another well-known demonstrative method: single switching off (blockade) of receptor in any regulatory system. Blockade of one subtype of receptors is accompanied with reorganization of all other regulatory systems activity, which are involved in concrete function control along with the blocked one. Such functional and dysfunctional reorganizations in the brain should be taken into account in clinical practice. For example, single blockade of NMDA-receptors in order to lower excitotoxicity of excitatory amino acids leads to disinhibition of other subtypes of glutamate receptors and increasing of glutamate toxicity. The clinician should know the mechanisms and causes of dysfunction and excitotoxicity development in order to disturb the balance of brain regulatory system preventing at the same time adverse effects of drugs, which act selectively on one of the elements of regulatory system.

Using this line of reasoning the one may draw contrary conclusions. Pessimist may conclude that the clinician provides facilities for disturbance of information processing by nerve cells due to imbalance in neurochemical brain homeostasis as the result of medicinal substance intake. But he does not consider high brain plasticity. Optimist will take into account adaptive capacities of the brain. He will also consider dose-dependent and adverse effects of psycho- and neurotropic drugs intake. In any case, traditional crush into psycho- and neurotropic drugs use should be limited by reasonable physiological bounds. Higher use of synthetic ligands and antagonists of nerve tissue receptors is accompanied with increased frequency of neurodegenerative and neurodestructive processes [3, 4, 7]. It is necessary to consider the fact of neurotransmitter imbalance intensification during the modeling of various pathological conditions and study the role of signal molecules. Effective correction protocols should be developed to manage this imbalance at the experimental and non-clinical stages.

The problem of impairment of signal molecules interaction in nerve tissue after synthetic medicinal drugs intake is discussed [4, 8] Due to significance of organ, tissue, cellular and subcellular levels of regulation in living organisms, it is necessary to take stock of this issue in other functional systems besides central nervous one.

Thus, carried experiments showed role of dielectrics in neutralization of free radicals in hypoxia. Carbon in organic molecules is natural dielectric [8]. Therefore, regulatory role of transmitters in the brain will manifest in different ways under conditions of normoxia and hypoxia according to dielectric features of natural or synthetic dielectrics.

Conclusion

Understanding the mechanisms involved in the role of dielectrics in neutralization of free radicals in hypoxia in the brain will require the attention and cooperation of scientists in the theoretical and applied areas of expertise. Resolving this issue will restore conditions of interneuron communication in the brain.

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FP5. IN THE WORLD OF BONES, CELLS AND MOLECULES

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FP6. НАКРАТКО ЗА СРЕБРОТО И НЕГОВАТА БИОЛОГИЧНА АКТИВНОСТ

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Резюме

В представения обзор са обобщени данни за биологичната активност на среброто и неговите съединения. Вниманието е насочено към приложенията на среброто в медицината, антимикробните и антитуморните му свойства, неговата токсичност.

Химични и физични свойства

Среброто (Ag, от латинската дума *argentum*, което означава лъскав или блестящ) е метал с бял цвят и красив блясък. Намира се в 1 Б група, V период, на периодичната система. То е с атомен номер 47, атомна маса 107.86 g/mol, плътност 10.5 g/cm^3, температура на топене 961.78^{o}C и температура на кипене 2162 ^{o}C. Среброто има много добра термична и електирческа (на първо място сред металите) проводимост. То е доста меко и ковко и може да се обработва механически. При обикновени условия не се окислява и спада към групата на „благородните метали". В химичните съединения е от +1 степен на окисление [1, 44].

Разпространение

Среброто се среща в природата както в самородно, така и (по-често) в свързано състояние. Открива се като сплав със златото, много често е примес на сулфидни руди (на Fe, Cu и Ni) [1,44]. Основна суровина за получаване на сребро са медни, медно-никелови, оловни и оловно-цинкови руди [33]. В Перу и Мексико среброто се добива от 1546 г. насам и тези страни, заедно с Полша, Австралия, Канада и Русия, и днес са сред най-големите производители в света [12].

Концентрацията на сребро в морска вода със соленост 3.5% възлиза на около 0.00028 ppm [17]. Съдържанието му в реки, езера и устия на реки е около 0.01 μg /L в незамърсени райони и 0.01-0.1 μg /L в градски и индустриални зони [36].

В човешкото тяло съдържанието на този метал е ниско (< 2.3 μg). Среброто се абсорбира чрез белите дробове, стомашно-чревния тракт, лигавиците и кожата [80]. Физиологичната му функция не е напълно изяснена.

Исторически данни

Металът е добит за първи път около 2500 г.п.н.е. в Мала Азия [1]. Доказателствата за прилагането му с декоративни цели датират от 4000 пр.н.е., а сплав на сребро и злато е била използвана за направа на монети 800 години пр.н.е. [6]. В древността среброто е било смятано за по-ценно дори от златото.

Приложение на среброто в медицината

Хипократ вярвал в целебните свойства на този метал и го препоръчвал при различни страдания. Алхимиците свързвали среброто с луната и смятали, че то е подходящо за лечение на мозъчни заболявания [6]. Разтворими сребърни съединения (сребърни соли) са били включвани в терапията на някои психични заболявания, епилепсия, зависимост от никотин, гастроентерит, стоматит [4, 73] и предавани по полов път заболявания, включително сифилис и

гонорея [28]. В продължение на дълги години сребърният нитрат ($AgNO_3$) е накапван в очите на новородени непосредствено след раждането в случаи на майки с гонококова инфекция с цел предотвратяване на заразяването в хода на родовия акт [37]. Добре известно е, че т.нар. ophthalmia neonatorum, причинявана от *Neisseria gonorrheae*, може да доведе до слепота. Днес този подход все по-често се поставя под въпрос, като причините за това са поне три: 1) доказана е способността и на други патогени (напр. *Chlamidia trachomatis*) да причиняват този здравен проблем; 2) прилагането на $AgNO_3$ се свързва с развитието на химически-индуцирани конюнктивити; 3) $AgNO_3$ би могъл да представлява проблем за климата, собено в тропическите зони [21, 59].

Антимикробна активност на сребро

Антимикробните свойства на среброто са известни от векове. За тях са знаели и финикийците, които са съхранявали хранителни продукти в сребърни съдове. То е било прилагано в древна Гърция и Рим като дезинфектант, а македонците са го използвали с цел подпомагане заздравяването на рани. Гърците покривали чиниите и чашите със сребро, за да спрат разпространението на болестите, а в кофите с вода поставяли сребърни монети, за да я запазят по-дълго време годна за пиене. На малките деца давали да смучат сребърна лъжичка, като вярвали, че по този начин ще ги предпазят от зарази. Преди ерата на антибиотиците, сребърни съединения са били прилагани за предотвратяване на инфекции по време на Втората световна война [3, 6, 44].

С появата на съвременните антибиотици през 20ия век, „позициите" на среброто в медицината започват постепенно да „отслабват". През последните години, заедно с нарастването на проблемите, произлизащи от устойчивостта на микроорганизмите към използваните в клиничната практика антибиотици, интересът към среброто отново започва да нараства. Поради високата си антимикробна активност и добра поносимост, то влиза в състава на редица медицински и други продукти, като мазила, превързочни материали, но също в тъкани, мобилни телефони, перални машини, бои и дори в спрейове срещу изпотяване. Използва се в системите за стерилизиране на питейна вода в орбиталните космически станции (руската станция „Мир", полетите на Аполо на НАСА). Топлата вода в голяма част от съвременните болници преминава през медно-сребърни филтри, за да се осигури защита срещу устойчивите на метицилин щамове *Staphylococcus aureus* (MRSA) [2, 3, 13].

Среброто (под формата на Ag+ йони, както и създадени на основата на Ag съединения) има бактерицидно действие по отношение на широк кръг Грам-положителни и Грам-отрицателни микроорганизми. Нещо повече, установено е, че то е ефективно и спрямо устойчиви към действието на антибиотици щамове, каквито са метицилин - резистентни щамове *Staphylococcus aureus* (MRSA), мултирезистентни щамове *Pseudomonas aeruginosa*, устойчиви на ампицилин щамове *Escherichia coli* O157: H7 и резистентни към действието на еритромицин щамове на *Streptococcus pyogenes* [50, 70, 72]. Особено полезни в това отношение се очаква да бъдат сребърните наночастици (AgНЧ) [64], чиито механизъм на действие привлича интереса на все повече учени [24, 27]. Установено е, че механизмът на действие на AgНЧ е подобен на този на Ag+ йони [22], но ефективните концентрации са значително по-ниски.

Металът се използва при пречистване на води, лечение на рани, изработка на устройства (напр. костни протези) за нуждите на ортопедичната реконструктивна хирургия, кардиологията, урологията, както и при създаването на хирургически инструменти. Със сребро се покриват изкуствени сърдечни клапи, както и сърдечни и пикочни катетри, за да бъде намалена или предотвратена опасността от инфекции, предизвикани от различни микроорганизми [11]. Металът намира приложение и като алтернативен дезинфектант в случаите, при които използването на традиционните дезинфектанти, напр. хлоридите, може да доведе до

образуването на токсични продукти или да причинят корозия на повърхности. Доказано е, че среброто има синергичен ефект в комбинация с някои дезинфектанти.

Сребърният сулфадиазин е най-широко използваният лекарствен продукт, съдържащ сребро. На фармацевтичния пазар се предлага под наименованията Silvadene и Flamazine. Използва се при лечение на изгаряния, като освобождава сребърни йони в мястото на нараняването. Доказано е, че в раната се абсорбират около 10% от отделеното сребро, като процентът е по-висок при по-добре кръвоснабдените рани [47].

Механизмът на антимикробното действие на среброто не е напълно изяснен. Смята се, че важна роля играе освобождаването на Ag(I) йони [46, 65]. Доказано е, че Ag+ йони предизвикват промени в ДНК и РНК молекулите, в митохондриалното дишане и в цитозолните белтъци, което води до смъртта на бактериалната клетка [46].

Предполага се, че зарядът на Ag+ йони има важно значение за антимикробната активност поради възникването на електростатично привличане между тях и отрицателно заредената клетъчна стена на микроорганизмите [43. Според други автори бактерицидното действие на сребърните наночастици по отношение на грам-отрицателни бактерии се свързва с изменения в клетъчната стена на бактериите и нарушения в пропускливостта ѝ, което в крайна сметка предизвиква гибелта на клетките [5, 72]. При *Candida albicans* Ag(I) потиска действието на ензима фосфоманоза изомераза, като се свързва с тиоловите групи в цистеиновите остатъци [57, 82]. Този ензим е абсолютно необходим за изграждането на клетъчната стена и в резултат на дефектите в него се губят жизненоважни вещества (напр. фосфати и глутамин). Не бива да се подценява и способността на сребърните наночастици да генерират образуването на свободни радикали [20]. Проведени от Lara и сътр. изследвания [50] показват, че бактерицидното действие може да се дължи и на възпрепятстване изграждането на клетъчната стена и потискане синтеза на белтъци (особено на процесите, свързани с участието на 30S-субединицата на рибозомите) и нуклеинови киселини. Резултатите от други проучвания сочат, че сребърните наночастици могат да дестабилизират външната мембрана, да доведат до промени в плазмения потенциал и да повлияят върху нивата на вътреклетъчния АТФ [22, 54]. Съобщено е за антимикробното действие на комплекси на Ag(I) с кумарини [18, 19]. Те нарушават дишането и блокират синтезирането на цитохроми [74].

Сребро и вируси

Предполага се, че сребърните наночастици могат да взаимодействат със суперкапсида на т.нар. „облечени" вируси и по този начин да предпазят клетките от инфекция [51]. Така например, сребърни наночастици се свързват с участъка от гликопротеин (gp120) върху суперкапсида на човешкия имунодефицитен вирус (HIV-1), който е отговорен за свързването с CD4 рецептора на клетката-гостоприемник [25, 51, 52]. Съобщено беше за създаване на покрит със AgНЧ полиуретанов кондом. Материалът, от който е изработен, не проявява токсичност за култивирани в негово присъствие в продължение на 3 часа клетки от линии HeLa (човешки карцином на шийката на матката), 293T (клетки от човешки ембрионален бъбрек, трансформирани с големия антиген на вирус SV40) и C8166 T (човешки CD4+ T-лимфоидни клетки). В същото време той ефективно инактивира инфекциозността на HIV-1 и HSV-1/2 (човешки херпесни вируси тип 1 и тип 2). Най-вероятно антивирусното действие се дължи на AgНЧ. Заслужава да се отбележи фактът, че към покрития с AgНЧ полиуретан са чувствителни щамове HIV-1, които проявяват тропизъм както по отношение на макрофагите (т.нар. M-тропни щамове), така и към T-лимфоцитите (T-тропни щамове) [58].

Има данни и за антивирусен ефект на AgНЧ по отношение на хепатит B вируса (HBV). Установено е, че наночастиците се свързват с висок афинитет с ДНК на HBV и извънклетъчните вириони. В култивирани в лабораторни условия клетки AgНЧ потискат синтеза на вирусната РНК и появата на извънклетъчни вириони [55]. Проучване, проведено от Xiang и сътр. [83] показва, че

AgНЧ проявяват антивирусна активност при култивирани в клетки от линия MDCK (кучешки бъбрек) грипни вируси (H1N1).

Предполага се, че антивирусният ефект на среброто (в различните му форми) се дължи на способността му да денатурира ензими, взаимодействайки с техните сулфхидрилни-, амино-, карбоксилни-, фосфатни- и имидазолови групи [7, 9, 13, 63, 67]. Тази негова способност обаче поражда и необходимостта от задълбочено изучаване на евентуалните странични ефекти на този метал.

Сребро и лечение на рани

През последните години бяха създадени редица превръзки за рани, които постепенно освобождават сребро за продължителен период от време. Целта е с тяхна помощ да бъде подобрен контролът върху бактериални, гъбични и вирусни инфекции. Смята се, че въвеждането им в клиничната практика ще подпомогне борбата с вътреболничните инфекции, ще предотврати някои от усложненията при диабетно болните, ще възпрепятства инфектирането и ще облекчи зарастването на рани (например от тежки изгаряния). Някои от проведените през последните години проучвания обаче показват, че съдържащите сребро превръзки не превъзхождат и дори отстъпват в някои отношения на използваните за сравнение превръзки, несъдържащи този елемент [8, 15, 26]. Една от причините е, че в определени концентрации среброто потиска клетъчната пролиферация и по този начин забавя епителизацията [14, 60]. Poon и Burd [62] установиха, че сребро от разтвор на сребърен нитрат или комерсиална превръзка за рани проявява изразен токсичен ефект върху отглеждани в монослойни култури кератиноцити и фибробласти. Предполага се, че това се дължи на прекъсване на дихателните вериги в митохондриите с последващо производство на реактивни кислородни видове и потискане на синтеза на АТФ, водещи до възникване на увреждания в ДНК молекулите. Проучванията показват, че цитотоксичният ефект на среброто за бозайническите клетки зависят от концентрацията на сребърни йони и показва вариации в зависимост от разтворимостта на сребърни соли, средата на освобождаване, типа на превръзката [14, 79].

Антитуморна активност на сребро

Към днешна дата данните за антитуморна активност на среброто са доста ограничени. Редица изследователски групи съобщават за обещаващи антинеопластични свойства на съдържащи Ag(I) съединения при различни експериментални тумрни модели. Сред тях са сребърни комплекси с различни лиганди - кумаринови производни, карбоксилови киселини, аминокиселини, донори на азот, фосфор или сяра [10, 75, 76]; сребърни карбоксилатни димери [85] и др. До момента ролята на координационното число, както и на хидрофилност/ипофилността на комплексите не е напълно изяснена [10].

През 2013 г. е съобщено за цитотоксичен ефект на сребърни наночастици при клетки от рак на гърдата у човек (линия MCF-7) [40]. Доказана е способността на AgНЧ да предизвикват апоптоза в клетки от линия HeLa (карцином на шийката на матката при човек) [78] и да намаляват преживяемостта на клетъчни линии от остра миелоидна левкемия (SHI-1, THP-1, DAMI) [29]. В последния случай цитотоксичният ефект на наночастиците е много по-слабо изразен при нормалните хемопоетични клетки. Сребърни наночастици убиват остеосаркомни клетки независимо от състоянието на протеина p53 в тях [45]. Смъртта настъпва чрез апоптоза вследствие на митохондиален стрес. Комплекси на сребро с хинолин потискат пролиферацията на клетки от линия HepG2 (рак на черния дроб у човек), като блокират клетките във фази G1 и S [84]. Комбинацията от сребърни наночастици и Alisertib (селективен инхибитор на киназата Aurora A) потиска растежа на глиобластомни клетки от линия U87MG [53]. Натоварени с Imatinib (Gleevek – тирозинкиназен инхибитор, прилаган в лечението на различни ракови заболявания) наночастици предизвикват апоптоза в клетки MCF-7 [68].

Специален интерес представлява възможността за получаване на сребърни наночастици с антитуморна активност чрез използване на растителни екстракти [16, 39, 42, 56, 69]. Подобни, получени с помощта на зелената химия наночастици, потискат растежа на клетки от линия H1299 (рак на белия дроб у човек), както *in vivo* - в мишки, характеризиращи се с тежък комбиниран имунодефицит (SCID), така и в култура. Установено е, че те инхибират активността на ядрения фактор NF-kB, намаляват експресията на гена bcl-2 и усилват активността на каспаза-3 [34].

Устойчивост към сребро

Устойчивостта на микроорганизмите към тежките метали е добре известна [71]. Среброто не прави изключение от тази тенденция, но се смята, че устойчивостта на микроорганизмите към него към момента не е сериозен клиничен проблем [77]. Доказателства за устойчивостта на микроорганизмите към сребро и други метали са получени с помощта на молекулярнобиологични методи и секвенционен анализ. Най-често тя се дължи на осъществяван чрез активен транспорт износ на токсични йони (с пмощта на т.нар. ефлукс помпи) или на наличието на плазмиди [49]. И двата механизма предотвратяват натрупването на нежелани молекули/йони в клетката. Устойчивостта към сребро е известна от много години. Обикновено тя се открива в среда, в която има високо съдържание на сребро или в области, където честотата на използване на съдържащи сребро продукти е голяма. През 1984 г. в сребърна мина в щата Юта, САЩ, е изолиран щам *Pseudomonas*, който носи плазмид, обуславящ устойчивост към сребро [31, 32]. Механизми на устойчивост, харакетрни за среброто, са открити при анализ на устойчив към този метал щам *Salmonella typhimurium*, изолиран от пациент с изгаряне в болница в щата Масачузетс, САЩ. Този щам се е предал към пациентите в съседни помещения и е причинил тежка септицемия и смърт, както и последващо затваряне на отдела за тежки изгаряния. Щамът съдържа кодиран от плазмид Ag-свързващ белтък (SilE), който се свързва със среброто върху повърхноста на клетката и по този начин я предпазва. Доказано е и присъствието на други гени (SilA, SilB, SilC, SilP, SilR, SilS), които кодират две ефлукс помпи - те отстраняват сребърните йони, успели да се изплъзнали от Ag-свързващия белтък [30, 31]. Познати са и други бактериални щамове, които са устойчиви към сребро – включително на *E. Coli, Enterobacter cloacae, Klebsiella pneumonia*, *Acinetobacter buamannii* [35, 47, 61]. Тези щамове се изолират в редки случаи и се смята, че рискът от разпространяване на гените за устойчивост към сребро е нисък.

Токсичност

Интересът към антимикробната и антитуморната активности на среброто е напълно заслужен, тъй като досегашните наблюдения са показали, че металът проявява слаба токсичност и се понася сравнително добре дори във високи концентрации, без да проявява кумулативна токсичност [80]. Разтворимите сребърни съединения се абсорбират по-лесно в сравнение с неразтворимите форми [38, 66] и затова именно те биха представлявали опасност като причинители на странични ефект [81]. Свръхвисокият прием на сребърен нитрат се свързва с понижено кръвно налягане, диария, раздразнен стомах и затруднено дишане. Продължителният прием на ниски дози сребърни соли може да доведе до мастна дегенерация на черния дроб и бъбреците и изменения в кръвните клетки. Продължителното вдишване или поглъщане на разтворими сребърни съединения или колоидно сребро може да причини аргирия и/или аргирозис. Разтворими сребърни съединения може да се натрупат в малки количесва в мозъка или в мускулите. Смята се, че среброто във всичките си форми не е токсично за имунната, сърдечносъдовата, нервната и репродуктивната системи и не е канцерогенно [23, 48].

Установено е, че в организма се абсорбират не повече от 10% от погълнатите сребърни съединения, като само 2-4% се задържат в тъканите. Сребро се открива в урината, кръвта и изпражненията. Елиминирането на метала става основно чрез фецеса [23, 80].

По-високата усвояемост (абсорбция) на разтворимите сребърни съединения се дължи на способността им да се свързват с белтъци, ДНК и РНК. Те бързо постъпват в кръвния ток [41], отлагат се в различни тъкани и се редуцират от светлината до метално сребро. Впоследствие акумулираното сребро може да се окисли до сребърен сулфид или сребърен селенид, което може да доведе до синьо-сива пигментация. Металното сребро не се разтваря във вода и други физиологични течности, затова се абсорбира много слабо и се екскретира от организма много по-лесно в сравнение с разтворимото сребро [38, 47].

Аргирия

Най-известното последствие от продължително излагане на въздействие със сребро е появата на характерна, необратима синя или синьо-сива пигментация на кожата (аргирия) или очите (аргирозис). Описните промени са по-интензивни в областите, изложени на влиянието на слънчевите лъчи. Най-силно засегнатите зони обикновено са ръцете, очите и мукозните мембрани [23, 47]. Състоянието не представлява съществен здравословен проблем и се приема за предимно козметичен проблем.

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