PROCEEDINGS

OF THE TENTH WORKSHOP

ON BIOLOGICAL ACTIVITY OF METALS, SYNTHETIC COMPOUNDS AND NATURAL PRODUCTS

17 – 19 NOVEMBER, 2015 , SOFIA, BULGARIA

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17-19 November 2015

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at the Bulgarian Academy of Sciences

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THE TENTH WORKSHOP

"BIOLOGICAL ACTIVITY OF METALS, SYNTHETIC COMPOUNDS AND NATURAL PRODUCTS"

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

Tuesday, 17 November 2015

8.30 – 9.00 REGISTRATION
9.00 – 9.30 OPENING CEREMONY

Session A.

Chairpersons:

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

Assoc. Prof. Neli Koseva, PhD
Institute of Polymers, Bulgarian Academy of Sciences

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

9.30 – 10.00

AO1. AN OVERVIEW ON THE PROTECTIVE ROLE OF VASOACTIVE INTESTINAL PEPTIDE - DEDICATED TO THE 10TH ANNIVERSARY

Reni Kalfin[1], Maria Lazarova[1], Nina Ivanovska[2], Federica Pessina[3], Giampietro Sgaragli[3], Nilanjana Maulik[4], Dipak Das[4]
[1]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Department of Neuroscience, Siena University, Siena, Italy*
[4]*Department of Surgery, University of Connecticut Health Center, Farmington, USA*

10.00 – 10.30

AO2. CHEMICALLY MODIFIED ALGINIC ACID AND CHITOSAN – SYNTHESIS AND APPLICATIONS

V. Mitova[1], E. Stoyanova[1], T. Tamer[2], A. Omar[2], M. Mohy Eldin[2], N. Koseva[1]
[1]*Institute of Polymers, Bulgarian Academy of Sciences,*
[2]*Polymer Materials Research Department,*
Advanced Technologies and New Materials Research Institute, MUCSAT
New Borg El-Arab City 21934, Alexandria, Egypt

10.30 – 11.00

AO3. ROLE OF THE AMINO GROUP IN THE STRUCTURE AND DESIGN OF POTENTIAL INHIBITORS OF AMINOPEPTIDASE A

V. Petrova[1], T. Aleksandrova[2], M. Dimitrova[1], V. Pavlova[1], D. Tasheva[2], I. Iliev[1], V. Mitev[3], I. Ivanov[3]

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

[2]University of Sofia "St. Kl. Ohridski", Faculty of Chemistry and Pharmacy, Bulgaria

[3]Department of Medical Chemistry and Biochemistry, Medical University of Sofia, Bulgaria

11.00 – 11.20 COFFEE BREAK

11.20 – 11.50

AO4. *HABERLEA RHODOPENSIS* METHANOL EXTRACTS REVITALIZE YEAST CELLS

Milena Georgieva[1], Dessislava Staneva[1], Daniela Moyankova[2], Dimitar Djilianov[2] and George Miloshev[1]

[1]Laboratory of Yeast Molecular Genetics, Institute of Molecular Biology "Roumen Tzanev", Bulgarian Academy of Sciences, Bulgaria

[2]Abiotic stress, Agrobioinstitute, Sofia, Bulgaria

11.50 – 12.05

AO5. ЦИКЛООКСИГЕНАЗНИ ИНХИБИТОРИ – ОТ ФАРМАКОЛОГИЯТА ДО КЛИНИЧНИТЕ ПРАКТИКИ

Лора Дякова[1], Радостина Александрова[2]

[1]Институт по невробиология, Българска академия на науките

[2]Институт по експериментална морфология, патология и антропология с музей, Българска академия на науките

12.05-12.20

AO6. SELECTIVE SILENCING OF GAD65-SPECIFIC B LYMPHOCYTES DELAYS DISEASE ACTIVITY IN MICE WITH STZ – INDUCED T1D

Gabriela Boneva[1, 2], Iliyan Manoylov[1], Andrey Tchorbanov[1]

1. *The Stefan Angelov Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
2. *Sofia university "St KlimentOhridski", Sofia, Bulgaria*

12.20 – 12.35

AO7. SELECTIVE ELIMINATION OF ALLERGEN-SPECIFIC B LYMPHOCYTES WITH CHIMERIC PROTEIN-ENGINEERED MOLECULES

Kiril Valentinov Kolev**,** Nikola Stoyanov Kerekov, Andrey Ivanov Tchorbanov
Laboratory of Experimental Immunology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

12.35 – 13.00

Poster presentation and Discussion

13.00-14.00 LUNCH TIME

Session B.

Chairpersons:

Assoc. Prof. Stefka Valcheva-Kuzmanova, MD, PhD, DSc
Medical University, Varna

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, Bulgarian Academy of Sciences

14.00– 14.30

BO1. БИОЛОГИЧНО АКТИВНИ КОМПОНЕТИ НА РАСТЕНИЕТО АРТИШОК И ПРИЛОЖЕНИЕТО ИМ ВЪВ ВЕТЕРИНАРНАТА И ХУМАННАТА МЕДИЦИНА

Ваня Младенова
Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria

14.30-14.45

BO2. АНТИВИРУСНИ СВОЙСТВА НА МЕДИЦИНСКОТО РАСТЕНИЕ *MELISSA OFFICINALIS*

Мирослава Дерменджиева
Институт по биология и имунология на размножаването „Акад.Кирил Братанов” - БАН

14.45-15.00

BO3. ANTI-VIRAL ACTIVITY OF MEDICAL BULGARIAN PLANTS AGAINS HUMAN HERPES VIRUS TYPE 1 AND 2

Venelin Tsvetkov, Petia Angelova, Kalina Shishkova, Anton Hinkov, Stoyan Shishkov
Laboratory of Virology, Faculty of Biology, University of Sofia ''St. Kl. Ohridski'', Sofia, Bulgaria.

15.00-15.15

BO4. THERAPEUTIC STRATEGY FOR SURVIVAL OF MICE INFECTED WITH INFLUENZA VIRUS BY COMBINATION OF S-ADENOSYL-L-METHIONINE AND OSELTAMIVIR

A. Dimitrova[1], M. Mileva[1], D. Krastev[2], G. Gegova[1], A.S. Galabov[1]
[1]*The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Medical University of Sofia, Medical Colleague "Jordanka Filaretova", Sofia, Bulgaria*

15.15-15.30

B05. АКТИВНОСТ НА HUMAN POLYOMAVIRUS 1 /BK ВИРУС/ ПРИ БЪБРЕЧНО- ТРАНСПЛАНТИРАНИ ПАЦИЕНТИ В БЪЛГАРИЯ

Георги Тошев[1], Златко Кълвачев[2]
Медицински факултет, СЕ „Св. Кл. Охридски", София, България, УМБАЛ „Софиямед"

15.30 – 15.50 COFFEE BREAK

15.50 – 16.05

BO6. ПРИЛОЖЕНИЕ НА НАНОБИОТЕХНОЛОГИИТЕ ПРИ РАЗЛИЧНИ ВИРУСНИ ЗАБОЛЯВАНИЯ

А. Павлова[1], Д. Пенчева[2], П. Генова-Калу[3], Ст. Крумова[3], Т. Кантарджиев[3]
[1]*СУ „Св. Климент Охридски", Биологически факултет,*
[2]*Бул Био – НЦЗПБ, София,*
[3]*Национален Център по Заразни и Паразитни Болести, София*

16.05-16.20

BO7. VITAMIN D DEFICIENCY IS CONSIDERED TO BE A RISK FACTOR FOR MULTIPLE SCLEROSIS

Vera Kolyovska[1], Velichka Pavlova[1], Dimitar Maslarov[2]
[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, 1113 Sofia, E-mail: verakol@abv.bg
[2]Medical University of Sofia, Neurology Clinic, First MHAT-Sofia

16.20-16.35

BO8. VITAMIN D AND AUTOIMMUNE DISEASES

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

16.35-16.50

BO9. FATTY ACID COMPOSITION OF DIFFERENT TYPES FLOUR

S. Ivanova, N. Mihalkova, G. Marinova, V. Batchvarov, P. Parvanova
Institute of Cryobiology and Food Technologies, Sofia, Bulgaria

16.50-17.05

BO10. TRACE ELEMENT COMPOSITION OF CHEESE FROM EWE'S MILK KARAKACHAN BREED RHODOPE TSIGAY AND MIDDLE RHODOPE BREED SHEEP

S. Ivanova[1*], D. Gadjev[2], L. Angelov[1], T. Odjakova[2], B. Blajev[3]
[1] Institute of Cryobiology and Food Technologies, Sofia, Bulgaria
[2]Experimental Station of Stockbreeding and Agriculture- Smolyan, Bulgaria
[3]Central laboratory for chemical testing and control, Sofia, , Bulgaria

17.05-17.20

BO11. POTENTIALLY HARMFUL EFFECTS OF GRAPEFRUIT

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

17.20-17.35

BO12. ЗДРАВОСЛОВНО ХРАНЕНЕ. ХРАНЕНЕ ПРИ ПОЛИКИСТОЗЕН ОВАРИАЛЕН СИНДРОМ

Надежда Стоянова, Стефани Димитрова
Медицински факултет, Медицински университет, София

17.35-17.50

BO13. POLYTETRAFLUOROETHYLENE (TEFLON) AND ITS IMPACT ON PEOPLE, ANIMALS AND ENVIRONMENT

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

17.50 – 18.30 Poster Session and Discussion

Wednesday, 18 November 2015

Session C.

Chairpersons:

Prof. Todor Dudev, PhD
Faculty of Chemistry and Pharmacy, Sofia University "St. Kl. Ohridski"

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

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

9.00-9.30

CO1. GALLIUM AS THERAPEUTIC AGENT: COMPETITION BETWEEN GA^{3+} AND FE^{3+} IN METALLOPROTEINS

Valia Nikolova[1], Silvia Angelova[2], Nikoleta Markova[1], Todor Dudev[1]
[1]Faculty of Chemistry and Pharmacy, Sofia University "St. Kl. Ohridski"
[2]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences

9.30-10.00

CO2. LET US SPEAK ABOUT COPPER

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

10.00 – 10.15

CO3. GOLD COMPOUNDS AS ANTITUMOR AGENTS

Tanya Zhivkova, Radostina Alexandrova
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

10.15 – 10.45

CO4. PROPERTIES OF LANTANIDES COMPLEXES OF MONENSIN

Ahmed Nedzhib, Ivayla Pantcheva
Laboratory of Biocoordination and Bioanalytical Chemistry, Faculty of Chemistry and Pharmacy, "St. Kl. Ohridski" University of Sofia

10.45 – 11.00

CO5. LEAD, CADMIUM AND NICKEL IN HOMEMADE WINE FROM STARA ZAGORA REGION

P. Gidikova, G. Sandeva, R. Deliradeva
Medical Faculty, Trakia University, Stara Zagora, Bulgaria

11.00 – 11.20 COFFEE BREAK

Session D.

Chairpersons:

Assoc. Prof. Anna Tolekova, MD, PhD
Medical Faculty, Trakia University, Stara Zagora, Bulgaria

Assoc. Prof. Boryana Ruseva, MD, PhD
Medical University, Pleven

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

11.20-11.50

DO1. EFFECTS OF CHLOROGENIC ACID, FERULIC ACID, GALLIC ACID AND QUERCETIN ON LEARNING AND MEMORY IN THE ONE-WAY PASSIVE AVOIDANCE TASK IN YOUNG/HEALTHY RATS

S. Valcheva-Kuzmanova[1], A. Georgieva[1], I. Belcheva[2], S. Belcheva[2,3], R. Tashev[2,4]
[1]*Department of Preclinical and Clinical Pharmacology, Medical University Prof. Dr. Paraskev Stoyanov, Varna, Bulgaria*
[2]*Department of Behavior Neurobiology, Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Faculty of Pre-School and Primary School Education, SU "Sv. Kl. Ohridsky",*
[4]*Department of Pathophysiology, Medical University of Sofia, Bulgaria*

11.50 – 12.05

DO2. INTERACTION BETWEEN ANGIOTENSIN II RECEPTORS OR INTERVENTION OF ANGIOTENSIN II DERIVATIVES FOR CONTRACTILE ACTIVITY OF VISCERAL SMOOTH MUSCLES?

P. Hadzhibozheva, A. Tolekova, Ts. Georgiev
Dept. of Physiology, Pathophysiology and Pharmacology, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

12.05 – 12.20

DO3. IN VITRO STUDY OF ROSEMARY OIL EFFECT ON SMOOTH AND STRIATED MUSCLE

Plamen Zagorchev
Department of Biophysics, Faculty of Pharmacy, Medical University-Plovdiv

12.20 – 12.50

DO4. INTERRELATIONS BETWEEN HEPCIDIN AND HOMEOSTASIS OF COPPER AND IRON

Tsvetelina Petkova – Marinova[1], Boryana Ruseva[1], Bisera Atanasova[2]
[1]*Department of Physiology, Medical University – Pleven*
[2]*University Hospital "Alexandrovska", Department of Clinical and Immunological Laboratory, Medical University – Sofia*

12.50 – 14.00 LUNCH TIME

14.00-14.30

DO5. APIPUNCTURE OR BEE VENOM IN ACUPUNCTURE-LITERATURE REVIEW

Y. Staykova-Pirovska[1], N.Pirovski[2], N.Dimitrov[2]
Medical Faculty, Trakia University, 11 Arsenalska str., 6000 Stara Zagora, Bulgaria

Session E.

Chairpersons:

Prof. Ivo Grabchev, PhD
Faculty of Medicine, Sofia University "St. Kl. Ohridski"

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

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

14.30-15.00

EO1. MODIFIED WITH 1,8-NAPHTHALIMIDE METALLODENDRIMERS AS ANTIMICROBIAL AGENTS

Ivo Grabchev[1], Desislava Staneva[2], Evgenia Vasileva-Tonkova[3], Paula Bosch[4]

[1]Sofia University "St. Kliment Ohridski", Faculty of Medicine, Sofia, Bulgaria
[2]University of Chemical Technology and Metallurgy, Sofia, Bulgaria
[3]Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[4] Institute of Science and Technology of Polymers, CSIC, Madrid, Spain

15.00-15.30

EO2. ANTIMICROBIAL ACTIIVITY OF SYNERGISTIC COMBINATIONS OF BIOLOGICALLY ACTIVE COMPOUNDS

I. Lazarkevich, A. Sotirova, T. Avramova, D. Galabova

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

15.30 – 15.50 Coffee Break

15.50 – 16.05

EO3. MECHANISMS OF BACTERIAL COAGGREGATION

Ivo Gantchev

The Stephan Angelov Institute of Microbiology, Bulgarian Academy of Sciences Sofia, Bulgaria

16.05-16.20

EO4. COAGGREGATION BETWEEN *Bacillus subtilis* and *Escherichia coli* K-12 STRAINS

Ivo Gantchev

The Stephan Angelov Institute of Microbiology, Bulgarian Academy of Sciences Sofia, Bulgaria

16.20 – 16.35

EO5. METABOLIC SYNDROME AND CHANGES IN GUT MICROBIOTA

R. Sandeva[1], B. Chakarova[2], G. Sandeva[2], A. Dimitrova[3]

[1]*Trakia University, Medical Faculty, Department of Physiology, Pathophysiology and Pharmacology, Stara Zagora, Bulgaria*
[2]*Trakia University, Medical Faculty, Department of Hygiene, Epidemiology and Infectious Diseases, Stara Zagora, Bulgaria*
[3]*Medical University - Pleven, Department of Physiology and Pathophysiology*,Pleven

16.35 – 17.05

EO6. COMPOSITE MATERIAL COTTON FABRIC-HYDROGEL-NANOPARTICLES WITH POTENTIAL APPLICATION AS WOUND DRESSING

Desislava Staneva[1], Evgenia Vasileva-Tonkova[2], Tatyana Koutzarova[3], Ivo Grabchev[4]

[1]*University of Chemical Technology and Metallurgy, Sofia, Bulgaria*
[2] *Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Institute of Electronics, Academy of Sciences, Sofia, Bulgaria*
[4]*Sofia University St. Kliment Ohridski, Faculty of Medicine, Sofia, Bulgaria*

17.05-18.00 Poster Session and Discussion

Thursday, 19 November 2015

Session F.

Chairpersons:

Assoc. Prof. Diana Rabadjieva, PhD
Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences

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

Secretary: Abdulkadir Abudallech, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

9.00 – 9.15

FO1. HYDROGEL WOUND DRESSINGS

B. Yerusalimova, E. Vassileva

Laboratory on Structure and Properties of Polymers, Faculty of Chemistry and Pharmacy, Sofia University "St. Kl. Ohridski", Sofia, Bulgaria

9.15 – 9.45

FO2. INNOVATIVE NEW MATERIALS FOR WOUND HEALING

Radostina Alexandrova[1], Orlin Alexandrov[2]

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

[2]*Health Service, Gorna Malina, Bulgaria*

9.45-10.15

FO3. BRIEF OVERVIEW OF NEW MATERIALS FOR BONE IMPLANTS

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

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

[2]*Institute of Neurobiology, Bulgarian Academy of Sciences*

[3]*Health Service, Gorna Malina, Bulgaria*

[4]*Tomas Bata University in Zlin, Czech Republic*

10.15 – 10.30

FO4. BRUSHITE BONE CEMENTS BASED ON AMORPHOUS CALCIUM PHOSPHATE AND TARTARIC ACID

D. Rabadjieva[1], S. Tepavitcharova[1], R.Ilieva[1], R. Gergulova[1], K. Sezanova[1], A. A. Apostolov[2]
[1]*Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Faculty of Chemistry and Pharmacy, Laboratory on Polymers, Sofia University, Sofia, Bulgaria*

10.30 – 11.00

FO6. ANTIOXIDANT STATUS AND HISTOLOGICAL STUDIES AFTER IMPLANTATION OF MODIFIED HYDROXYAPATITE IN RAT CALVARIA

Vasileva R.[1], E. Dyulgerova[1], R. Ilieva[2], M. Gabrashanska[3], M. Alexandrov[3], V. Nanev[3], I. Vladov[3], N. Tsocheva-Gaytandzhieva[3], P. Dimitrov[3]
[1]*Medical University, Faculty of Dental Medicine, Sofia, Bulgaria*
[2]*Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*

11.00 – 11.20 COFFEE BREAK

11.20-11.35

FO7. PHOSPHORILATED CHITOSAN: SYNTHESIS AND BIOMEDICAL APPLICATIONS

R. Rikova, E. Vassileva
Laboratory on Structure and Properties of Polymers, Faculty of Chemistry and Pharmacy, Sofia University "St. Kl. Ohridski", Sofia, Bulgaria

11.35 – 11.50

FO8. MEDICAL ADVANCEMENTS IN THE TREATMENT OF DIABETES MELLITUS TYPE 1

Plamen Slavov, Kalin Stoyanov
Medical Faculty, Sofia University "St. Kl. Ohridski"

Session G.

Chairpersons:

Assoc. Prof. Julia Radenkova-Saeva, MD, PhD
Clinic of Toxicology, Department for Adult, Emergency University Hospital "N.I.Pirogov"

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

Secretary: Delka Salkova, DVM, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

11.50 - 12.20

GO1. AN EPIDEMIOLOGICAL STUDY OF ACUTE POISONING BY PHARMACEUTICAL AGENTS WITH FATAL OUTCOME

Radenkova – Saeva J.
Toxicology Clinic, Emergency University Hospital «Pirogov», Sofia, Bulgaria

12.20-12.35

GO2. ПИЛОТНО ПРОУЧВАНЕ СРЕД ПАЦИЕНТИ НА ПЕРОРАЛНА АНТИКОАГУЛАНТНА ТЕРАПИЯ ОТНОСНО ТЯХНАТА ИНФОРМИРАНОСТ ЗА БЕЗОПАСНО И ЕФИКАСНО ПРОВЕЖДАНЕ НА ЛЕЧЕНИЕТО

Сияна Георгиева[1], Георги Христов[1], Георги Семовски[1], Татяна Димитрова[1], Алия Сабах[1], Павлина Андреева- Гатева[1,2]
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[2]Медицински университет – София, Медицински факултет

12.35-13.30 LUNCH TIME

13.30-14.00

GO3. *IN VIVO* ANTITUMOR EFFECT OF THE NOVEL ALKYLPHOSPHOCHOLINE ERUFOSINE APPLIED ALONE OR IN COMBINATION WITH DOXORUBICIN AGAINST GRAFFI MYELOID TUMOR IN HAMSTERS

Ani Georgieva[1], Reneta Toshkova[1], Veselina Uzunova[2], Martin R. Berger[3], Rumiana Tzoneva[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

14.00 – 14.15

GO4. IS SUGAR ACTUALLY TOXIC? CUTTING OUT SUGAR DRASTICALLY IMPROVES OVERALL HEALTH

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

14.15-14.45

GO5. ASSESMENT OF THE HEAVY METAL POLLUTION LONG-TERM EFFECTS ON POPULATION GENETIC STRUCTURE OF COMMON DANDELION *TARAXACUM OFFICINALE*

Borislava Kukurina, George Miloshev
Laboratory of Molecular Genetics, Institute of Molecular Biology, BAS, Sofia, Bulgaria

15.45 -15.00

GO6. GENOTOXIC POTENTIAL OF SOIL SAMPLES COLLECTED IN THE REGION OF KCM PLOVDIV

Zhana Mitrovska,[1], Daniela Miteva[1], Radostina Hristova[1], Stephka Chankova[1], Nadezhda Yurina[2]
[1]*Institute of Biodiversity and Ecosystem Research, BAS, Sofia*
[2]*Bach Institute of Biochemistry, Russian Academy of Sciences, Moscow*

15.00 – 15.30

GO7. HONEY BEES AND THEIR PRODUCTS AS INDICATORS OF ENVIRONMENTAL POLLUTION WITH PESTICIDES

D. S. Salkova, M. S. Panayotova-Pencheva
Institute of experimental morphology, pathology and anthropology with museum, Bulgarian Academy of Sciences

15.30 – 15.50 COFFEE BREAK

15.50-16.05

GO8. ОТРАВЯНЕ С ТЕЖКИ МЕТАЛИ

Благовеста Георгиева[1], Алексей Митев[2], Анри Аструг[1]
[1]*Фармацевтичен факултет, Медицински университет;* [2]*Медицински факултет, Медицински университет, София*

16.05-16.20

GO9. ANTIOXIDANTS AS NOVEL POTENTIAL THERAPY AGAINST MEHG-INDUCED NEUROTOXICITY

Vladimir P. Veselinov, Radoslav F. Todorov
Faculty of Medicine, Sofia University "St. Kl. Ohridski", Sofia, Bulgaria

GO10. ВЛИЯНИЕТО НА УОЗ ПЕСТИЦИДИ ВЪРХУ ЗДРАВЕТО НА ЖИВИТЕ ОРГАНИЗМИ. ОЦЕНКА, АНАЛИЗ И УПРАВЛЕНИЕ НА РИСКА ОТ ТЯХНОТО ЗАМЪРСЯВАНЕ

Юлия Караиванова
Институт по биология и имунология на размножаването „Акад. К. Братанов”, БАН, София

16.20-16.50
Poster Session and Discussion

16.50-17.00 Closing Remarks

Posters

BP1. КАКВО (НЕ) ЗНАЕМ ЗА КАРДАМОНА?

Абдулкадир Абудаллех и ученици
ИЕМПАМ-БАН

CP1. THE MANY FACES OF GOLD COMPOUNDS

Radostina I. Alexandrova[1], Tanya Zhivkova[1], Abdulkadir M. Abudalleh[1], Lora Dyakova[2], Gabriela Marinescu[3], Daniela-Cristina Culita[3], Luminita Patron[3]
[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Institute of Physical Chemistry, Romanian Academy, Bucharest, Romania*

CP2. METAL (ZN/AG, ZN/AU) COMPLEXES WITH SCHIFF BASES: INITIAL STUDY OF POTENTIAL ANTIHERPESVIRAL ACTIVITY

Abedulkadir Abudalleh[1], Tanya Zhivkova[1], Lora Dyakova[2], Petya Genova-Kalou[3], Konstantin Simeonov[4], Gabriela Marinescu[5], Daniela-Cristina Culita[5], Luminita Patron[5], Radostina Alexandrova[1]
[1]*Institute of 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 Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria*
[4]*National Diagnostic and Research Veterinary Medical Institute, Sofia, Bulgaria*
[5] *Institute of Physical Chemistry "Ilie Murgulescu", Bucharest, Romania*

FP1. VITAMIN A AND WOUND HEALING

Abedulkadir Abudalleh[1], Desislav Dinev[1], Boyka Andonova-Lilova[1], Tanya Zhivkova[1], Lora Dyakova[2], Radostina Alexandrova[1]
[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

FP2. VITAMIN C AND WOUND HEALING

Tanya Zhivkova[1], Abedulkadir Abudalleh[1], Desislav Dinev[1], Boyka Andonova-Lilova[1], Lora Dyakova[2], Radostina Alexandrova[1].
[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

FP3. VITAMIN E AND WOUND HEALING

Lora Dyakova[1], Tanya Zhivkova[2], Boyka Andonova-Lilova[2], Abedulkadir Abudalleh[2], Desislav Dinev[2], Radostina Alexandrova[2]

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

FP4. VITAMIN K AND WOUND HEALING

Boyka Andonova-Lilova[1], Lora Dyakova[2], Tanya Zhivkova[1], Abedulkadir Abudalleh[1], Desislav Dinev[1], Radostina Alexandrova[1]

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[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

FP5. SILVER AND WOUND HEALING

Tanya Zhivkova[1], Abedulkadir Abudalleh[1], Desislav Dinev[1], Boyka Andonova-Lilova[1], Lora Dyakova[2], Radostina Alexandrova[1]

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[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

FP6. ZN AND WOUND HEALING

Lora Dyakova[1], Boyka Andonova-Lilova[2], Tanya Zhivkova[2], Abedulkadir Abudalleh[2], Desislav Dinev[2], Radostina Alexandrova[2]

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

FP7. HONEY AND WOUND HEALING

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

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

[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

FP8. AMORPHOUS CALCIUM PHOSPHATE AND VIABILITY AND PROLIFERATION OF MOUSE EMBRYONAL FIBROBLASTS.

B. Andonova-Lilova[1], A. Abudalleh[1], T. Zhivkova[1], L. Dyakova[2], D. Rabadjieva[3], S. Tepavitcharova[3], R. Alexandrova[1]

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[2]*Institute of Neurobiology, Bulgarian Academy of Sciences*

[3]*Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*

FP9. IN VITRO BIOCOMPATIBILITY ASSESSMENT OF NEW MATERIALS FOR BONE IMPLANTS: SOME FACTORS THAT CAN INFLUENCE CELL VIABILITY AND GROWTH.

R. Alexandrova[1], B. Andonova-Lilova[1], A. Abudalleh[1], T. Zhivkova[1], L. Dyakova[2], D. Rabadjieva[1], S. Tepavitcharova[3]

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GP1. ЛЕКАРСТВЕНИ ВЗАИМОДЕЙСТВИЯ НА ЧЕСТО СРЕЩАНИ ВЕЩЕСТВА

Ангелина Банковска
Медицински факултет, СУ „Св. Кл. Охридски”, София, България

Session A.

Chairpersons:

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

Assoc. Prof. Neli Koseva, PhD
Institute of Polymers, Bulgarian Academy of Sciences

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

AO1. AN OVERVIEW ON THE PROTECTIVE ROLE OF VASOACTIVE INTESTINAL PEPTIDE - DEDICATED TO THE 10TH ANNIVERSARY

Reni Kalfin[1], Maria Lazarova[1], Nina Ivanovska[2], Federica Pessina[3], Giampietro Sgaragli[3], Nilanjana Maulik[4], Dipak Das[4]
[1]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Department of Neuroscience, Siena University, Siena, Italy*
[4]*Department of Surgery, University of Connecticut Health Center, Farmington, USA*

AO2. CHEMICALLY MODIFIED ALGINIC ACID AND CHITOSAN – SYNTHESIS AND APPLICATIONS

V. Mitova[1], E. Stoyanova[1], T. Tamer[2], A. Omar[2], M. Mohy Eldin[2], N. Koseva[1]
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Abstract

Alginic acid and chitosan are natural polymers widely investigated for various biomedical applications due to their inherent properties such as biocompatibility, excellent biodegradability, low toxicity, as well as abundant availability and considerably low production cost. Since last two decades, increasing attention has been focussed on delivery systems derived from their polyelectrolyte complexes, formed via electrostatic interactions between the two oppositely charged polysaccharides. In order to enhance specific properties methods for their chemical modifications have been developed.

The article briefly reviews specific methods for introduction of additional functional groups – phosphate groups in the alginic acid and additional amino groups in chitosan. Changes in the properties as a result of the modification are also discussed. Examples of oral drug delivery systems are also presented.

Key words: alginate, chitosan, phosphorylation, amination, polyelectrolyte complex, drug delivery

1. Introduction

Alginic acid belongs to the family of polysaccharides. It is a linear copolymer composed of two monomer units – saccharide epimers, namely β-D-mannuronic acid (M) and α-L-guluronic acid (G) coupled through (1-4)-glycosidic linkages. The monomers are arranged in homopolymeric sequences G-blocks (..GG..) and M-blocks (..MM..) or in heteropolymeric MG-blocks with alternating M and G-residues (..MGMG..). The polyacid has a pKa around 3.5 depending on the content of M and G blocks [1]. Alginic acid, also called alginate, is found in the cell walls of brown algae. Industrially, it is also produced by two bacterial genera *Pseudomonas* and *Azotobacter*.

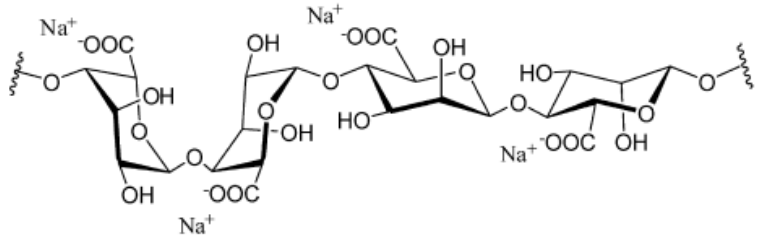

Fig. 1. Structure of a sodium alginate fragment.

Modern tendencies in biomaterials design are focused on mimicking many functions of the extracellular matrices of body tissues. Materials derived from natural polymers have recently been regaining attention due to their inherent biocompatibility. Alginate has been extensively investigated and used for many biomedical applications, due to its biocompatibility, low toxicity, relatively low cost, and mild gelation by addition of divalent cations such as Ca^{2+} [2]. Therefore, alginates are typically used in the form of hydrogels including wound healing, drug delivery and tissue engineering applications.

Alginate hydrogels can be prepared by various cross-linking methods. The most common one among them is the ionic cross-linking method. Calcium chloride ($CaCl_2$) is the frequently used agent to obtain ionically cross-linked alginate. The guluronate blocks allow a high degree of coordination of the divalent ions which favours the formation of junctions between the guluronate fragments of different alginate chains. This interaction is termed the egg-box model of cross-linking resulting in a gel structure [3].

Alginate gels have been investigated for the delivery of a variety of low molecular weight drugs. Typically, alginate gels possess nanoporous structure with pore size about 5 nm [4] which causes rapid diffusion of small molecules out of the gel. Therefore, chemical modifications have been used to introduce new functions and control properties of the alginate-based materials.

Alginate has also been widely exploited in drug delivery applications in combination with oppositely charged synthetic and natural polymers. Among polysaccharides, chitosan is a cationic polymer, derivative of chitin which is the second most abundant natural polymer in the world. Usually, commercially available chitosan is composed of 80% β-D-glucosamine

and 20% N-acetyl-β-D-glucosamine [5]. Chitosan has a pKa value of 6.1–6.5 depending on the degree of deacetylation and its molecular weight. Due to its biocompatibility, chitosan has been widely used in the areas of food industry, cosmetics, medicine and pharmacy [6].

Chitosan exhibits antimicrobial activity against a wide range of microorganisms. Being a positively charged molecule, its target is the negatively charged cell wall of bacteria, where it binds and disrupts its normal functions [7, 8].

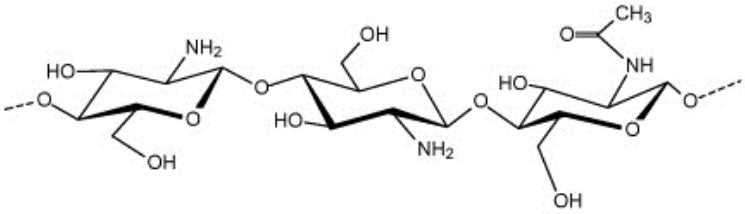

Fig. 2. Chemical structure of chitosan.

2. Chemical modification of alginate – introducing phosphate groups

Alginate-based biomaterials have been designed for use in cell encapsulation, drug delivery, wound healing and tissue engineering. The variety of applications has induced research on property enhancement and introduction of new functionalities through chemical modification. Coleman et al. [9] prepared phosphorylated alginate derivatives (PAlg) using the urea/phosphate method, which had been applied to cellulose phosphorylation [10]. The reagents used in the reaction were urea and phosphoric acid (Fig. 3). Adduct was formed due to strong hydrogen-bonding which catalyzed the condensation of phosphoric acid into ammonium polyphosphates followed by phosphorylation of the alginate. The degree of substitution (DS) was defined as the mole fraction of the phosphorylated alginate subunits. DS from 5 to 26% was achieved depending on the amount of urea and phosphoric acid added in the reaction. The molecular weight of the phosphorylated product was reduced by a factor of 2-4 while the molar-mass dispersity was increased. A detailed NMR investigation provided data that phosphorylation occurred mainly at the equatorial hydroxyl groups of mannuronic units (M3 site) and to a smaller degree at the axial hydroxyl groups (M2 site) being more sterically hindered. Hydroxyl groups of guluronic units also underwent conversion but the NMR data did not evidenced regioselectivity for the G2/G3 sites.

Applying the same procedure we have performed phosphorylation of alginate which was lyophilized prior modification. Depending on the reagents ratio the phosphorus content (determined by inductively coupled plasma optical-emission spectroscopy) varied from 5.5 w% to 16 w%, i.e. degrees of substitution from 10 to 30 mol%. It was observed that the products with the highest degree of substitution contained about a 34 w% insoluble fraction.

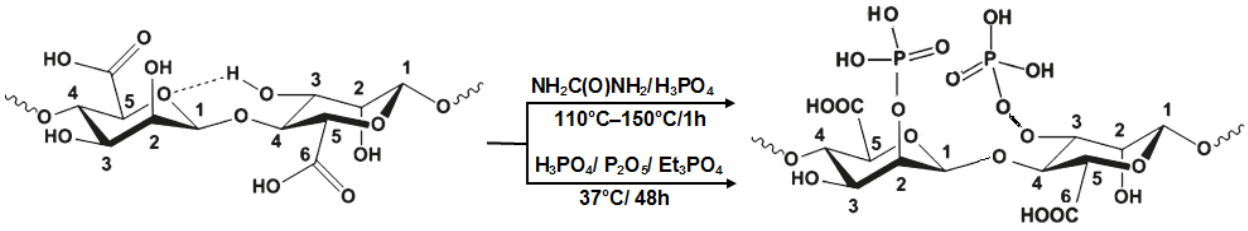

Fig. 3. Phosphorylation reaction conditions and possible sites for modification of the mannuronate block (MM) in the alginic acid macromolecule.

The phosphorylated alginates did not produce cross-linked beads upon extrusion of their 3 w/v% solutions into a 0.1 M $CaCl_2$ solution [9]. This behavior was attributed to the reduced molecular mass of the modified polymer and conformational changes due to introduction of the phosphate groups. Nevertheless, ionically cross-linked hydrogel beads were prepared when blends of PAlg and alginate (in ratios from 1:1 to 5:1, respectively) were extruded into a 0.1 M $CaCl_2$ solution. Moreover, The Ca-Alg/PAlg beads displayed higher stability toward calcium extraction which implied that the phosphate functions of the modified polymer participated in the inter-chain cross-linking. In vitro mineralization of Ca-Alg and Ca-Alg/PAlg capsules was tested in simulated body fluid (SBF), however, mineralization was not induced. The capsules displayed increase in calcium content after immersion in a $Ca(OH)_2$ solution. This pretreatment facilitated the mineralization at the surface of the Ca-Alg beads. The ratio calcium to phosphorus was close to the mineral deposit consistent with the formation of hydroxyapatite.

Kim et al. [11] applied modified approach for phosphorylation of alginate. In order to avoid the elevated temperatures and degradation of the macromolecules as a consequence of that, another set of reagents $H_3PO_4/P_2O_5/Et_3PO_4$ was used in the phosphorylation procedure. The reaction was performed at 37 °C. Degree of substitution of 12.5 mol% for PAlg was achieved. Comparing the three available methods for phosphorylation of polysaccharides applying three different combinations of phosphorylating agents H_3PO_4/urea, $P_2O_5/MeSO_3H$ [12] and $H_3PO_4/Et_3PO_4/P_2O_5$, the latter one is carried out under milder conditions yielding less degraded product with satisfactory degree of substitution. Phosphorylated alginic acid calcium complex (CaPAlg) was prepared by dissolving of PAlg powder in a 1.0 N $Ca(OAc)_2$ solution at pH ~ 8 followed by dialyses and freeze-drying. A DS value of 112.5 mol% of Ca ion for the CaPAlg complex was found, i.e. approximately 1.13 mol of Ca element per 1 mol of the cyclic monosaccharide units. These results evidenced that all carboxylate and phosphate functional groups were involved in salt formation without any obvious gelation and the calcium ions were evenly distributed along the polysaccharide chain. The phosphorylated alginic acid calcium complex CaPAlg was used in gel formation with sodium alginate as an alternative approach to the traditional method using $CaCl_2$ as cross-linking agent. The optimum results were obtained from the CaPAlg/NaAlg combination in ratio 40:60 showing effective gelation within 3–10 min. Moreover, cell culture assay revealed that CaPAlg hydrogels provide favorable microenvironment for 3 day encapsulation of cells. These experimental facts supported the assumption that the CaPAlg complexes would be potentially suitable for the preparation of injectable hydrogels.

Phosphorylated alginate membranes demonstrated great potential in another interesting application - dehydration of various solvents. A membrane obtained from sodium alginate by casting and drying, followed by crosslinking with phosphoric acid was suitable for separation of ethanol–water mixtures at 30°C. [13]. Due to their good affinity towards water molecules, polysaccharides are promising materials for dehydration membranes. Alginate membranes have gained special attention as they demonstrated the highest flux and separation factor among the hydrophilic materials tested for dehydration of solvents by pervaporation technique [14].

3. Chemical modification of chitosan – introducing of additional amino groups

The antimicrobial activity of chitosan increases with decreasing of pH [8, 15, 16] which is due to the fact that its amino groups become ionized at pH below 6. The antimicrobial activity of chitosan rises also with increasing degree of deacetylation which is a consequence of the increased number of ionisable amino groups [17].

Mohy Eldin et al. [18] proposed a method for introducing additional amino groups into the backbone of chitin, affording chitosan with high antibacterial activity. In the first step chitin was modified using parabenzoquinone (pBQ) as activation agent and ethylene diamine (EDA) as source of amino groups. The aminated chitin was treated with 40 % aqueous solution of NaOH at 120 °C -150 °C to obtain chitosan with higher content of amino groups (Fig. 4).

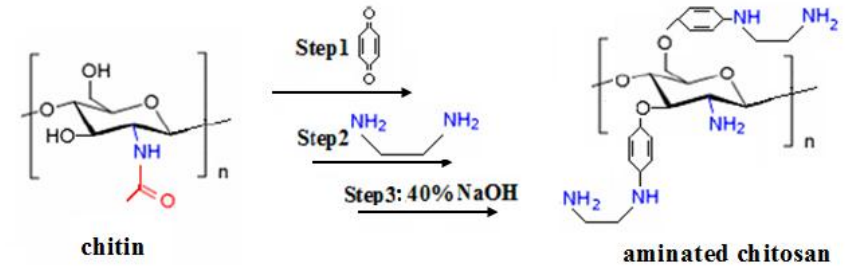

Fig. 4. Reaction steps for introduction of additional amino groups in chitosan.

The antibacterial activity of the modified chitosan was tested on four different bacterial strains: two of them were gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*), and other two – gram positive (*Bacillus cereus*, *Staphylococcus aureus*) [18]. It was found that the antimicrobial activity of chitosan was dependent on the degree of grafting, degree of deacetylation, the molecular weight and the pH of the tested media. Similarly to the unmodified chitosan, the modified one exhibited bactericidal activity against all of the treated strains examined, especially on the gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*). The antibacterial activity enhanced with increasing the number of introduced amino groups. According to the cytotoxicity assay, the toxicity of aminated chitosan was negligible. Modification improved also the solubility of the polymer at pH ranging from 5 to 6.

4. Interpolymer complexes between alginate and chitosan and their applications

Ionic interactions between the anionic carboxyl residues of alginate and the cationic amino groups of chitosan lead to polyelectrolyte complex (PEC) formation, schematically presented in Fig. 5. In the last decade, different PEC forms based on alginate and chitosan have been studied as carriers for proteins and drugs [19]. The interaction between alginate and chitosan is known to be pH-dependent and strong complexes are obtained at pH around 4.0–6.0. Using phosphorylated alginate it was possible to broaden the acidic pH region (pH<4.0) in which stable interpolymer complex with chitosan was formed. The pH sensitivity of alginate/chitosan PEC has been studied for the development of oral delivery of low molecular drugs, proteins or peptides [20].

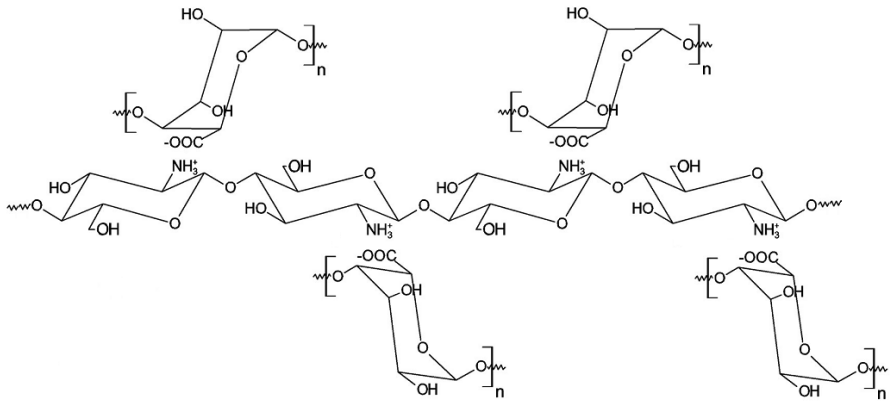

Fig. 5. Ionic interaction between alginate and chitosan

Metronidazole was entrapped into chitosan-treated alginate beads via an ionotropic gelation method, and the resulting beads were effective in eradication of *Helicobacter pylori* when orally administrated into mice [21].

Abdelbary et al. [22] demonstrated that chitosan interpolymer complexes with sodium alginate allowed a more extended release of nicorandil, with respect to those observed with each polymer alone. The addition of Imwitor_900 K, a monoester rich glyceryl stearate, allowed obtaining a prolonged release over 8 h.

The drug β-lapachone (β-lap) represents a powerful antineoplastic agent yet with limited pharmaceutical use. In view of overcoming its limitations, controlled delivery systems of β-lap in simulated gastric fluids *in vitro* from chitosan (CS) and alginate (ALG) hydrogel beads with purpose for oral administration were investigated. The ALG-CS hydrogel beads were obtained by coacervation with sizes of approximately 1 mm, and demonstrated good stability and low porosity. The *in vitro* drug release profile was with low burst effect, especially in an acid medium, allowing a prolonged release of ~ 72 h. The beads were resistant to the acid medium and might be considered for development of β-lap therapy of colorectal cancer [23].

One of the most important characteristics of alginate/chitosan polyelectrolyte complex is the swelling behaviour which always affects the drug release profile from loaded systems. We prepared alginate beads coated with polyelectrolyte complex by immersing the preformed ionically cross-linked ALG beads into solutions of chitosan or aminated chitosan (ACS). After drying the swelling profile and equilibrium degree of swelling of the three types of beads were compared at different pH of the solution. It was observed that polyelectrolyte coating influenced the degree of swelling and the effect of the aminated chitosan was more expressed when ASC solutions with concentration below 1% were used for the beads coating. The data for the swelling degree of non-coated beads and treated with 0.25% solution of chitosan or aminated chitosan are presented in Fig. 6.

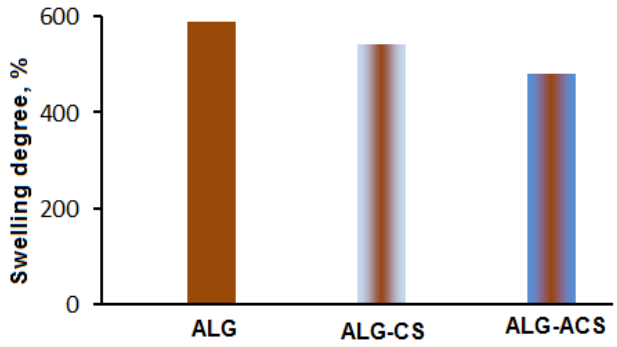

Fig. 6. Degree of swelling of non-coated alginate beads (ALG) and coated with polyelectrolyte complex formed in 0.25% solution of chitosan (ALG-CS) or aminated chitosan (ALG-ACS) at pH 5 and 37 °C.

The swelling behaviour of chitosan grafted alginate (CS-g-ALG) hydrogel microcapsules was evaluated under physiological conditions and compared with the alginate-chitosan mixed polyelectrolyte complex (PEC) [24]. The mechanism of pH-sensitive swelling involves the protonation of amine groups of chitosan under low pH and deprotonation of carboxyl groups of alginate at high pH. The sensitivity of beads toward swelling depended on many factors: chitosan concentration and molecular weight, pH and temperature of the medium. It was observed that the highest values of the equilibrium swelling degree for grafted and mixed beads 120% and 100%, respectively, were obtained at 0.3 % CS and pH 6.8. The beads preserved their shape for 6 h from the initial swelling time and then all beads started to disintegrate.

5. Conclusions

Polysaccharides have been extensively studied and widely used for biomedical applications, such as tissue engineering, drug and protein encapsulation and delivery. Both chemical and physical methods have been developed to modify their structure and properties with the aim to broaden the range of biomaterials derived from polysaccharides. Alginate and chitosan, and combinations of them are particularly appropriate for the design of oral delivery systems based on their biocompatibility, biodegradability, pH sensitiveness, mucoadhesiveness, etc. Detailed studies on the formation and properties of the alginate–chitosan complexes provide information to facilitate the large-scale production of microcapsules/beads as drug or peptide oral delivery systems.

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AO3. ROLE OF THE AMINO GROUP IN THE STRUCTURE AND DESIGN OF POTENTIAL INHIBITORS OF AMINOPEPTIDASE A

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Aminopeptidase A (Glutamyl aminopeptidase, APA; EC 3.4.11.7) is a zinc-dependent membrane-bound peptidase of the M1 family, which catalyzes the cleavage of glutamic or aspartic amino acid residues from the N-terminus of polypeptides. It is activated by Ca^{2+} and inhibited by chelating agents and heavy metals such as Pb^{2+}. The enzyme is a part of renin angiotensin system (RAS). It degrades angiotensin II (AngII) into angiotensin III (AngIII), a substance which has been recently proposed to be the main effector of the brain RAS in the regulation of vasopressin release. Thereby, APA participates in the control of blood pressure. Furthermore, the enzyme is involved in blood vessels formation, and is a putative target for angiogenesis in cancer.

Specific APA inhibitors are regarded as potential central antihypertensive agents as an alternative of the current treatments based on the suppression of AngII formation by angiotensin-converting enzyme (ACE, EC 3.4.15.1) inhibitors.

Recently, the synthesis of a novel class of APA inhibitors - β-aminotiols, was described. Two of those compounds - (*S*)-4-amino-5-mercaptopentanoic acid (**1**) and (*S*)-3-amino-4-mercaptobutane-1-sulfonic acid (**2**) - are effective ($K_i \leq 0.1$ μM) inhibitors of the enzyme. However, the expensive multistep synthesis of (**1**) and (**2**) limits their application in practice. This is a prerequisite for further studies on the design of new specific inhibitors of APA.

The purpose of this work was to determine the importance of amino group in the structure of potential APA inhibitors representing compounds similar to (**1**) and (**2**) in terms of their effectiveness. For this purpose, two compounds were synthesized - 3-((diethylcarbamothioyl)thio)propanoic acid (**3**) and 2-(4-carboxybutyl)isothiouronium

chloride (**4**). In our experiments, the substance (**3**) did not inhibit the enzyme, whereas (**4**) was a weak APA inhibitor with K_i> 0.01 M. These results point out the central role of NH_2-group in the structure of this type of inhibitors in terms of effectiveness of inhibiting the enzyme. Furthermore, binding of (**2)** and (**4)** in the active site of APA was modeled by the methods of molecular mechanics. The results proved that NH_2-group plays a crucial role for the inhibitor binding and correct orientation in the enzyme active center.

In conclusion, the above findings about the crucial role of amino group in the structure of potential APA inhibitors, give valuable information for the design and development of new inhibitors of the enzyme.

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AO4. *HABERLEA RHODOPENSIS* METHANOL EXTRACTS REVITALIZE YEAST CELLS

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Abstract

The endemic plant *Haberlea rhodopensis* is known for its ability to withstand drought and to revitalize when returned to optimal conditions after a long time in desiccation. It is a mere fact that this plant not only can completely resurrect from a dried state but is also able to bring back the natural biochemical compositions of its cells. Therefore, *H. rhodopensis* offers interesting possibilities for investigation of the exact mechanisms of the revitalization process. Moreover, this plant creates broad horizons for search of unique bioactive chemical substances in its cells.

Here, by using the yeast *Saccharomyces cerevisiae* as a model we have demonstrated that methanol extracts from the plant *H. rhodopensis* possess specific properties to revitalize and ameliorate cellular growth as well as to balance intracellular metabolic states. Our results add valuable knowledge on the effects of natural compounds on ageing and reinforce the idea of using yeast as a model organism in the development of rapid tests for studying the efficacy of different bioactive substances.

Keywords: resurrection plant, *Haberlea rhodopensis*, *Saccharomyces cerevisiae*, ageing, cellular vitality, metabolism, model organism

АО5. ЦИКЛООКСИГЕНАЗНИ ИНХИБИТОРИ – ОТ ФАРМАКОЛОГИЯТА ДО КЛИНИЧНИТЕ ПРАКТИКИ

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Откриването на аспирина от Феликс Хофман през 1899 г. поставя началото на най-широко използваната и до днес група лекарства- нестероидните противовъзпалителни средства (НПВС). Широкият спектър на действие на тези лекарствени средства ги прави подходящи за лечението на редица патофизиологични състояния- профилактика на сърдечно-съдови заболявания, облекчаване на болката, в резултат на редица възпалителни процеси и дегенеративни ставни заболявания. Механизмът на действие на НПВС става известен чак през 1971 г., когато Д. Вейн открива общата мишена на тези лекарства- ензимът циклооксигеназа (COX). Нещо повече, противовъзпалителният им ефект се дължи на понижаване на активността на този ензим, а от там и потискане синтезата на медиаторите на ексудативното възпаление. По-късно става ясно, че ензимът има две изоформи- COX 1 (конститутивна оксигеназа, участваща в редица физиологични процеси) и COX 2 (индуцируема оксигеназа, чиято концентрация нараства в резултат на възпалителна реакция, онкогенни и митогенни стимули). Откриването на COX 2 през 1990 г. води до редица лабораторни изследвания, които доказват, че действието на тези средства се дължи основно на потискането активността на тази изоформа, а страничните ефекти- основно от потискане активността на COX 1. Това води до появата на пазара на лекарства, чиято мишена е единствено COX 2 (celecoxib, rofecoxib, valdecoxib, lumiracoxib и др.). Наличието на кардиоваскуларни странични ефекти при употребата на коксибите е причина голяма част от тях да бъдат свалени от пазара. Все още се търси връзката на традиционните НСПВ (ибупрофен, аспирин, наприксен и др.) и рискът от сърдечно-съдови странични ефекти.

Различният механизъм на действие на парацетамола в сравнение с останалите НСПВ води до откриването на COX 3 през 2002 г. от Симънс и сътр. Все още се знае малко за тази изоформа на циклооксигеназите. Известно е, че се кодира от същия ген, който кодира COX 1 (PTGS1), но продуктът му не е функционално активен при човека.

Въпреки нежеланите странични ефекти и борбата на учените да се справят с тях, НСПВ остават едни от най-широко използваните лекарствени средства в световен мащаб. Все още се търси ролята на тези лекарства в профилактиката на сърдечно-съдовите заболявания и болестта на Алцхаймер. В последните десетилетия интересът на учените е насочен и към тяхната потенциална антитуморна активност.

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AO6. SELECTIVE SILENCING OF GAD65-SPECIFIC B LYMPHOCYTES DELAYS DISEASE ACTIVITY IN MICE WITH STZ – INDUCED T1D

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Autoimmune disease develops when the immune system attacks self-antigens. Type 1 diabetes (T1D) is associated with pancreatic β cell destruction as a result of infiltration of activated immune cells. Self-specific B cells play a main role in the disease pathogenesis by generation of autoantibodies as well as by serving as important autoantigen-presenting cells. B lymphocytes also modulate T cell mediated immune responses[1] and are one of the major contributors for T1D. Therefore, targeting autoreactive B cells can be used as apotential treatment to prevent the disease onset.

The co-crosslinking of BCR with FcγRIIb (the IgG-inhibitory receptor), regulates negatively BCR signaling and inhibits BCR-induced cellular proliferation and other downstream biological responses through immunoreceptor tyrosine-based inhibition motifs (ITIMs) in the cytoplasmic tail[2]. Thus, FcγRIIb is an attractive target for down-regulation of autoimmunity.

GAD65(glutamic acid decarboxylase) is an enzyme required for the synthesis of γ-aminobutyric acid and is a key antigen in T1D. We designed a chimeric molecule using antibody against FcγRIIb, conjugated with copies of a peptide containing epitopes of GAD65. The chimeric antibody should bind specifically to the inhibitory receptor on B lymphocyte's surface leading to cell suppression.

Treating rodents with multiple low doses of streptozotocin (STZ) is a model for studying T1D disease development[3]. STZ treatment induces production of antibodies against nucleic acids and insulin in healthy Balb/c mice[4]. We are going to test the constructed chimeric antibody on STZ – treated Balb\c mice and determine its efficacy. Hence, we intend to examine the presence of antibodies against GAD65 in mice sera. Pancreatic tissue from the experimental mice will be used for histochemistry and immunohistochemistry, where we are going to observe infiltration of mononuclear cells and β cells death.

Our expectations are amelioration of disease symptoms like decrease in the levels of anti-GAD65 antibodies, stabilization of glucose levels in the blood and reduction in pancreatic islets destruction.

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AO7. SELECTIVE ELIMINATION OF ALLERGEN-SPECIFIC B LYMPHOCYTES WITH CHIMERIC PROTEIN-ENGINEERED MOLECULES

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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 murine B cells in mouse model of allergy by administration of chimeric molecule, containing a monoclonal antibody against the inhibitory B-cell receptor FcγRIIb coupled to a B and a T cell epitopes from the Der p1 allergen. Co-crosslinking of the immunoglobulin receptors and FcγRIIb by this molecule is expected to deliver higher affinity and suppressive signal selectively silencing these B cells and the subsequent allergic response.

Methods: A synthetic peptide, Der p1 p52-71 and anti- mouse FcγRIIb monoclonal antibody will be used for the construction of Der p1 chimera. We have already analysed the effects of the chimeric molecule *in vitro* and *in vivo* - against human B-cells using PBMC from allergy patients; Der p1-specific IgE and IgG antibody production by ELISA; B-cell proliferation by ELISpot; apoptosis by flow cytometry using AnnexinV-FITC/PI staining. We intend to perform the same group of experiments in mouse model of house dust allergy.

Conclusion: These results from human model bring us up the idea to examine the effect of the chimera in murine model where the whole set of lymphocytes are available and we can monitor more specifically the allergic response. The constructed chimeric molecule could bind Der p1 specific B-lymphocytes and could suppress their proliferation and production of anti-Der p1 IgE antibodies.

Session B.

Chairpersons:

Assoc. Prof. Stefka Valcheva-Kuzmanova, MD, PhD, DSc
Medical University, Varna

Assoc. Prof. Radostina Alexandrova, PhD
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ВО1. БИОЛОГИЧНО АКТИВНИ КОМПОНЕТИ НА РАСТЕНИЕ АРТИШОК И ПРИЛОЖЕНИЕТО ИМ В ВЕТЕРИНАРНА И ХУМАННА МЕДИЦИНА

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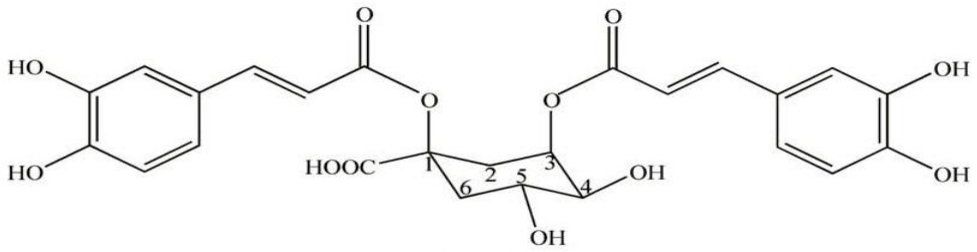
Cynara scolymus / артишок/ , принадлежащ към семейство Сложноцветни, има вид на неразцъфнала цветна пъпка на бодливо растение. Артишокът е използван още от древни времена за лечение на чернодробни и хранителни заболявания при хора и животни. Ядлива част, която се използва в кулинарията, са главите, а за производство на хранителни добавки и екстракти се използват листата и стъблата (1,7,8).
При изследванията на химичния състав е доказано, че артишока има богат набор от биологичноактивни компоненти - феноли, флавоноиди, витамини и минерали(2,3, Табл.1).

Polyphenol(mg/100 g edible portion)	Raw		Cooked	
	Mean	SD	Mean	SD
Chlorogenic acid	276·1	30·9	307·5	10·3
Total monocaffeoylquinic acids	16·9	1·0	66·7	0·6
Cynarin	0·0	0·0	18·3	0·5
Total dicaffeoylquinic acids	309·7	21·8	396·3	8·4
Luteolin-glycoside	7·5	0·2	7·9	0·3
Apigenin-glycoside	10·0	0·7	9·8	0·1

Таблица 1. Избрани полифеноли за анализ на сурови и варени глави от Cynara scolymus L (сорт Violetto di Provenza) (7)

Цинаринът е едно от основните и уникални химични вещества на артишока, като по-високата му концентрация се определя в листата на растението(4). Неговата химична структура е 1,3-О-дикафеоилхинова киселина, която притежава изразени

антиоксидантни свойства. Хепатопротективните свойства на артишока се дължат предимно на цинарина,който има жлъчогонно действие и с това обезпечава по-добро функциониране на черния дроб,тъй като забавянето на отделяне и транспортиране на жлъчката от жлъчния мехур крие риск за увреждане на черния дроб..Жлъчно-стимулиращи вещества като цинарина допринасят за по-ефективното отделяне на токсините от жлъчката (1,13). Хепатопротективният ефект на цинарина се наблюдава при индуцирана токсичност с въглероден тетрахлорид (CCL4) в изолирани хепатоцити на плъх (1,8,фиг. 1).



Cynarin

Фигура 1. Структурата на цинарина(4)

Инулинът е друга неразделна част от състава на растението, това е водоразтворим въглехидрат от групата на фруктаните. В химично отношение инулинът представлява фруктан с линейно полидисперсна въглехидратна част, състоящ се главно, но не само от β(2←1) фруктозил-фруктозни връзки(4,10,11,12). Фруктаните от типа на инулина подпомагат функциите на стомашно-чревния тракт, стимулирайки минералната абсорбция , главно, на калций и магнезий. Инулин-подобните фруктани се усвояват в дебелото черво и по този начин превъзходят повечето диетични фибри. Също така те стимулират отделянето на стомашно-чревни пептиди,които помагат за регулирането на липидния метаболизъм и подобряването на чревната микрофлора. Изследванията при човека показват, че инулинът има много добър пробиотичен ефект, което намалява риска от канцерогенеза на дебелото черво(11).

Други фенолни съединения на артишока, които са от голямо значение за медицината поради антиоксидантните си свойства, са апигенин, лутеолин , различни позиции на изомери на кафеевата киселина и естери на хининовата киселина. Действието на лутеолина е да защитава липопротеините с ниска плътност от окисление,(5,6). Екстрактите от артишока имат благоприятен ефект върху сърдечно-съдовите заболявания , провокирани от високите нива на холестерол. В опитите с хепатоцитите на плъх е доказано, че прилагането на големи дози от тези екстракти води до инхибиране на биосинтезата на холестерол (4).

Освен това , артишокът съдържа витамини-А,Е,К, С, В1, В2,В3 ,Р и минерали-желязо, селен, магнезий и цинк.

Всяко растение има своя уникална комбинация от биологично активни компоненти, които са полезни за организма и се използват от народната медицина за лечение. Артишокът е едно растение с богата комбинация от вещества с полезно действие за различни системи на организма.Терапевтичните качества на растението се дължат не само на едно съединение, а на комбинацията от няколко биологичноактивни компоненти, които се съдържат в него.Благоприятното му действие върху храносмилателния тракт и сърдечно-съдовата система е доказано. Артишокът има жлъчогонно действие, оказва пробиотичен ефект и инхибира холестероловата синтеза. Обаче,вземайки предвид съдържанието на голям набор от силни антиоксиданти в състава му, може да се предположи, че той има ефекти и върху другите системи на

организма. На това предположение се дължи изследователският интерес към растението артишок от страна на биохимици, фармаколози, биолози и диетолози.

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BO2. АНТИВИРУСНИ СВОЙСТВА НА ЛЕЧЕБНОТО РАСТЕНИЕ *MELISSA OFFICINALIS* L.

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Резюме

През последните години силно нарастна търсенето на вещества от растителен произход, които да бъдат успешно прилагани за лечението на различни инфекциозни и неинфекциозни заболявания при животните и човека. *Melissa officinalis* L. или маточина е растение, което е много разпространено в България и има добре известни лечебни свойства. До момента е доказано, че то притежава антивирусна, антигъбна, антибактериална, седативна и др. ефективност. Сред вирусите за които има сведение, че маточината проявява антивирусно действие са *Newcastle disease virus, Vaccinia virus, Herpes simplex virus, Semliki Forest virus* и *Pseudorabies virus*. Към момента изследванията в тази област са насочени към изясняване на механизмът на антивирусно действие на маточината, както и към разработването на лечебни препарати, които да бъдат употребявани с превантивна или терапевтична цел.

Ключови думи: маточина, антивирусен ефект, лечебни растения

Въведение

Вирусите са едни от най-честите причинители на инфекциозни заболявания с различна степен на тежест при човека и животните. Това налага търсенето на ефктивни антивирусни препарати от синтетичен или естествен произход, които да бъдат прилагани с превантивна или терапевтична цел.

Лечебните растения са източник на богат набор от вещества с антивирусни свойства, които освен това имат по-ниска степен на токсичност и развитие на резистентност в сравнение с синтетичните препарати [9,24].

Маточината (*Melissa officinalis* L., lemon balm) е обект на изледване на редица изследователи тъй като има многостранно позитивно действие върху здравето на човека и животните. Доказано е, че маточината има антигъбно, антибактериално, седативно и антиспастично действие [6,11,15]. Алкохолният екстракт на маточината има и *in vitro* антимутагенен ефект [22]. Въпреки, че е добре позната като лечебно растение натрупването на познания за ползите и рисковете от употребата ѝ подължават [5].

Едно от най-важните биологични свойства на маточината е антивирусната и активност. Антивирусната активност на водните екстракти от маточина срещу *Herpes simplex virus* и *Vaccinia virus* е била установена още през 1965 година от Kucera et al [19]. Въз основа на данните за антивирусният ефект на *M. officinalis*, учените смятат, че екстракти от това растение може да послужат като основа за развитие на нови антивирусни препарати [1,6,12,15].

Обши данни за *Melissa officinalis* L.

Добре познато от билковите чайове за масова консумация, това многогодишно тревисто растение има бели цветове и тъмнозелени листа с характерен лимонен аромат, оказва редица ползи върху здравето (Фиг.1). *Melissa officinalis* L. е член на семейство Lamiaceae и произхожда от западна Азия и източното Средиземноморие. В наши дни данни за нейното разпространение има в цял свят - от Северна Америка до Нова

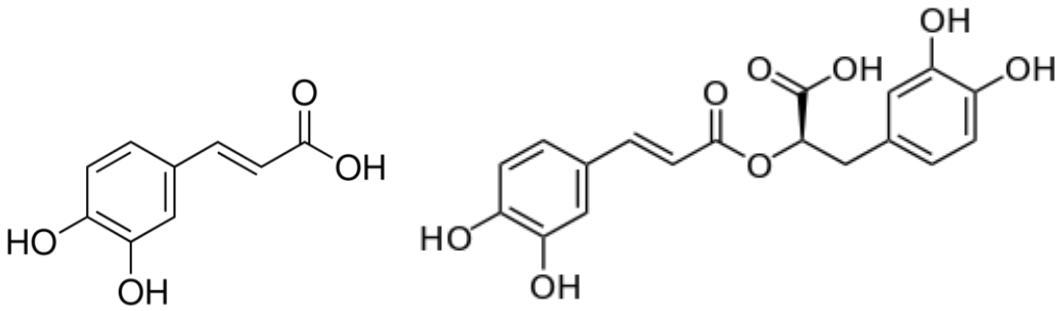
Зеландия [10,28]. Маточината се среща и в България. У нас тя е известна още като маточник, лимонче, пчелник. От медицинско значение са листата и етеричните масла добивани от растението [6];

Фиг.1 *Melissa officinalis* L. Показано е схематично изображение съдържащо всички части на растението и снимка по какъв начин изглежда в природата. (Източници:
http://bilki.bg/userfiles/editor/image/matochina_Melissa-Officinalis.jpg;
www.sector39.co.uk/garden/plants/lemonbalm_600w.jpg)

Основни биологично активни вещества съдържащи се в етеричното масло и екстрактите от *M. officinalis*.

Фитохимичните изследвания върху *M .officinalis* са показали наличието на многобройни съставки, включително полифенолни съединения, етерични масла, дъбилни и слузни вещества, монотерпеноидни алдехиди, сесквитерпени, флавоноиди и танини [13,16,17]. Главните компоненти на етеричното масло са 39% цитронелал, 33% цитрал (цитронелол, линалол) и 2% гераниол [8].

Счита се, че основната роля в антивирусното действие на маточината се дължи на танин-подобните кафеена и розмаринова киселини (Фиг.2) получени от извлеци на това растение [6,11,13]. Според Tóth, J et al. (2003) розмариновата киселина, като активно вещество в маточината, има важна роля при лечение на *Herpes simplex virus* [25].



Фиг.2 Кафеена киселина (ляво) и розмаринова киселина (дясно).(Източници: http://www.mpbio.com/images/product-images/molecular-structure/02104797.png;https://upload.wikimedia.org/wikipedia/commons/thumb/4/4f/Rosmarinic_acid_acsv.svg/320pxRosmarinic_acid_acsv.svg.png)

Антивирусен ефект на маточината

Доказано е, че екстракти от *M. officinalis* имат антивирусен ефект върху *Newcastle disease, Vaccinia virus, Herpes simplex, Semliki Forest virus* и *Pseudorabies virus* [4,12,20,23]. Установено е, че при различни тест-системи (клетъчните култури и оплодени кокоши яйца) водният и алкохолният екстракт от билката имат различен ефект върху вирусите [12,14,19].

Newcastle disease virus , Vaccinia virus и *Semliki Forest virus*

Проучвайки антивирусния ефект на *M. officinalis,* Kucera et al. (1965) установяват, че въвеждането на воден екстракт от маточина в пилешки ембриони, преди заразяване със смъртоносна доза от тези вируси, води до протекция на ембриона. Ефектът обаче бил обратим, ако в пилешките ембриони се инжектира желатин. При прилагане на екстракта върху монослой от пилешки фибробласти, и трите вируса били успешно инактивирани. Според авторите ефектът на екстракта от маточина върху вирусите се дължи на инхибиране на хемаглутинационната им способност. Предполага се, че активният фактор на защита е танин, тъй като вирусите се активизират при прибавяне на желатин или при допълнително разреждане на екстракта, при което антивирусната активност се блокира изцяло. Авторите заключват, че освен неутрализационна активност в процеса на антивирусна защита на екстракта от маточина важна роля има и повърхността на клетката гостопиемник [19].

Herpes simplex virus

Кожните инфекции причинени от *Herpes simplex virus* 1 (HSV-1) и *Herpes simplex virus* 2 (HSV-2) се характеризират с болезнено парене съпроводено със сърбеж, както и характерни струпвания на везикули по различни части на тялото - устните, устната лигавица и гениталната област [24]. Има данни, че тежки форми на инфекция като кератоконюнктивити и енцефалити причинени от HSV са наблюдавани при пациенти с намален клетъчен имунитет, реципиенти с трансплантация на костен мозък или пациенти с придобит синдром на имунна недостатъчност [3]. След първоначалното инфектиране с вирусът на *Herpes simplex* тип 1, той достига до нервните ганглийни клетки и остава там в летално състояние. Смята се, че различни фактори отслабващи

защитните сили на организма като например треска, стрес, умора, менструация или излагане на ултравиолетови лъчи могат да доведат до реактивация на инфекцията на първоначалното място [3]. Заразяването с HSV-2 при хората, е причина за неонатални и фатални инфекции като менингит и рак на маточната шийка [20].

Нуклеозидният аналог ацикловир (ACV) е сред най-разпространените препарати за лечение на инфекции предизвикани от HSV. Поради нарастващата устойчивост и развитие на резистентност към лекарства от този тип, има все по-голямата нужда от разработване на нови терапевтични средства за лечение и превенция от HSV инфекции [3].

Множество експерименти с *M. officinalis* показват, че екстрактите от това растение притежават антивирусна активност срещу HSV [4,14,23].

Растението под формата на кремове за локално приложение има много добър лечебен ефект при системно третиране срещу херпес лабиалис. При 5-дневно третиране с крем с 1:70 екстракт от листата на маточина се отчита незначителна тенденция при облекчаването на симптомите в полза на активното лечение [18]. В друго проучване на пациенти с остра изява на симптоми на HSV, обаче, по-продължителното лечение (5-10 дни) със същия крем е имало "много добър" лечебен ефект [26,27].

При проучване антивирусният ефект на воден екстракт от *M. officinalis* срещу HSV-1, Astani et al. (2012) също установяват висока антивирусна активност на екстракта, дори при много ниски концентрации (1.5 μg/ml), като подобен ефект е бил наблюдаван и при самостоятелно третиране с фенолни съединения, получени от същото растение, но в 100 кратно по големи дози [7].

Механизмът на действие, който използват антивирусните лекарства основно зависи от тяхната способност да инхибират вирус-специфични ензими, тимидин киназа, и ДНК полимераза [19]. Екстрактът от маточина инхибира намножаването на HSV-1 в *in vitro* условия, но неговата антивирусна дейност при HSV-2 още не е напълно дефинирана [20].

Pseudorabies virus (PsRV)

Pseudorabies virus принадлежи към семейството Herpesviridae Основен гостоприемник са свинете, а като вторични гостоприемници се явяват много други видове селскостопански, домашни и диви животни, като коне, крави, овце, кози, кучета, котки и др. Хората и човекоподобните маймуни са неподатливи [21]. Вирусът причинява болестта на Ауески, известна още като лъжлив бяс. Освен, че е остропротичащо заразно заболяване, което уврежда централната нервна система болестта води и до тежки икономически загуби в свиневъдството. При експерименти проведени *in vitro,* с възприемчиви към вируса клетъчни линии от говежди и заешки бъбрек е установено, че вирусната репликация се повлиява при директно третиране на *PsRV* с екстракта, преди заразяване на клетъчните култури едновременното добавяне на *PsRV* и екстракт към клетъчните култури, води до блокиране на вирусната адсорбция [2]. Същият екип установява и добра преживяемост на свински ооцити след третиране с различни концентрации на воден екстракт от маточина [2].

Заключение

Натрупаните литературни данни свързани с *M. officinalis* все още не са достатъчни както по отношение на конкретния начин на действие, така и по отношение на възможността за използване на екстрактите и етеричното масло от *M. officinalis* срещу

други вируси. Допълнителни проучвания фокусирани върху създаването на по-ефективни лекарства чрез комбиниране на етеричните масла с вече познатите антивирусни препарати би увеличило техния положителен ефект върху човешкото здраве.

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BO3. ANTI-VIRAL ACTIVITY OF MEDICAL BULGARIAN PLANTS AGAINS HUMAN HERPES VIRUS TYPE 1 AND 2

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Human Herpes Virus (HHV) type 1 and 2 are cause of hidden pandemics in global scale, as well as sever clinical symptoms associated with active replication in the human host. For ethological treatment of those diseases are used different drugs, mostly used are nucleoside analogues. As until now there are 11 license anti-herpes drugs. Most of them are based on acyclovir and his derivative. Although the large number of available drugs, their usage leads to development of drug resistance strains and also to unwanted side effects. On the other side of the coin are the plant based drug products. The benefits of their usage are shown in higher tolerance from the side of organism, combined with lower cytotoxicity.
In the current report we present summarized overview of our results connected with established anti-herpes effect of isolated extracts from representatives of *Genera*: *Lamiaceae*, *Asteraceae, Adoxaceae* and *Gesneriaceae*. The plant extracts are obtained from in vivo and in

vitro cultured plants, using different extraction techniques. We established their antiviral and cytotoxic activity. For that purpose we used MDBK (cell line). For visualization of the results we used number of methods, which includes direct contact assay, modified MTT assay for cytotoxicity and inhibiting concentration 50 (IC50). Obtained data shows the potential of some of the extracts to be taken under further examination, in conjunction with their medical application. Gained results are due to long term working by the team of laboratory of Virology, department of Biology, Sofia university.

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BO4. THERAPEUTIC STRATEGY FOR SURVIVAL OF MICE INFECTED WITH INFLUENZA VIRUS BY COMBINATION OF S-ADENOSYL-L-METHIONINE AND OSELTAMIVIR

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Influenza is a highly contagious viral infection of the respiratory system. Many studies provide compelling evidence that the excessive production of reactive oxygen species are crucial mediators of the acute lung injury in influenza A virus infection.. Therefore antioxidants are potentially useful against this ongoing clinical problem. Our studies show that S-adenosyl-L-methionine (SAM) has a protective effect in a model of influenza infection in mice. This substance converts in glutathione - the main antioxidant in the body, through multistep biochemical cycle. In the present study, we report the effect of combined treatment with SAM and the antiviral agent oseltamivir. SAM was given as a single daily doses of 50 and 100 mg/kg in different mice groups starting from 5 days before infection until day 4 after infection. Oseltamivir was given twice at daily dose of 1.25mg/kg for 5 days, starting from 4 h before infection. End-point evaluation was 14 day survival. Survival was 70% with Oseltamivir and raised to 90% with oseltamivir and SAM in both of doses. SAM alone does not show any antiviral activity. The present findings suggest that therapy with molecules converted in antioxidants in the body increases survival by modulating the host defense mechanisms, and by a direct antioxidant effect against oxidative stress associated with viral infections. This study demonstrated the effectiveness of combining agents that act through different mechanisms - antiviral drug oseltamivir as specific NA infibitor of influenza virus, and SAM as precursor of most important antioxidant glutathione.

B05. АКТИВНОСТ НА HUMAN POLYOMAVIRUS 1 /BK ВИРУС/ ПРИ БЪБРЕЧНО- ТРАНСПЛАНТИРАНИ ПАЦИЕНТИ В БЪЛГАРИЯ

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BO6. ПРИЛОЖЕНИЕ НА НАНОБИОТЕХНОЛОГИИТЕ ПРИ РАЗЛИЧНИ ВИРУСНИ ЗАБОЛЯВАНИЯ

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Резюме
Нанотехнологиите са приложими и притежават висок биологичен потенциал, който е полезен в лабораторната диагностика на редица заболявания, включително и вирусни инфекции. Честата използване на лекарствени препарати и страничните ефекти, които се получават след продължителната им употреба могат да бъдат занижени драстично чрез поставянето на активен биоагент в съответния орган в не по-висока от нужната доза. Потенциално това може да се постигне чрез прилагане на молекулно прицелване чрез наноустройства. Изследванията и приложенията се различават в голяма степен, вариращи от конвенционалната физика до съвсем нови подходи, базирани на молекулно самосъбиране, от развитие на нови материали с размери в наноскалата до директен контрол на материята на атомно ниво. Наносензорите са новият похват за детекция на потенциални биотерористични инфекциозни агенти. Разработват се спектроскопски изследвания с висока чувствителност и специфичност за бърза детекция на следи от вируси, базирани на рамановата спектроскопия, използвайки сребърни наносонди, които значително увеличават сигнала.
Контролът на вирусни инфекции като HBV, HIV и др. е предизвикателна задача, особено в развиващите се страни, където е ограничен достъпът до ранно диагностициране и ефективно антивирусно лечение, поради високите цени и неефикасно здравеопазване. Въпреки че настоящите диагностични технологии могат надеждно да детектират HBV, HIV и др. те са относително трудоемки, непрактични и изискват скъпи ресурси. Предимствата на нанотехнологиите са пионерни за развитието на нова генерация лабораторни методологии за диагностицирането редица вирусни заболявания. Чрез използването на миниатюрни сензори, благодарение на комбинацията от наноматериали (метални/неорганични наночастици, въглеродни нанотуби и др.) и технологиите на микро/нанониво, би могла да се реализира ранната диагностика на HBV и други вируси от много малък обем кръв, серум или плазма.

Нанотехнологията може да създаде много нови материали и устройства с много приложения както в медицината, така и в електрониката, продукцията на енергия и др. От друга страна обаче, нанотехнологиите повдигат много теми относно токсичността, влиянието на наноматериалите върху природата и техния потенциален ефект за икономиката.

Ключови думи: нанобиотехнологии, вирусни заболявания, ранна диагностика, приложение

BO7. VITAMIN D DEFICIENCY IS CONSIDERED TO BE A RISK FACTOR FOR MULTIPLE SCLEROSIS

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Abstract

Vitamin D deficiency (VDD) is believed to be a global epidemic. Vitamin D for humans is obtained from sun exposure, food and supplements. Humans with multiple sclerosis (MS) and other autoimmune deceases have a higher Vitamin D deficiency. Preliminary evidence suggests that persons with high circulating levels of vitamin D are at lower risk of MS, thus, vitamin D supplementation may reduce the risk of developing MS, may also reduce the relapse rate among patients with relapsing-remitting MS. VDD has increasingly been diagnosed in patients and consequently vitamin D supplementation has been prescribed. Low vitamin D may be associated with clinical MS breakthrough within 2–3 years. Associations were detected in a large international sample of patients with MS between latitude, deliberate sun exposure and vitamin D supplementation and health outcomes including disability, relapse rate and quality of life. Vitamin D is likely to have a pivotal role in these associations. Its role in MS health outcomes urgently requires detailed exploration with well-designed clinical trials.

Key words: vitamin D, vitamin D deficiency, sun exposure, multiple sclerosis

In October at the Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) 2015 in Barcelona few publications about Vitamin D deficiency and low sunlight exposure have been presented.

Multiple sclerosis (MS) is a progressive neurodegenerative disease with a complex aetiology. MS is a familial disease, and genetic background plays a significant role in disease development, although of lesser importance than environmental and lifestyle factors. Previous research has examined potential environmental and lifestyle risk factors for MS disease development and progression. The longest studied of these factors has been the association of increasing latitude with MS incidence [4].

The researchers detected significant associations between latitude, deliberate sun exposure and vitamin D supplementation and health outcomes of this large group of people with MS.

Vitamin D is likely to have a key role in these associations and its role in the health outcomes of people with MS urgently requires further study [4].

Multiple sclerosis (MS) is the eighth most common neurologic disorder in Europe, affecting more than 500,000 people, most of them young adults aged 20 to 40 years, and costing the economy some 14.6 billion euros (that's $16.45 billion) each year. MS is also the second leading cause of disability in Europe, but as traffic accidents become less common in an ever more safety-conscious society, it's now the leading cause of disability in some countries, Xavier Montalban, MD, PhD, professor, neurology, Autonomous University of Barcelona, Spain, and president, European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), told a press conference factors influencing risk for MS that will be discussed during the congress include:

- Low sunlight exposure and vitamin D deficiency;
- Viral infections;
- Hygiene;
- Salt intake;
- Cigarette smoking;
- Individual gut microbiome;
- Intake of fatty acids [1].

Vitamin D was named in 1922 by American biochemist Elmer McCollum (1879 - 1967), who performed experiments to understand the contents of fish liver oil. It was named “D” because it was the fourth substance he identified.

Vitamin D is a group of fat-soluble secosteroids responsible for enhancing intestinal absorption of calcium and phosphorus in our bones and aid in cell to cell communication throughout the body. Five forms of vitamin D have been discovered, vitamin D_1 - D_5. In humans, the most important compounds in this group are vitamin D_3 (also known as cholecalciferol) and vitamin D_2 (ergocalciferol). Cholecalciferol and ergocalciferol can be ingested from the diet and from supplements. The body can also synthesize vitamin D (specifically cholecalciferol) in the skin, from cholesterol, when sun exposure is adequate (hence its nickname the "sunshine vitamin"). Vitamin D_3 is made in the skin when 7-dehydrocholesterol reacts with ultraviolet light at 270-300 nm wavelengths - peak vitamin D_3 production occurs between 295-297 nm. It is only when the UV index is greater than 3 that these UVB wavelengths are present [5].

Vitamin D inhibits the development of autoimmune diseases such as diabetes, rheumatoid arthritis, lupus and multiple sclerosis. Vitamin D for humans is obtained from sun exposure, food and supplements. Preliminary evidence suggests that persons with high circulating levels of vitamin D are at lower risk of MS, thus, vitamin D supplementation may reduce the risk of developing MS, may also reduce the relapse rate among patients with relapsing-remitting MS. The results of previous studies suggested that MS risk is related to vitamin D status at different ages, possibly starting in utero and extending through early childhood, adolescence and adult life. Independent data may argue for potential additional mechanisms associated with a 25-OH-D decrease immediately prior to disease manifestation. Here are referred evidence for the relationship between sun exposure, vitamin D, and the data of MRI in patients with multiple sclerosis.

Vitamin D deficiency is often misdiagnosed as fibromyalgia. Higher 25-OH-D serum levels were reported with lower risk to develop MS later in life [5].

Results from epidemiological and clinical studies clearly suggest that changes in vitamin D serum concentrations are correlated with the magnitude of the risk of developing MS, the phases of relapsing-remitting MS and with gender differences in vitamin D metabolism. Experimental and clinical studies also have established that 25-hydroxy vitamin

D (25(OH)D) and 1,25-dihydroxy vitamin D (1,25(OH)2D) exert an immunomodulatory effect in the central nervous system and peripheral organs of the immune system [5].

The scientists investigate both 25-OH-D serum levels and Immunoglobulin G (IgG) response against Epstein-Barr virus (EBV) before the first clinical MS manifestation in individuals who had donated blood prior to disease onset [5].

In some studies when considering vitamin D as a key environmental factors were not taken into account or excluded the effect of other proven risk factors (infection with the EBV, smoking). In the study of insolation is important to remember that the relationship between the amount of vitamin D formed and the level of insolation is not direct, it contributes to the presence of clothing, use of sunscreens, skin type and color, as well as the time of day. In addition, there are indications that the insolation has independent immunomodulatory effect of vitamin D. Thus further studies on possible interactions between different environmental factors and these factors' role in the disease pathogenesis are justified and necessary [5].

The association between Body Mass Index (BMI), sunlight and age at onset supports the theory that childhood and adolescence could be a particularly vulnerable period in the development of MS. Moreover, the scientists suggest that sunlight avoidance and being overweight trigger the disease [3].

Although several vitamin D-associated environmental and genetic factors were included in the study, none were associated with the age of onset of MS. The findings suggest that the protective effects of Ultraviolet B (UVB) light and the harmful effects of adipose tissue may be independent and not necessarily related to vitamin D. Both types of vitamin D supplements, 25(OH)D and 1,25$(OH)_2$D, demonstrated anti-inflammatory immune response modulation, noted the investigators. In addition, recent mouse studies have suggested the importance of vitamin D in de- and remyelination [3].

UVB irradiation is also immunosuppressive, and works indirectly via vitamin D–induced immunomodulation, and through both systemic mechanisms and local signaling pathways in the skin. This finding parallels the recent EnvIMS case control study in a Norwegian population that showed an association between infrequent summer outdoor activities in adolescence and increased risk of MS [3].

Also, results from an epidemiologic study of French farmers has shown that UVB exposure was markedly associated with MS prevalence, and also supports the hypothesis of an influence of sun exposure or vitamin D on the pathogenesis of MS [3].

Vitamin D, which can be obtained through exposure to the sun or fortified foods, plays a role in the pathogenesis of MS. It has been shown to improve physical function and decrease inflammation. Evidence also links vitamin D to cognitive performance in older adults. That vitamin D affects cognition makes some biological sense. We know there are vitamin D receptors in the brain on both animals and humans. This suggests a function in cognition. The low and normal vitamin D groups were similar in terms of marital status, income, and employment level. The researchers noted that both groups were highly educated, with many having at least a college degree [1].

As for lifestyle, those with low vitamin D are engaged in less physical activity than the normal vitamin D group, and they smoked more and drank more alcohol. This low vitamin D group also tended to participate in fewer leisure activities. Cognitive performance and anxiety in MS seem to be affected by low vitamin D level and improve after vitamin D replacement. The things that vitamin D does is increase a number of factors, including growth factors, and one of these is BDNF [brain-derived neurotrophic factor], which we know plays a role in cognition as well as in inflammation. One of the first things patients with MS are told to do in the clinic is start taking vitamin D supplements at a recommended dose of 4000 to 5000 IU a day. For Canadian patients with MS, "there's no point" in even getting serum vitamin D checked because almost all will have low levels [1].

Vitamin D insufficiency is believed to be a worldwide epidemic.

But the Vitamin D toxicity also known as hypervitaminosis D was previously believed to be rare. But with an increase in vitamin D supplementation several cases have been reported in literature. Fat soluble vitamins like Vitamin D, due to their ability to accumulate in the body, have a higher potential for toxicity than water soluble vitamins. The main clinical consequence of vitamin D toxicity is hypercalcemia. Critically discussed is the mechanism of toxicity and hypothesize the possible molecular/metabolic factors which might have been responsible for this non toxic presentation. This case study highlights the fact that physicians need to consider the risk of medication errors while prescribing Vitamin D therapy. Clinical trials to study Vitamin D toxicity in humans is not possible ethically. Thus the evidence base regarding the safety profile of Vitamin D supplementation in humans has been build through case reports. This review of the paradoxical clinico-laboratory manifestation of hypervitaminosis D could possibly contribute to existing literature [2].

Epidemiological data over many years has confirmed the striking latitude gradient of MS incidence, both world-wide and within countries. Australia is a good example, with the incidence of MS varying around seven-fold between cities in northern versus southern Australia. This has long been postulated to be due to sun exposure, and elegant Australian studies have confirmed that both recalled time in the sun, particularly winter sun during childhood and solar skin damage relate inversely to incidence of MS [4].

There has been a long delay in testing the effects of vitamin D supplementation on disease course in MS, presumably due to little commercial incentive to test this non-patentable naturally occurring agent however a number of large international studies are now underway to examine this issue. Trials to date suggest a benefit, with markedly reduced conversion of optic neuritis to MS and fewer lesions when added to existing disease-modifying medication. While the effect is biologically plausible, it is possible that higher serum vitamin D levels are just a marker of increased sun exposure, and it is both the direct effect, and other as yet unknown indirect effects of sun exposure that mediate beneficial immunomodulatory improvements in disease course for MS patients [4].

The researchers sought to shed more light on whether latitude was associated with disease activity not only disease incidence, and whether we could detect a signal of a beneficial effect on disease course and other health outcomes for MS patients who supplemented with vitamin D. Our data showed that disability was indeed related to latitude with increasing disability the further away from the equator people lived, with a 2–3 % increase in odds of a higher disability category for each degree further from the equator. Similarly, there was some association between lower disability and intentional sun exposure and increasing dose of vitamin D supplementation. Taking vitamin D supplements was also associated with around a third lower annualised relapse rate, while increasing latitude was associated with a small increase in the odds of more relapses. Intentional sun exposure was not associated with relapse rate in our sample [4].

Data suggest a complex relationship between the variables of interest of latitude, sun exposure and vitamin D supplementation, and the additional variables controlled for in the study, namely exercise levels and frequency of fish consumption. The latter two are both likely to affect serum vitamin D levels, as well as having associations with the outcome variables of interest in their own right [4].

Therefore, until further high quality evidence is available, clinicians may wish to consider relevant MS guidelines on vitamin D supplementation when making decisions about the care of people with multiple sclerosis. Adequately powered, multi-centre trial with a focus on clinical as well as immunological and MRI outcomes that are meaningful to people with MS, and are able to provide insight into the benefits of vitamin D in people with MS, are still required.

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BO8. VITAMIN D AND AUTOIMMUNE DISEASES

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Vitamin D is a fat-soluble steroide, holesterole derivate, mainly synthetized in skin by UV radiation. Its main forms are vitamin D2 (ergocalciferol) and vitamin D3 (also known as cholecalciferol). It has well-known control function of calcium and phosphate metabolism. But this hormone rather than a vitamin has other roles. It has important place for maintaining homeostasis of organism confirmed by expression of vitamin D receptors (VDR) in different tissues, such as brain, heart, skin, bowel, gonads, prostate, breasts and the immune cells. Hypovitaminosis D is associated with latitude and reduced sun exposure, urban lifestyle, smog and microparticles in air, night work, dark skin. Hypovitaminosis shifts immune answer in direction of Th1, which is present in most autoimmune diseases like MS, rheumatoid arthritis, psoriasis, diabetes type 1. Some studies show that high levels of vitamin D enhance risk of development of asthma and allergies, which are mediated by Th2. In conclusion vitamin D maintain balance between Th1 and Th2, and in this way is important in pathogenesis of autoimmune diseases.

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BO9. FATTY ACID COMPOSITION OF DIFFERENT TYPES FLOUR

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Summary

The objective of the present investigation was to study the fatty acid composition of flour from naked oat variety "Mina", rye, barley and triticale variety "Vihren".

The saturated fatty acids (SFA) of analysed flour has the highest concentration at barley - 48,44 g / 100 g fat and lowest in rye flour- 16,27 g / 100 g fat. The monounsaturated fatty acids (MUFA) on the examined sample have the greatest amount in the naked oat 37,44 g / 100 g fat and lowest in the flour from tritikale- 17,80 g / 100 g of fat, while the content of polyunsaturated fatty acids (PUFA) is highest in rye flour- 65,07 g / 100 g fat and lowest in barley- 29,81 g / 100 g fat. Barley's flour has the highest content of saturated fatty acids compared to the other three types of flour, making it less favorable a healthy perspective when used for animal and human nutrition. The biologically active monounsaturated fatty acids are in the highest concentration in the oat flour. Studied flours are poor of omega-3 fatty acid. The proportion of omega-6 and omega-3 fatty acids is most favorable to the animal and human nutrition with barley flour.

Keywords: flour, oats, rye, barley, triticale, fatty acids

Introduction

The cereals food provide half of the daily energy intake of people in developed countries and about 80% in developing countries.

The cereals foods occupy about 60% of food production and are cheap and accessible source of nutrients. They are the main source of carbohydrates, but contain protein, fat, minerals and vitamins of group B. The total fat in cereals food varies between 1 and 10% depending on the type of culture, variety and growing conditions.

Fatty acid composition of oat it consists of three main fatty acids - palmitic (C16: 0), oleic (C18: 1) and linoleic (C18: 2) which amount exceed of 95% and an insignificantly amount of fatty acids such as stearic (C18: 0), linolenic (C18: 3) etc. (Leonova et al., 2010).

The major fatty acids in rye are representatives of unsaturated - 74.6%, of which 56.5% are of linolenic acid (Body & Hansen, 1978). Rocha et al., established in their research when to combining the flour from corn and rye and the use of yeast that palmitic (C16: 0), oleic (C18: 1) and linoleic (C18: 2) acids are main fatty acids in various combinations for the preparation of bread, such as the content of C18: 2 amounts to 52% of the total fatty acids (Rocha et al., 2012).

Yousef et al. (2012) establishes the following fatty acid composition of barley - saturates 25.03%, of which palmitic-16.72%, lauric - 2.73% and stearic- 1.82% and unsaturated fatty acids 71.06%, which as 49,23% are essential fatty acids.

The fatty acid composition of triticale established by Khan & Eggum (1978), include linoleic (C18: 2) -59.3, oleic (C18: 1) - 15.0 and palmitic (C16: 0)- 18,6 g / 100g fat. Morrison (1977), determine the content of total fat in triticale from 3,2 to 4,6 %, linoleic from 57- 59 g / 100g fat and linolenic from 3- 4 g / 100g fat.

The objective of the present investigation was to study the fatty acid composition of flour from naked oat variety "Mina", rye, barley and triticale variety "Vihren".

Materials and methods

The determining of fatty acid composition are used four types of flour: flour from naked oat variety "Mina" and triticale flour variety "Vihren" received laboratory roller mill with separation of bran part wholegrain rye flour produced by the company ,,Tehra "and wholegrain barley flour produced by" My Organic market ".

The extraction of total lipids was carried out by the method of Bligh&Dyer (1959), by means of methanol and chloroform. The fatty acid methyl esters /FAME/ were analysed with the aid of gas chromatograph Shimadzu-2010 (Kyoto, Japan). The analysis was carried out on capillary column CP7420 (100m x 0,25mm i.d., 0,2µm film, Varian Inc., Palo Alto, CA), with carrier gas- hydrogen and make- up gas- nitrogen. A five step temperature of the furnace was programmed.

Results and Discussion

The fatty acid composition of analysed flour from naked oat variety "Mina", rye, barley and triticale variety "Vihren" is presented mainly of unsaturated fatty acids (Figure 1).

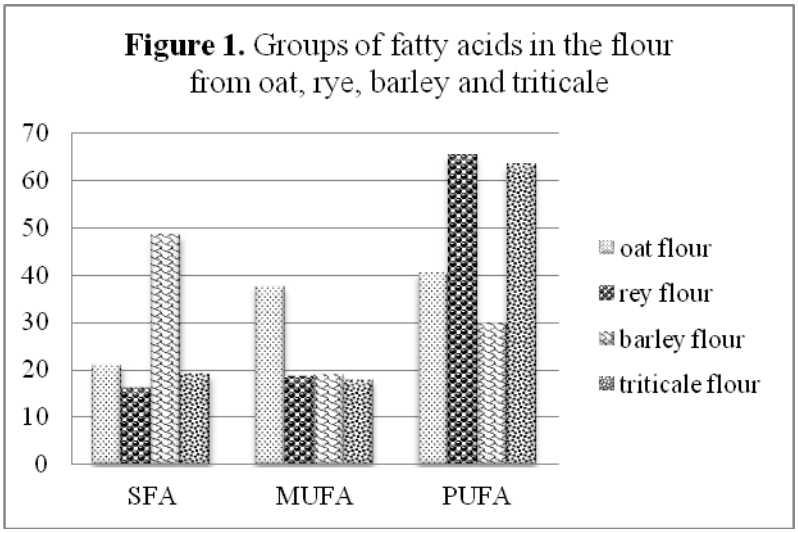

Figure 1. Groups of fatty acids in the flour from oat, rye, barley and triticale

The saturated fatty acids (SFA) from the examined flours have the highest concentration in barley- 48.44 g / 100 g fat and lowest in rye flour-16.27 g / 100 g fat. Monounsaturated fatty acids (MUFA) on the examined samples have the greatest amount in the naked oat 37,44 g / 100 g fat and lowest in the flour from tritikale- 17,80 g / 100 g of fat, while the content of polyunsaturated fatty acids (PUFA) is highest in rye flour- 65,07 g / 100 g fat and lowest in barley- 29,81 g / 100 g fat.

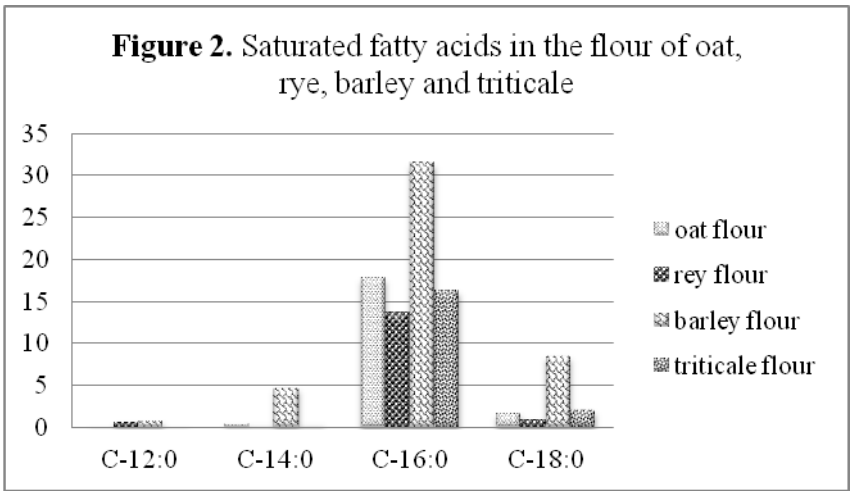

Figure 2. Saturated fatty acids in the flour of oat, rye, barley and triticale

Of the saturated fatty acids, have a portion of palmitic (C16: 0) and stearic (C18: 0) fatty acids and the four types of flour. Palmitic (C16: 0) acid have a highest concentration in the barley flour- 31,44 g / 100g fat and lowest in the rye flour- 13,56 g / 100g fat. Similar is the case in the stearic acid- barley flour- 8,43 g / 100g fat and rye flour- 0,95 g / 100g fat (Figure 2). In the barley flour was detected a higher concentration of myristic (C14: 0) acid- 4.64 g/ 100g fat, while in other types of flour is in the range from 0.31 to 0,41 g / 100g fat. The other representatives of the saturated fatty acids are in quantities below 1 g / 100g fat.

The representatives of monounsaturated fatty acids, which are relevant for human nutrition are oleic acid C18: 1cis9 and vaccenic acid C18: 1trans11. Oleic acid (C18: 1cis9) is in the highest concentration in the oat flour- 34,59 g / 100 g fat, while the flour of triticale is the lowest- 15,67 g / 100 g fat. The flour of rye and barley are similar of content of oleic acid, respectively with a content of 16.31 and 16,41 g / 100 g fat. The amount of vaccenic acid (C18: 1trans11) ranges from 0.02 (oat flour) g / 100 g fat to 0,08 g / 100 g fat (triticale flour).

The total content of cis and trans isomers in the analyzed flour is present in the figure 3. The trans isomers are in highest amount in the rey flour -1,04 g/100g fat and lowest in the barley flour-1,26 g/100g fat, while on the cis isomers was found the highest contents in oat flour 34,60 g/100g fat and lowest in triticale flour-15,78 g/100g fat.

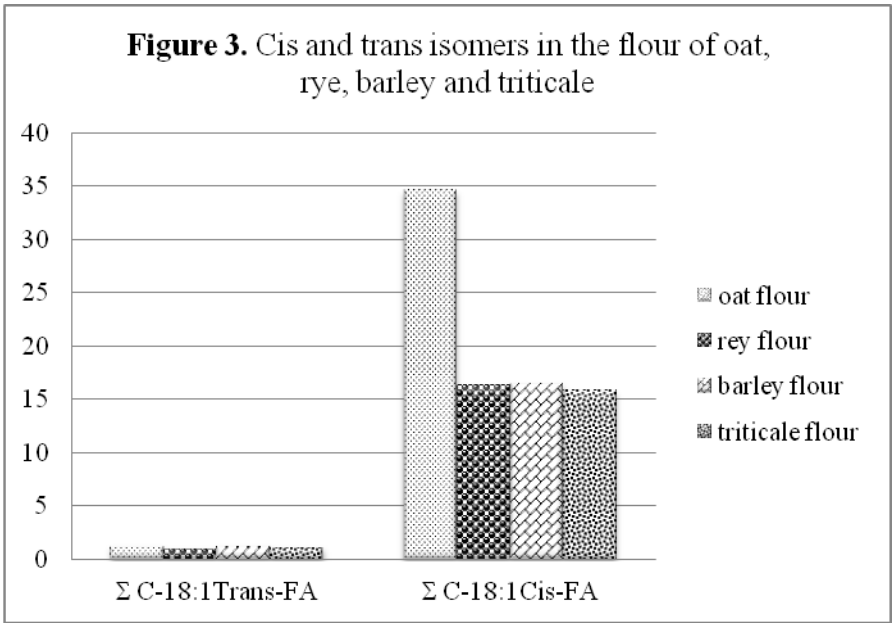

Figure 3. Cis and trans isomers in the flour of oat, rye, barley and triticale

The PUFA in the analyses flour was present mainly of linoleic (C18:2) acid from 21,37 (barley flour) to 58,81 (triticale flour) g/100 g fat and α- linolenic(C18:3) from 1,24 g/100 g fat in the oat to 6,70 g/100 g fat in the barley (Figure 4).

Solely in the rye flour is established the highest content of dihomo- γ- linolenic acid (C20:3n6)- 11,29 g/100 g fat.

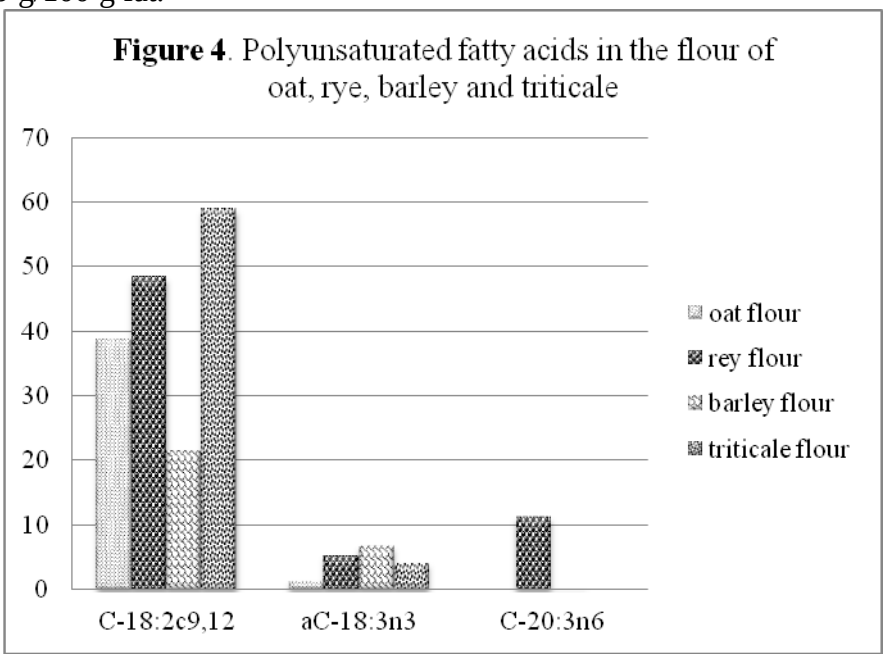

Figure 4. Polyunsaturated fatty acids in the flour of oat, rye, barley and triticale

The total content of the omega-3 fatty acid is lowest in the oat flour- 1,30 g / 100g fat, followed by the tritikale flour - 4,12 g / 100g fat, rye flour- 5,28 g / 100g fat and lowest in barley flour. In terms of the amount of omega-6 fatty acids in the analysed samples of flour rye and triticale have a highest concentration- 59.77 and 59,08 g / 100g fat, in oat flour is 38,91 g / 100g fat and barley flour is the lowest content-22,55 g / 100g fat (figure 5).

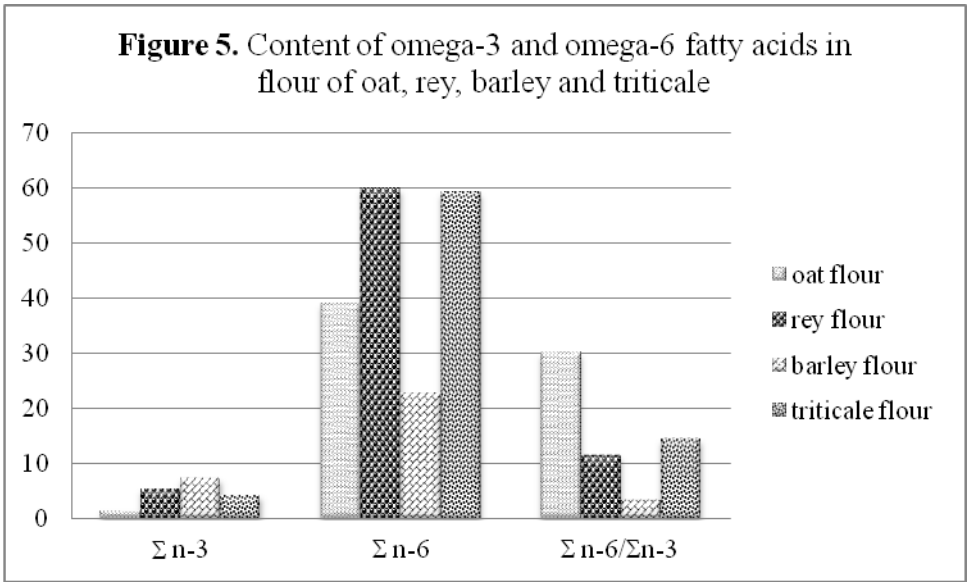

Figure 5. Content of omega-3 and omega-6 fatty acids in flour of oat, rey, barley and triticale

On the base of survey we are found the low content of omega-3 fatty acid in the studied flour, as a result of which the ratio between the two groups of fatty acids - omega-6 and omega-3 is a relatively high coefficient from 11.32 in rye flour to 29.93 in oat flour, with the exception of the flour of barley, wherein the ratio is 3.14.

Conclusions

From studies conducted on the fatty acids composition of oat flour, rye, barley and triticale can made the following conclusions:

- Barley's flour has the highest content of saturated fatty acids compared to the other three types of flour, making it less favorable a healthy perspective when used for animal and human nutrition.
- The biologically active monounsaturated fatty acids are in the highest concentration in the oat flour.
- Studied flours are poor of omega-3 fatty acid. The proportion of omega-6 and omega-3 fatty acids is most favorable to the animal and human nutrition with barley flour.

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BO10. TRACE ELEMENT COMPOSITION OF CHEESE FROM EWE'S MILK KARAKACHAN BREED RHODOPE TSIGAY AND MIDDLE RHODOPE BREED SHEEP

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ABSTRACT

The purpose of this study was to investigate the trace element composition of white brined cheese made from ewe's milk from Karakachan breed, Rhodope Tsigay and Middle Rhodope breed sheep on the pasture grass during the May-July period in the region of Smolyan (Middle Rhodopes).

The cheeses are tested for essential trace elements copper, iron and zinc and ultra trace elements manganese, chromium and strontium. During the lactation concentration of copper is higher in milk from Karakachanian breed (average for the period 0,314 mg/l), iron and zinc in the milk of Rhodope Tsigay (average 1,401 mg/l and 6,248 mg/l), manganese, chromium and strontium in milk from Karakachan breed respectively- 0,173 mg / l, 0,153 mg / l and 1,002 mg / l. In the production of white brined cheese from ewe's milk from three breeds are found the highest concentrations of copper and iron in the breed Rhodope Tsigay (respectively 0,637 mg / kg and 19,667 mg / kg) of iron in the Rhodope Tsigay (3,405 mg / kg), for manganese and strontium in Middle Rhodope breed sheep (respectively 1,053 and 4,860 mg / kg) and chromium in Karakachan breed- 0,776 mg / kg.

Keywords: ewe's milk, white brined cheese, Karakachann breed, Rhodope Tsigay, Middle Rhodope breed sheep, trace elements

INTRODUCTION

The milk is a complex of biologically active components, which promotes the growth and development of small mammals. It is believed that is a complete food, which is a good source of protein, fats, sugars, vitamins and minerals. For this reason, milk and dairy products are important components in human nutrition, consumed in different ages throughout the world. Dairy products are an important source of minerals, which is determined by the growing conditions, breed and forage. Technological treatment of milk to dairy products also had an effect, due to the loss of the mineral components in the whey. The trace elements are essential constituents of milk necessary for the vital activity of adolescent subjects and in human nutrition, as well as subsequent technological processing to dairy products.

Milk and dairy products are an important source of mineral salts in many European countries and represent 10-20% of the daily intake. The content of macro and trace elements in milk depends on the content in the soil and feedingstuffs to feed ruminants (Malbe et al., 2010).

Abdulkhaliq et al. (2012), explore the cow milk and dairy products containing metals and establish cadmium content from 0.022-0.057 μg / g, lead by not established to 0.93 μg / g, coper from 0.62-0.85 μg / g and iron from 3.2-12.91 μg / g. Borys et al., (2006) in his research on ewe's milk the following results for trace elements chrome- 0,024, zinc- 6.66, iron- 0.69 and copper-0.09 mg / kg and in different types of cheeses for chromium in the range from

0,048 to 0,084 mg / kg, zinc- 17.95 to 27,77 mg / kg, iron from 1.94 to 3,28 mg / kg, copper from 0.18 to 0,35 mg / kg.

Levkov et al. (2015), established the concentration of copper in the ewe's milk varied from 0.66 to 1.47 mg / kg, Fe from 1.52-3.82 mg / kg, Mn from 0.04-0.13 mg / kg and Zn from 2.90-6.27 mg / kg. In the white brined cheese content of Cu (2.49 to 8.08 mg / kg) showed slightly higher concentration of all samples collected in comparison with the literature data. The iron content is in the range of 3.81-12.09 mg / kg, Mn from 0.12 - 0.70 mg / kg, Zn-4.21-18.33 mg / kg and Cr from 0.04-0.14 mg / kg.

Zamberlin et al., (2012) found in different types of cheese iron content from 0,1 to 0,8 mg / 100g, zinc from 0,9 to 5,3 mg / 100g, manganese traces and copper traces to 0 07 mg / 100g. Yılmaz (2012), established in the white cheese copper in the range from 1,2 to 6,4 mg / 100g and zinc from 0,4 to 3,69 mg / 100g. Mustafa et al., (2013), in the cheese from different regions in Sudan detected concentration of manganese from 0,3 to 0,13 mg / 100g, zinc- 5,39 to 7,9 mg / 100g and iron from 0, 34 to 0,77 mg / 100g.

The objective of the present investigation was to study the trace element composition of white brine cheese made from ewe's milk from Karakachan breed, Rhodope Tsigay and Middle Rhodope breed sheep on the pasture grass during the May-July period in the region of Smolyan (Middle Rhodopes).

MATERIAL AND METHODS

The investigated 18 samples white brined cheese (3 x 6 samples) produced from buik samples of ewe's milk from (18 samples, 3 x 6 samples) by BDS 15-2010- Bulgarian white brine cheese from the Karakachan breed, Rhodope Tsigay and Middle Rhodope breed sheep on the pasture grass during the May-July period in the region of Smolyan (Middle Rhodopes) by BCS 15-2010- Bulgarian white cheese.

The mineral composition of the white brined cheese from the Bulgarian Rhodopean cattle breed was determined by dry ashing of the sample and its mineralization in a muffle furnace at 450ºC for 72 hours. Ash residue was dissolved with 6n HCl and diluted with double distilled water to a certain volume. The analysis of the trace elements is made of atomic emission photometer- AES-ICP "Varian- Liberty II", as follows Cu- 324,75 nm, Zn-213,85 nm, Fe- 259,94 nm и Mn- 257,61 nm.

The data obtained were processed statistically with software Statistic for Windows 2010.

RESULTS AND DISCUSSION

The copper is an essential element important for the absorption of iron and is a cofactor of the enzyme in glucose metabolism and the synthesis of haemoglobin, connective tissue and phospholipids. Copper deficiency in humans occurs only in long-term hunger. The copper content in the analysed ewe's milk on the lactation period varied within wide limits, which is due to the geological structure of the area and transfer it through a grassy associations consumed by test animals three breeds. Average for the May-July period of copper found in Karakachan breed of sheep is 0,314 mg / l, in the Rhodope Tsigay reported the lowest content of copper-0,154 mg / l and Middle Rhodope breed sheep accumulation is 0,230 mg / l. The copper content in the examined white brined cheese during the survey period is similar between Karakachan Rhodope sheep breed, respectively 0.407 and 0,404 mg / kg, while in the Rhodope Tsigay founded a highest concentration-0,637 mg / kg.

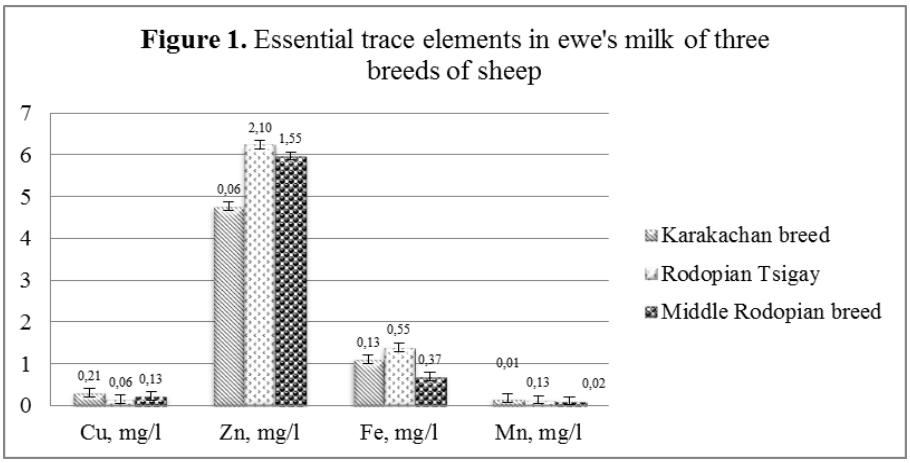

Figure 1. Essential trace elements in ewe's milk of three breeds of sheep

The zinc is an essential trace element for growth, sexual development, wound healing, and the normal functioning of the immune system and other physiological processes. Zinc is a component of the hormone insulin. It is a cofactor for many enzymes that are involved in most metabolic processes. Dairy products such as milk, cheese and yogurt are very important in human nutrition, but not a sufficient source of zinc. Milk from the target species is characterized by low zinc content in Karakachan breed sheep- 4,764 mg / l and high in the Rhodope Tsigay- 6,248 mg / l. In white brined cheese the content of zinc is similarly low at Karakachan breed-13,233 mg / kg, but the highest concentration is in the Middle Rhodope breed- average 19,667 mg / kg.

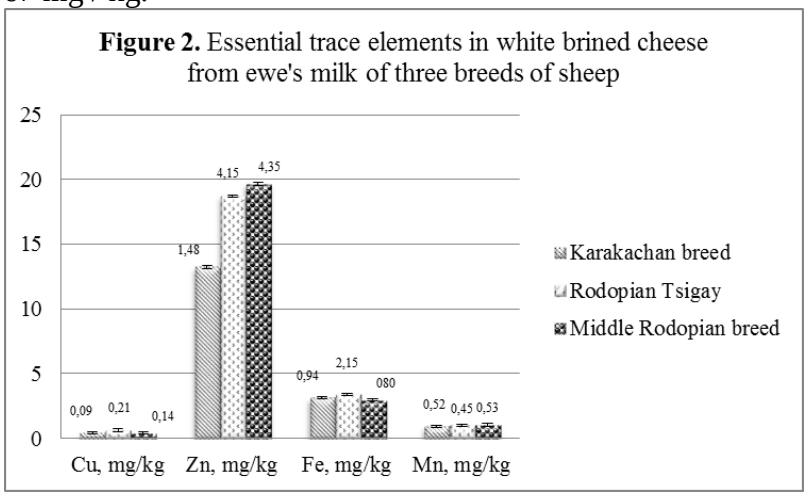

Figure 2. Essential trace elements in white brined cheese from ewe's milk of three breeds of sheep

Iron is an essential trace element and is involved as a catalyst in certain metabolic reactions. As a component of haemoglobin, cytochromes, and other proteins, iron plays an important role in the transport, storage and utilization of oxygen. It is also a cofactor for many enzymes. Milk and milk products are a poor source of iron. The iron content in the white brined cheese during the lactation has variables. Ewe's milk analysed during the period May-July three breeds with the highest concentration of iron in the Rhodope Tsigay -1,401 mg / l and the lowest average in the Middle Rhodope breed sheep-0,699 mg / l. The iron content in the white brined cheese in reared breeds are maintained as in the milk from which it is produced and does not suffer changes caused by the technological process. The amount is the highest in Karakachan breed - 3,405 mg / kg and the lowest average in the Middle Rhodope breed - 2,953 mg / kg.

Manganese is an essential trace element that is involved in the metabolism of carbohydrates, lipids and proteins. Manganese is a specific cofactor for enzymes involved in the synthesis of mucopolysaccharides and non-specific cofactor for many enzymes. It is in

significant amounts in all foods. Its deficiency has not been registered as a cause of disturbance or disease. From 3 to 5% of the total dietary intake of manganese absorbed successfully, the remainder is eliminated from the body through feces.

The concentration of manganese in the analyzed ewe's milk average for the period is 0,173 mg / l at Karakachan breed, 0,142 mg / l in the Rhodope Tsigay and 0,119 mg / l on Middle Rhodope breed sheep. In the production of white brined cheese, the amount of manganese is the highest average in the Middle Rhodope breed sheep - 1,053 mg / kg and the lowest at Karakachan breed- 0,926 mg / kg, which is probably conditioned by technological processing.

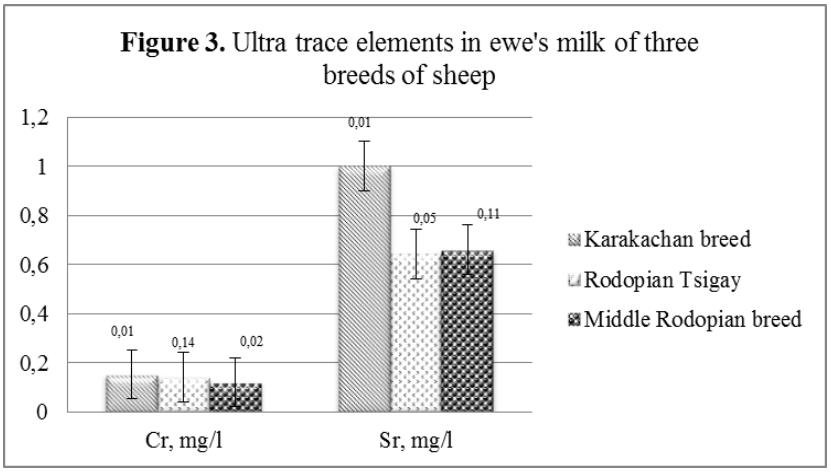

Figure 3. Ultra trace elements in ewe's milk of three breeds of sheep

Micronutrient chromium involved in regulating blood sugar levels, assists in the formation of insulin from the pancreas, the transport of certain proteins in the body, growth can overcome hypertension, prevents the development of diabetes mellitus. Shortage of chromium in the body need twice as much energy to maintain blood glucose levels within the normal range for humans. The physiological needs of the body of chromium are not strictly fixed. According to the requirements of the WHO, the recommended intake for adults ranges from 50-200 μg / day. With advancing age, the amount of chromium in the body decreases. The chromium content in the analysed milk from sheep of Karakachnska breed, Rhodope Tsigay and Middle Rhodope breed of sheep in the range from 0,121 to 0,153 mg / l. In the production of white brined cheese from milk obtained established that chromium is the lowest concentration in the Rhodope Tsigay- 0,690 mg / kg and highest in Karakachan breed- 0,776 mg / kg, therefore we have lost in the technological processing of the final product .

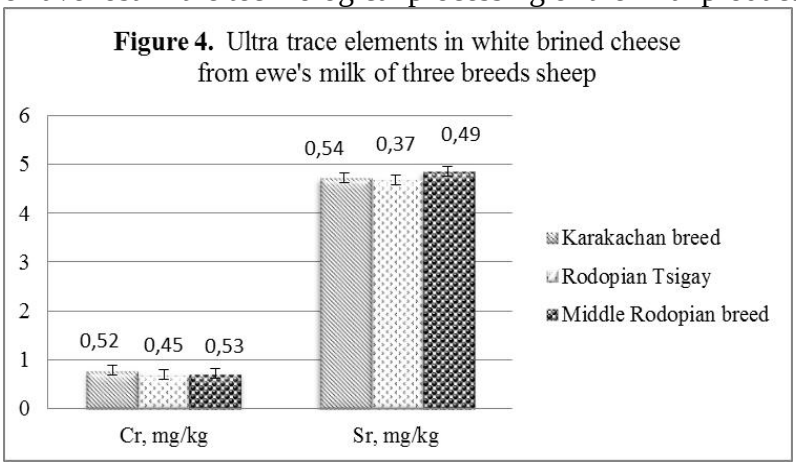

Figure 4. Ultra trace elements in white brined cheese from ewe's milk of three breeds sheep

Strontium in the human body is contained in an amount between 0,01 and 0,01 mg / kg body weight. Increased content strontium was observed in some areas (soil, water, plants) in which the observed softening of the bones of the skeleton of the animal, so-called. "Strontium rickets" not amenable to treatment with vitamin D, phosphorus and calcium

(Stoyanov, 1999). Shortage of silicon in the body, calcium is poorly absorbed and strontium begins to replace him in bone tissue. Strontium are retained in the body for a long time. After carrying out his destructive work in human bones is returned to nature, leaving the body. Wegener (1963), establishes the strontium content in sheep milk to 4% per liter. Total strontium content in the analyzed ewe's milk of three breeds for the period is as follows Karakachan breed- 1,002 mg / l, Rhodope Tsigay- 0,642 mg / l and Middle Rodope breed- 0,660 mg / l. Produced white brined cheese, after technological processing retain the amount of strontium from milk. The cheese of Karakachan breed sheep was containing- 4,712 mg / kg strontium from Rhodope Tsigay -4,680 mg / kg strontium and Middle Rhodope breed - 4,860 mg / kg strontium.

CONCLUSIONS

Processing to manufacture the white brined cheese from ewe's milk of three different breeds sheep led to the preservation of content amounts of copper, iron, chromium and strontium and losses of zinc and manganese.

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BO11. POTENTIALLY HARMFUL EFFECTS OF GRAPEFRUIT

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Grapefruit is a subtropical citrus tree originating as an accidental cross between two species - sweet orange and pomelo. Similar to other citrus fruits it is known with its good nutritional properties - a rich source of vitamin C, antioxidants, lowering cholesterol etc.

But in some cases using it can be dangerous. Grapefruit`s adverse effects are mainly due to drug interractions and photosensibilization.

Grapefruit contains a number of polyhenolic compounds, including flavanone naringin, bergamottin and dihydroxybergamottin. They inhibit the drug metabolizing enzyme CYP450 thus increasing the biovailability of at least 85 drugs. Some of this interractions can be rather dangerous for the patient. We will discuss its interraction with statins. They are among the best selling drugs and have very high risk for interraction with grapefruit. A daily glass of grapefruit juice increases blood levels of Simvastatin and Lovastatin by about 260%. That increases risk of side effects of statins, one of which is rhabdomyolyses. It can lead to kidney failure, liver injury, imbalance of electrolytes resulting in heart arrhythmias etc.

Citrus fruits contain psoralens and flurocoumarins which are photoactive compounds and make skin more sensitive to the sun. There are higher levels of psoralens in grapefruit than in other citrus fruits. Researchers discovered that melanoma risk is 36% higher in people who consume grapefruit and/or other citrus fruits every day compared to those who consumed them less than twice per week. But citrus fruit can't lead to melanoma without excessive sun exposure.

In conclusion - citrus fruit especially grapefruit should be utilised carefully from people who administer medicines which can interract with it and from those who have risk factors for developing melanoma.

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ВО12. ЗДРАВОСЛОВНО ХРАНЕНЕ. ХРАНЕНЕ ПРИ ПОЛИКИСТОЗЕН ОВАРИАЛЕН СИНДРОМ

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Начинът на живот и начинът на хранене обуславят човешкото здраве.Като здравословно се маркира това хранене, което отговаря на метаболитните потребности на организма.То трябва да бъде балансирано, разнообразно и да включва минимум 3 пъти прием храна за денонощие. Храните биха могли да се диференцират в следните категории в зависимост от биологичната си активност:
-Категория А- храни, най- богати на есенциални нутриенти и микронутриенти;
-Категория Б-високоенергийни храни, доставящи есенциални нутриенти;
-Категория В- почти несъдържащи есенциални нутриенти храни, но доставящи много калории.
Оптималното разпределение за храната през деня трябва да бъде на 4 приема: закуска-20%, обяд-40%, втора закуска-10% и вечеря-30%.
Специални диети се изискват при определени заболявания. Диета се препоръчва и при поликистозния овариален синдром. Той се причинява от хормонален дисбаланс в женското тяло, като резултат от неправилно функциониране на яйчниците, панкреаса, надбъбречните жлези, хипофизата, щитовидната жлеза и хипоталамуса.
В повечето случаи се свързва с хиперандрогенизъм, инсулинова резистентност и затлъстяване. При инсулинова резистентност тъканите на тялото са неподатливи към ефекта на инсулина, в отговор на което тялото произвежда повече инсулин. Високите нива на инсулин водят до увеличаване на производството на мъжкия полов хормон – тестостерон, което пречи на развитието на фоликулите и нормалната овулация. И тъй като инсулинът е необходим за усвояването наглюкозата от храната, при инсулинова резистентност глюкозата се натрупва под формата на мазнини.

Няма конкретно лечение на поликистозните яйчници, но симптомите могат да бъдат повлиявани медикаментозно, хирургично и с промяна в начина на живот.
Диетата трябва да съдържа храни, които предизвикват отделянето на инсулин и глюкагон, които участват в поддържането на нормална кръвната захар.Храната да включва здравословна комбинация от зеленчуци, плодове, пълнозърнести храни, протеини на растителна основа /пълнозърнести, бобови/ и здравословни мазнини.
Препоръчва се да са застъпени предимно въглехидрати с нисък и среден ГИ. Като хранителна добавка към балансираната диета се включва: инозитол, фолиева киселина, витамин А и витамин Е.
Използвана литература: Учебник по „Хигиена, хранене и професионални болести” , под редакцията на проф.д-р Божидар Попов

BO13. POLYTETRAFLUOROETHYLENE (TEFLON) AND ITS IMPACT ON PEOPLE, ANIMALS AND ENVIRONMENT

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Polytetrafluoroethylene (PTFE, teflon) is widespread plastics in industry and households. It is used as non-stick coating for pans.
When heated above 250 ^{0}C, toxic products like carbonyl fluoride and hydrofluoric acid formed. This products cause flu-like symptoms in humans known as polymer fume fever. They can be lethal to birds as they have much more sensitive to toxins respiratory tract.
Using scratched utensils hides additional risks. Toxic products start to form at lower temperature and in greater amount. Moreover Teflon often lies above aluminium which is dangerous when in contact with food.
Perfluorooctanoic acid (PFOA) is used as a surfactant in the emulsion polymerization of PTFE. It has cancerogenous properties.
Thermolysis of teflon can produce trifluoroacetate, chlorodifluoroacetate, polyfluoro- and polychlorofluorocarboxilic acids. Some of these products have recently been linked with destroying ozone and acting as greenhouse gases.

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BP1. КАКВО (НЕ) ЗНАЕМ ЗА КАРДАМОНА?

Абдулкадир Абудаллех и ученици
ИЕМПАМ-БАН

Session C.

Chairpersons:

Prof. Todor Dudev, PhD
Faculty of Chemistry and Pharmacy, Sofia University "St. Kl. Ohridski"

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

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

CO1. GALLIUM AS THERAPEUTIC AGENT: COMPETITION BETWEEN GA^{3+} AND FE^{3+} IN METALLOPROTEINS

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Gallium, an abiogenic metal, has been employed (in the form of soluble salts) to fight various forms of cancer, infectious and inflammatory diseases [1-3]. The rationale behind this lies in the ability of gallium cation, Ga^{3+}, to mimic closely in appearance the native ferric ion, Fe^{3+}, thus interfering with the biological processes requiring Fe^{3+}. However, Ga^{3+} cannot participate in redox reactions and, when substitutes for the "native" Fe^{3+} in the enzyme active site, renders it inactive. Although a significant body of information on the $Ga^{3+} \rightarrow Fe^{3+}$ competition in biological systems have been accumulated, the intimate mechanism of the process is still not well understood and several outstanding questions remain: (1) What are the basic physical principles governing the competition between the two trivalent cations in proteins? (2) What is the effect of different factors such as the pH of the medium and the composition, overall charge and solvent exposure of the binding site on its metal selectivity? (3) What type of metal centers are the most likely targets for Ga^{3+} therapy? (4) To what extent are the Fe^{3+}-binding sites in the key enzyme ribonucleotide reductase vulnerable to Ga^{3+} substitution?

Here, we endeavor to address these questions by studying the competition between Ga^{3+} and Fe^{3+} in model metal binding sites of various compositions and charge states. As the interactions between the metal ion and protein ligands play a key role in the Ga^{3+}/Fe^{3+} competition, they were treated explicitly using density functional theory (DFT). The region inside the metal binding site was represented by an effective dielectric constant, ε, varying from 4 to ~30, mimicking binding sites of increasing solvent exposure. The results obtained are in line with available experimental data and shed light on the intimate mechanism of the

Ga^{3+}/Fe^{3+} selectivity in few biological systems such as serum transferrin and ribonucleotide reductase (a putative target for anticancer therapy).

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CO2. LET US SPEAK ABOUT COPPER

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CO3. GOLD COMPOUNDS AS ANTITUMOR AGENTS

Tanya Zhivkova, Radostina Alexandrova

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CO4. PROPERTIES OF LANTANIDES COMPLEXES OF MONENSIN

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Monensin is a natural antibiotic produced by *Streptomyces cinnamonensis*. It is widely applied in stock farming and veterinary medicine due to its pronounced coccidiostatic and antibacterial properties. The main form of the ionophore is Monensin A (Fig. 1) (Monensic acid, MonH), accompanied by two minor factors, Monensin B and Monensin C, also produced by the *Streptomyces* bacteria. From a chemical point of view, Monensin A is a polyether derivative of a monocarboxylic acid. Its monohydrated form ($MonH \times H_2O$) exists in a pseudo-cyclic conformation (Fig. 2) secured by head-to-tail H-bonding between the carboxylic moiety and an alcoholic hydroxyl group. Oxygen atoms pointing inside the cavity ensure its hydrophilic character, while the alkyl-rich polyether backbone provides antibiotic lipophilicity and corresponding cell membrane activity.

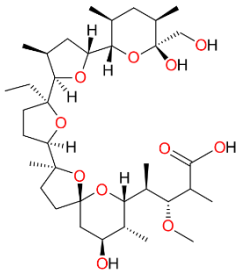

Fig. 1 Monensin A

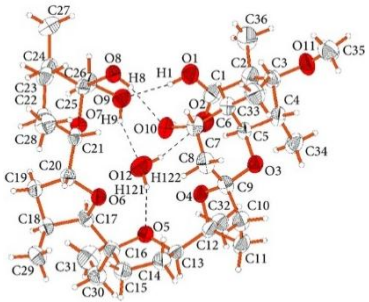

Fig. 2 Pseudo-cyclic conformation of $MonHxH_2O$

Monensin possesses high affinity to bind alkali cations but also forms various divalent metal-containing derivatives

depending on the nature of the metal(II) ion and reaction conditions. Generally, the metal complexes of Monensin enhances significantly biological activity of non-coordinated ionophore as the antibacterial and the anticancer studies revealed. Recent studies show that the anticancer activity of polyether ionophores may be a consequence of the induction of apoptosis leading to apoptotic cell death, arresting cell cycle progression, induction of the cell oxidative stress, loss of mitochondrial membrane potential, reversion of multidrug resistance, synergistic anticancer effect with other anticancer drugs, etc. Broad investigation of the mechanisms of action and development of new polyether ionophores derivatives may provide more effective therapeutic drugs for cancer treatment.

In the present research we focused our interest towards evaluation of coordination ability of MonH in the presence of trivalent rare-earth ions (Ln(III)). Lanthanides are not specified as biometals, but due to their specific spectral properties (partial occupation of the 4f-electron layer) they are intensively used in X-ray, fluorescent, MRI and NMR studies of biological systems.

In the course of the experimental work we optimized reaction conditions for the synthesis and isolation of 10 new complex species of Monensic acid as a ligand. The newly synthesized complexes have been characterized in the solid state using various spectroscopic methods, their behavior in solution was studied by circular dichroism. We performed an initial assay of their antibacterial activity against Gram-positive aerobic bacteria (*B. Subtilis, B. Mycoides, S. Lutea*) using the "agar diffusion method." The data revealed that the rare-earth complexes of Monensin potent the antibacterial activity of the ligand.

CO5. LEAD, CADMIUM AND NICKEL IN HOMEMADE WINE FROM STARA ZAGORA REGION

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Abstract

This paper examines the levels of lead, cadmium and nickel in 14 samples of homemade grape wine from two villages located near Stara Zagora and the military testing ground Zmeyovo. Ten control samples were collected from the most remote village of the region, situated in Sredna Gora mountain. The wines were from vintages 2005-2014. Direct measurements of the metals were carried out by flame atomic absorption spectroscopy. The mean lead concentrations in the two groups were identical – 0.19 mg/L. Four samples from the study group and three from the control slightly exceeded the permissible limit of 0.2 mg/L [4]. Four of the registered excessive samples came from vintage 2014. It should be noted that the norm of lead in wine from vintages 2016 onwards will be reduced to 0.15 mg/L. All tested samples, including controls, contained lead above this value. The mean cadmium concentration in the test group (0.028 mg/L) was significantly higher than the one in the control group (0.020 mg/L). The mean group concentrations for nickel were not significantly different, although its level was higher in the test group – 0.22 mg/L, compared to the control – 0.19 mg/L. None of the measured nickel concentrations exceeded the limit of 0.3 mg/L [2]. The results for lead and cadmium from our study were comparable with levels in wines from the FYR Macedonia [5], and were a lot higher than Argentine and Hungarian wines [3,6]. A Bulgarian study of wines from the region of KCM Plovdiv [1] found that after washing the

grapes concentrations of heavy metals in wine reduced by two to three times. The authors recommend popularizing among manufacturers of homemade wine the practice of grape washing.

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CP1. THE MANY FACES OF GOLD COMPOUNDS

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The application of gold in medicine is traceable for several thousand years, since the dawn of civilization. Even in the 19th century gold was considered to be a "cure-all' for diseases. The rational use of gold in medicine began in the early 20th century with the discovery of Robert Koch that gold cyanide can kill the bacteria that cause tuberculosis (*Micobacterium tuberculosis)* in cultures. In the 20th century following the observations of Jacques Forestier, gold compounds were successfully introduced in the treatment of rheumatoid arthritis. Recently research into gold-based drugs for a range of human diseases has seen a renaissance. Old as well as new Au(I) and Au(III) compounds have been investigated as potential anti-cancer, anti-parasitic and anti-HIV agents. Gold has been also used in reconstructive medicine and dentistry.

History

The application of gold in medicine, to treat a number of pathological problems, is traceable for several thousand years. It dates back to ancient Arabic and Chinese physicians. Even in the 19th century gold was considered to be a "cure-all' for diseases [21].

The rational use of gold in medicine began in the early 20th Century when Robert Koch discovered that gold cyanide $K[Au(CN)_2]$ could kill the bacteria that cause tuberculosis (*Mycobacterium tuberculosis*) in cultures, thus offering a scientific basis for the pharmacological applications of gold compounds. Treatment of pulmonary tuberculosis with gold compounds was popularized, particularly by Danish physicians, in the mid-1920s [2]. $K[Au(CN)_2]$ was changed by the less toxic Au(I) thiolate complexes.

Gold in the treatment of rheumatoid arthritis

In the early 1930's, the French Jacques Forestier was the first to use these thiolate complexes to treat rheumatoid arthritis (RA), a condition which he believed to be related to tuberculosis. Chrysotherapy - the treatment of RA patients with monovalent gold drugs possessing anti-inflammatory and other properties - has been used with some success for more than 70 years. However, the metabolites generated from gold drugs have not been identified positively and the mechanisms of action are not known with certainty [13; 25].

The role of gold in different phases of an immune reaction has been studied. The results obtained reveal that this metal gold plays an important role already in the initiation, namely the uptake and presentation of foreign antigens. Thus, gold is taken up by the macrophages and stored in the lysosomes (called aureosomes) where this metal inhibits antigen processing. The ability of Auranofin (the only approved oral gold complex of value in suppressing rheumatoid arthritis) to inhibit MHC-restricted antigen presentation in professional antigen-presenting cells has been reported [7; 19]. Especially peptide antigens, which contain sulfur such as cysteine and methionine, are important. There are data suggesting that chronic inflammatory diseases such as RA are caused by prolonged production of proinflammatory cytokines including tumor necrosis factor (TNF) and interleukin 1 (IL-1). The nuclear factor kappaB (NF-kappaB) plays an essential role in transcriptional activation of TNF and IL-1. NF-kappaB is induced by many stimuli including TNF and IL-1, forming a positive regulatory cycle that may amplify and maintain RA disease process. Therefore, NF-kappaB and enzymes involved in its activation can be considered to be possible targets for anti-inflammatory treatment [20].

It has been found that gold suppresses nuclear factor kappaB (NF-kappaB) binding activity as well as the activation of the I-kappa B-kinase. This mechanism results in a subsequently reduced production of pro-inflammatory cytokines, most notably tumor necrosis factor (TNF)-alpha, interleukin-1 (Il-1) and interleukin-6 (Il-6). On the subsequent T-cell level, gold has been shown to induce an upregulation of IL-4 mRNA, a potent antiinflammatory cytokine, resulting in a shift of the T-cell population to the Th2 profile. The activation of T-lymphocytes is also inhibited. On the effector level, gold inhibits proteolytic enzymes and can result in the destruction of synovial fibroblasts, their proliferation is a hallmark of the invasive pannus in the rheumatoid joint [3].

Gold compounds as antiviral and antiparasitic agents

Highly active antiretroviral therapy (HAART) has resulted in decreased mortality and morbidity from the acquired immune deficiency syndrome caused by the human immunodeficiency virus (HIV). Drug resistance and toxicity of HAART has led to the search for novel inhibitors of HIV infection. Gold-based inhibitors of HIV reverse transcriptase (RT), protease (PR) and viral entry of host cells have been reported [15; 17; 16]

Entamoeba histolytica is the causative agent of amoebiasis in humans and is responsible for an estimated 100,000 deaths annually, making it the second leading cause of death due to a protozoan parasite after Plasmodium [29]. The WHO considers amebiasis as one of the major health problems in developing countries; it is surpassed by only malaria and schistosomiasis for death caused by parasitic infection [28]. Treatment relies on metronidazole, which has adverse effects, and potential resistance of E. histolytica to the drug is an increasing concern [1]. It has recently been reported that auranofin is active against *E. histolytica* in culture. Auranofin was ten times more potent against E. histolytica than metronidazole. It has been suggested that auranofin targets the E. histolytica thioredoxin reductase, preventing the reduction of thioredoxin and enhancing sensitivity of trophozoites to reactive oxygen-mediated killing. In a mouse model of amebic colitis and a hamster model of amebic liver abscess, oral auranofin markedly decreased the number of parasites, the detrimental host inflammatory response and hepatic damage [8]. Some gold-containing compounds have been found to exhibit potent antileishmanial activity [6].

Gold as an implant in medicine and dentistry

Gold implants are used in various medical procedures, including reconstructive surgery of the middle ear, upper lid closure in facial nerve paresis-induced lagophthalmos, drug delivery microchips, use on the surface of voice prostheses, and endovascular stents. In order to achieve better therapeutic benefits, clinical reports have documented that the surface of gold implants have been modified or encased in biocompatible alloplastic materials, or they have been replaced by cheaper and more biocompatible materials. Gold is also applied to a long list of dental prostheses, including inlays, onlays, crowns, bridges, periodontal splints, and post and cores. It has sufficient strength and corrosion resistance, and it is relatively biocompatible. In addition, gold dental prostheses have a long life cycle. Gold was first used in dentistry over 2500 years ago, and its dental applications have increased steadily, especially during the past 100 years [11]. Esthetic concerns and cost make it a less desirable prosthesis today than in the past [9].

Gold compounds as anticancer agents

Studies of the anti-tumour activity of gold compounds were stimulated by at least two observations: i) Patients treated with gold for rheumatoid arthritis (Chrisotherapy) had lower rates of malignancy than other patients [18]; ii) The efficacy of cisplatin against cancer stimulated great interest because gold in the +III oxidation state is isoelectronic with platinum (II) and forms similar square-planar complexes [24;10 ; 22; 26].

Gold(I) and gold(III) complexes do interact with DNA via various chemical mechanisms to produce different conformational changes [25].

The precise mechanisms through which cytotoxic gold compounds produce their biological effects are still largely unknown. In a recent review the possible modes of action and the most probable biomolecular targets for some of the most extensively studied gold compounds were discussed: auranofin and analogues, gold(III) porphyrins and gold(III) dithiocarbamates. For these three families markedly distinct molecular mechanisms were invoked: a direct mitochondrial mechanism involving thioredoxin reductase inhibition in the case of the gold(I)

complexes, the influence on some apoptotic proteins—i.e. MAPKs and Bcl-2—for gold(III) porphyrins, and the proteasome inhibition for gold(III) dithiocarbamates. In a few cases the distinct mechanisms may overlap [4].

Nowadays, gold compounds constitute a family of very promising experimental agents for cancer treatment. Indeed, several gold(I) and gold(III) compounds were shown to manifest outstanding antiproliferative properties in vitro against selected human tumor cell lines and some of them performed remarkably well even in tumor models in vivo. Something more, some gold compounds (i.e. gold(III)-dithiocarbamato derivates) showing outstanding in vitro and in vivo antitumor properties have been found to express reduced, or even no, systemic and renal toxicity, compared to the reference clinically-established anticancer drug cisplatin [31] and have been suggested to be suitable candidates for clinical trials [23].

Side effects

Gold has been shown to have generally mild side effects (mostly kidney dysfunction and pulmonary that are not significant) and thus the treatment can be conducted for long periods in many cases [5; 31]. Contact allergy to gold (gold jewelry, dental restorations, implants) has been established [14; 12; 27].

In conclusion, gold remains one of the most fascinating antirheumatic agents and has been also found to express impressive anti-cancer, anti-HIV and anti-parasitic potencies. The future research in this field will lead to new fundamental knowledge of gold action, possible allowing development of new gold-based drugs for the treatment of various pathological conditions.

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CP2. METAL (ZN/AG, ZN/AU) COMPLEXES WITH SCHIFF BASES: INITIAL STUDY OF POTENTIAL ANTIHERPESVIRAL ACTIVITY

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Session D.

Chairpersons:

Assoc. Prof. Anna Tolekova, MD, PhD
Medical Faculty, Trakia University, Stara Zagora, Bulgaria

Assoc. Prof. Boryana Ruseva, MD, PhD
Medical University, Pleven

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

DO1. EFFECTS OF CHLOROGENIC ACID, FERULIC ACID, GALLIC ACID AND QUERCETIN ON LEARNING AND MEMORY IN THE ONE-WAY PASSIVE AVOIDANCE TASK IN YOUNG/HEALTHY RATS

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The phenolic acids chlorogenic (CA), ferulic (FA) and gallic (GA) and the flavonoid quercetin (Q) are polyphenols abundant in natural food sources. Polyphenols exhibit strong antioxidant properties. There are data that they cross the blood-brain barrier and accumulate in the brain. These substances have been tested in different models of impaired memory. The aim of the present study was to investigate their effects on learning and memory processes in young/healthy rats.
Male Wistar rats were treated in the course of 7, 14, 21 and 30 days. There were 5 groups for each treatment period. Control groups were treated with saline. The other 4 groups received CA, FA, GA or Q at an equal dose of 20 mg/kg. At the end of each period, learning and memory processes were evaluated using the one-way passive avoidance task (step-through). Step-through latency was recorded in two retention tests on the 3rd and 24th h after an acquisition trial. Administered for 7 days, the experimental substances had no significant effects on rat behavior. Applied for 14 and 21 days, GA and Q significantly prolonged the latency time in both retention tests. After 30 days of treatment all tested polyphenols significantly improved the recorded indices of memory. The onset of the effect after 14/30 days of treatment may be explained by the accumulation of polyphenols in the brain following a long-term consumption. Our results suggest that CA, FA, GA and Q applied subchronically improve memory and cognition of young/healthy rats.

DO2. INTERACTION BETWEEN ANGIOTENSIN II RECEPTORS OR INTERVENTION OF ANGIOTENSIN II DERIVATIVES FORCONTRACTILE ACTIVITY OF VISCERAL SMOOTH MUSCLES?

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Abstract:
This study aims to explore the role of AT1 and AT2 receptor subtypes for the development of Angiotensin II (Ang II) – induced visceral smooth muscle contractile activity. For in vitro experiments mature female Wistar rats were used. Longitudinal strips from stomach, uterus, urinary bladder and rectum were prepared and treated by Ang II in a dose of 10^{-6} M. The significance of Ang II receptors was investigated by application of selective inhibitors: AT1 antagonist Losartan or AT2 receptor blocker PD 123319. After AT1 or AT2 receptor blockade, the change in the response to Ang II of stomach and urinary bladder preparations was similar: AT2 receptor blockade led to development of significantly powerful contractions, while AT1 blockade suppressed them considerably. The administration of selective blockers significantly reduced the amplitude of Ang II-provoked uterine and rectal contractions in a similar pattern.
The study demonstrates that in Ang II - mediated contractile process of rat stomach and urinary bladder AT2 receptors antagonize the actions of AT1 receptors, while in the uterus and rectum both receptor subtypes act synergistically. The locally produced angiotensin derivatives probably also participate in Ang II – induced smooth muscle activity by interaction with AT2 or another receptor type, different from AT1.

Introduction:

Renin-angiotensin system (RAS) is a hormonal system, involved in blood pressure regulation and electrolyte balance in the body. The system consists of different in length and action peptides, named angiotensines, with octapeptide Angiotensin II (Ang II) being the main effector in it. The information about the physiological role of RAS was extended with the establishment of multiple effects of Ang II outside the cardiovascular system and kidneys. The discovery of the local expression of RAS components in a number of organs and tissues showed the presence of alternative pathways for Ang II formation [14]. This significantly changed the existing concept and nowadays RAS is regarded as multicomponent system, operating through different signal pathways, and having a great importance for the regulation of the physiological processes as well as the onset and progression of many diseases.

The classical antihypertensive therapy involves the application of different groups of drugs. Most of them are targeted on RAS and aim blocking of Ang II formation and its effects. However, this is only "the one side of the coin," because the significance of Ang II for the organs which are outside the cardiovascular system is still under investigation. It is known that Ang II induces contractile responses of visceral smooth muscle, mediated primarily by AT1 receptor [6,7]. However, the importance of AT2 receptors for smooth muscle contraction still remains a mystery [8]. The classical assumption, that activation of the AT2 receptor counteracts AT1-mediated responses is not enough to explain the role of AT2 receptors in the development of smooth muscle contraction. There is an accumulating data in the literature, revealing the significance of the two subtypes Ang II receptors in various processes. This is evidence that AT1 and AT2 could share common signaling pathways and presents the underexplored AT2 in a new light as an independent receptor with an important signaling [2].

This raises a number of questions about the change in contractile activity of visceral smooth muscle tissues as a result of antihypertensive treatment and influencing the formation

or the effects of Ang II. In many cases, the progression of an untreated cardio-vascular disease or the treatment of hypertension with conventional antihypertensive agents could lead to side effects, concerning the normal function of many smooth muscle organs. Therefore, the investigation of the effects and mechanisms of action of Ang II on visceral smooth muscle would help to elucidate the physiological role of this peptide for normal smooth muscle activity as well as for the pathogenesis and treatment of micturition disturbances, uterine and digestive disorders.

Materials and methods:

Female mature Wistar rats, weighting 250-300 g were used. The animals were anesthetized with Nembutal 50 mg/kg/ i.p. and exsanguinated. Abdominal cavity was opened and the stomach, uterus, urinary bladder and rectum were dissected out. The isolated organs were transferred immediately in cold Krebs solution (3°C). The experiment was carried out in accordance with the National regulations and European Directive of 22.09.2010 (210/63/EU) concerning the protection of animals used for scientific and experimental purposes.

The composition of Krebs solution, the preparation of the tissue samples and the isolated tissues setting were as it was previously described [9,10] The investigated preparations were divided into 3 groups: the first one was influenced only by Ang II (1μM); the second one – by the selective blocker for AT2 receptors - PD 123319 (100 nM) and 15 min after that - by Ang II (1μM) and the third one – by the selective blocker for AT1 receptors - Losartan (100 nM) and 20 min after that – by Ang II (1 μM). Mechanical activity was digitized and recorded by ISOSYS-Advanced 1.0 software (Experimetria Ltd., Hungary). The conversion of the data for later analysis was performed by KORELIA software [20,21]. All the chemicals and drugs *(*Ang II, PD 123319, Losartan and the reagents for the preparation of Krebs solution) were purchased from Sigma-Aldrich Chemie GmbH, Germany.

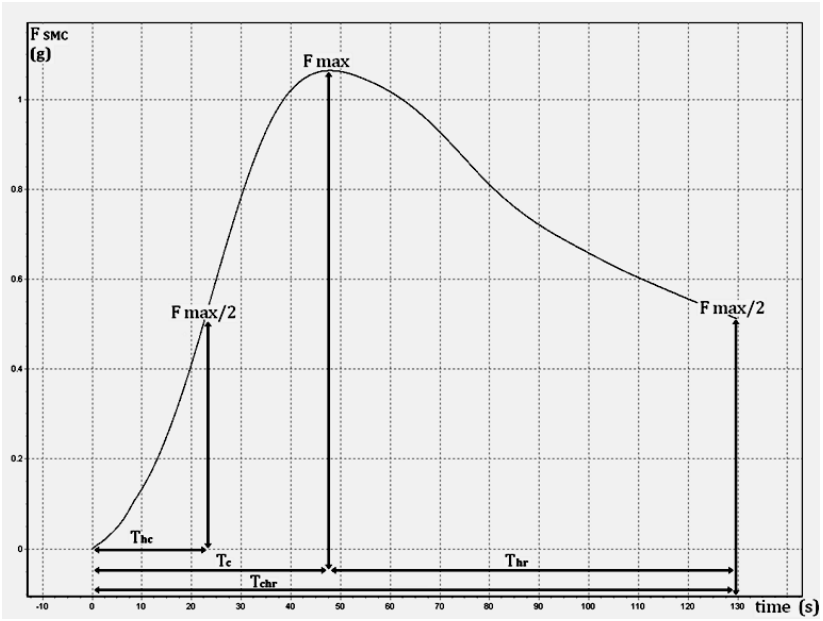

Figure 1. Smooth muscle contraction (SMC) - graph and time – parameters: F_{max} – maximal force of the SMC; $F_{max}/2$ – half of the maximal force of SMC

T_{hc} – half - contraction time: time interval between the beginning of the SMC and $F_{max}/2$

T_c – contraction time: time interval between the beginning of the SMC and F_{max}

T_{hr} – half-relaxation time: time interval between F_{max} and $F_{max}/2$

T_{chr} – contraction plus half-relaxation time: time between the beginning of the SMC and $F_{max}/2$

Analysis. The duration of the interval for analysis of tonic contraction was defined from the beginning of the contraction, until the amplitude fell to 50%. The recorded force - vs. time curves permit determination of amplitude and the integrated force (represented by the area under the curve - AUC). The different phases of the provoked contractions were clarified and analyzed by application of time-parameter analysis, similarly to that made in the study of Raikova & Alajov, 2004 [16]. Following time-parameters were introduced and examined: half

- contraction time (T_{hc}); contraction time (T_c); half-relaxation time (T_{hr}); contraction plus half-relaxation time (T_{chr}) (Fig. 1). The calculation of time-parameters was made by KORELIA-Dynamics program [19]. The program offers opportunities for interpolation and graphical visualization of experimental data with a cubic spline. Data obtained were processed by the statistical program Statistica 8.0, StaSoft, Inc. and presented as mean ± standard error, *P*-value ≤ 0.05 was considered to be statistically significant.

Results

After AT1 or AT2 receptor blockade, the change in the response of stomach and urinary bladder preparations to Ang II was similar: AT2 receptor blockade led to the development of significantly powerful contractions, while AT1 blockade suppressed them considerably (Fig. 2 and Fig. 3).

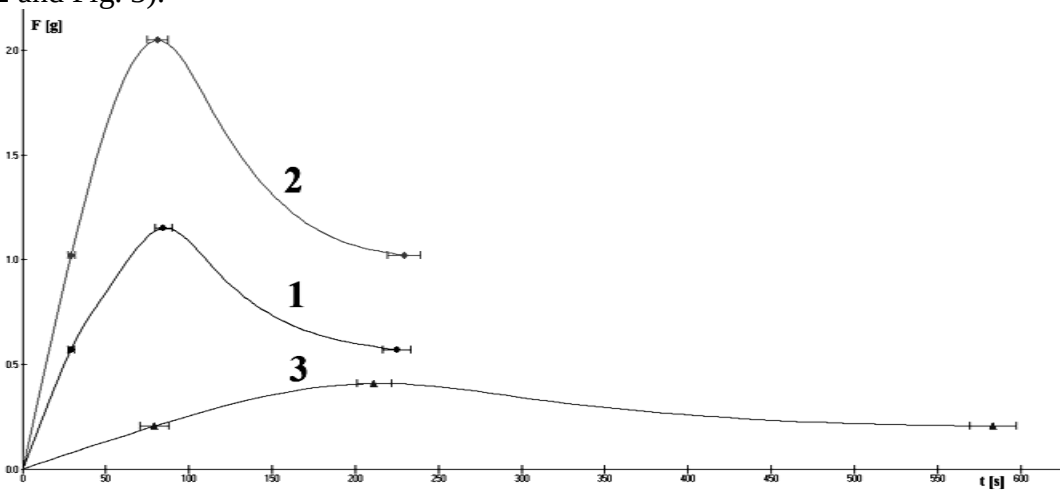

Fig. 2. Contractile activity of stomach preparations induced by Ang II alone (1); Ang II applied 15 min after PD 123319 (2); Ang II applied 15 min after Losartan (3).

When AT1 blockade was performed, the response of stomach and urinary bladder preparations to Ang II was developed for a longer period of time, evidenced by all statistically significantly extended time-parameters of the contraction. The time-parameters of the contractions induced by Ang II when AT2 receptors were inhibited, were not significantly different from those of the responses induced by Ang II only.

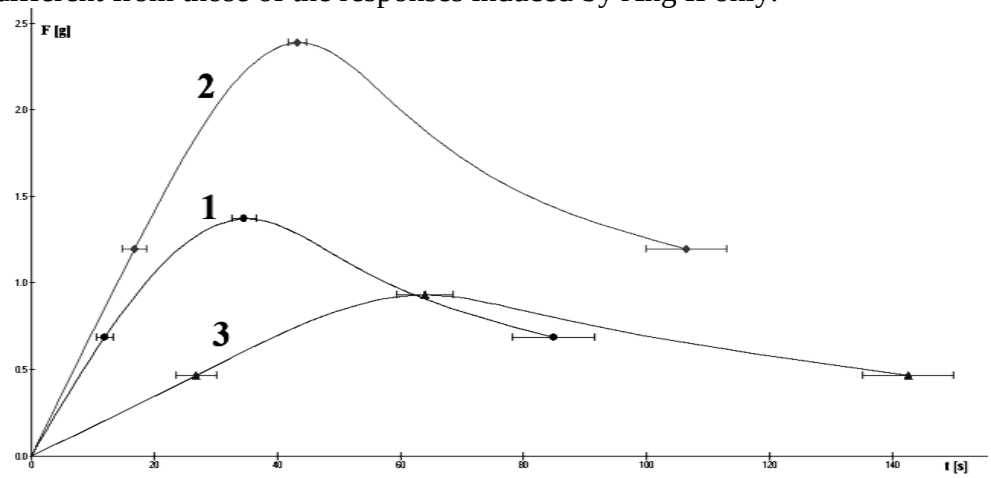

Fig.3. Contractile activity of urinary bladder preparations induced by Ang II alone (1); Ang II applied 15 min after PD 123319 (2); Ang II applied 15 min after Losartan (3).

There was statistically significant difference between the amplitudes of rectal contractions provoked only with Ang II (4.74 ± 0.44 g) and the contractions of the other two groups of preparations (Fig. …): 2.86 ± 0.33 g (PD 123319 + Ang II) and 2.79 ± 0.25 g (Losartan + Ang

II). The observed changes in the power of rectal contractions were in the similar pattern. AT_2 receptor blockade significantly prolonged all of the time - parameters of the contraction, comparing to those, calculated when Ang II was applied alone. The application of Losartan caused a development of a very rapid and short rectal relaxation (Fig.…).

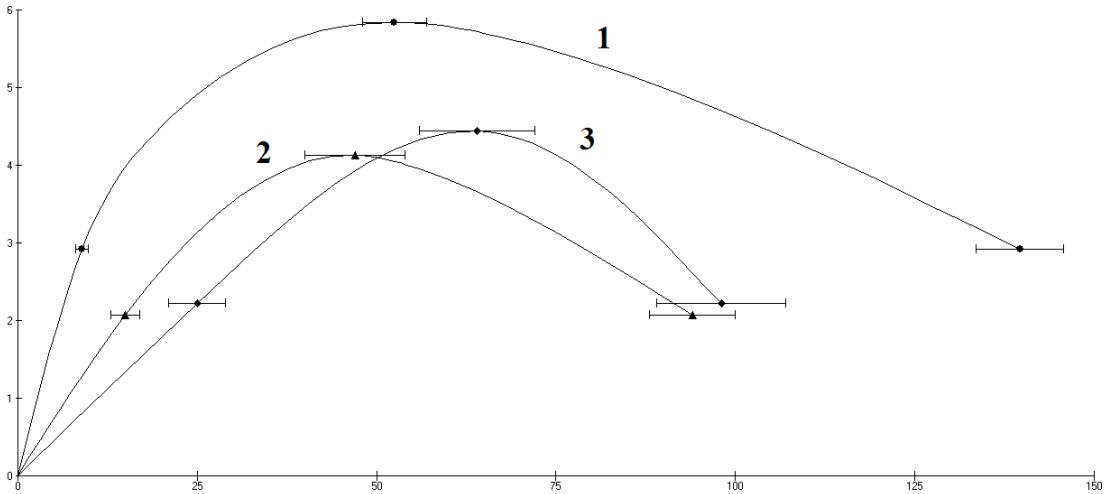

Fig.4. Contractile activity of uterine horn preparations induced by Ang II alone (1); Ang II applied 15 min after PD 123319 (2); Ang II applied 15 min after Losartan (3).

The administration of selective blockers caused a similar decrease in the amplitude of Ang2-induced uterine contractions to 4.13 ± 0.7g (PD+Ang II) and 4.44 ± 0.54 g (Los+Ang II) compared to the contractions induced with Ang II alone (5.84 ± 0.28 g). The application of AT1 or AT2 blocker led to faster decay process as Thr and Tchr in both groups were very similar.

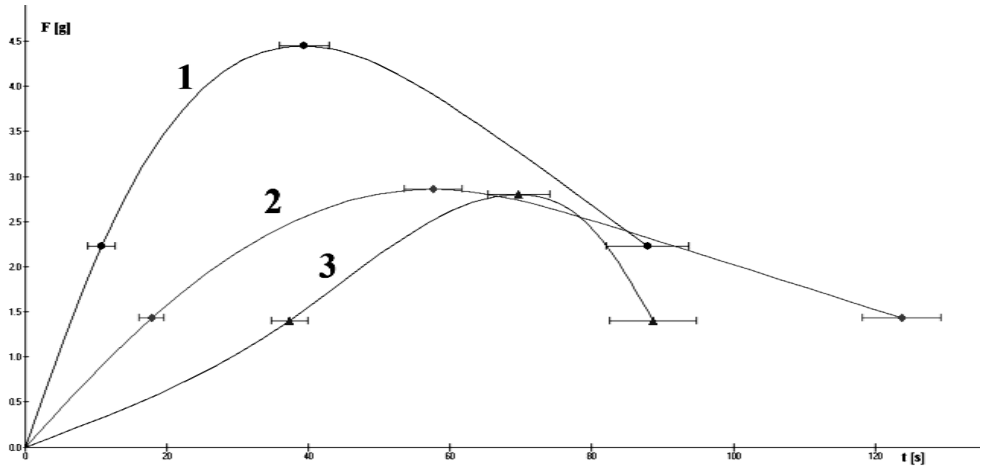

Fig.5. Contractile activity of rectal preparations induced by Ang II alone (1); Ang II applied 15 min after PD 123319 (2); Ang II applied 15 min after Losartan (3).

Discussion

Since Ang II exhibits equal affinity for binding to both AT1 and AT2 receptors, the reaction to the peptide of the given tissue is largely determined by the response of both types of receptors (13). Therefore, when AT1 receptors are blocked and AT2 are free to interact with Ang II, AT2-mediated effects become dominant [4,17].

The decreased power and prolonged phases of Ang II-mediated contractions of urinary bladder and stomach when AT1 were blocked, confirmed the leading role of these receptors for the development of Ang II-induced responses in these organs [1,12]. However, the registered increase in the strength of Ang II-provoked contraction when AT2 blocker was

applied, suggests a possibility for an antagonistic participation of AT2 receptors or active metabolites of Ang II, in the answer of the stomach and bladder to this peptide. It was found that shorter angiotensin fragments, formed from enzyme destruction of Ang II, may play role as endogenous ligands for AT2 receptors. Ang III, Ang IV and Ang (1-7) exhibit substantial selectivity for AT2 in comparison with AT1 [3]. This data provides directions to consider the possible involvement of AT2 receptors or locally formed angiotensines for the overall contractile process in stomach and urinary bladder.

Interestingly, the analysis myometrial contractions reveals that AT1 and AT2 possibly exhibit a synergistic effect and only the activation of both receptor subtypes could contribute to a maximal uterine response to Ang II. Similar results were obtained in the study of the rectal contractions. A possible explanation for this synergistic interaction could be searched in an activation of a common signaling pathway. Such a convergence of signal transduction chains was described from some autors [11,15], which indicate that there is an involvement of a common signaling pathway with participation of phospholipase A2, when activation of both angiotensin receptors occurs. A presence of additional pathways that may be activated when blockade of Ang-2 receptors was performed and lead to formation of active peptides could be suggested as well. In the smooth muscle of the uterus and rectum, the presence of all components of RAS, including the enzymes was demontrated [4,5,18]. Therefore, the existence of alternative pathways for defragmentation of Ang II in these organs is possible. The resulting fragments reduce smooth muscle tone by interacting with a different from AT1 and AT2 receptor [4].

In conclusion, the study demonstrates that in Ang II - mediated contractile process of rat stomach and urinary bladder AT2 receptors antagonize the actions of AT1 receptors, while in the uterus and rectum both receptor subtypes act synergistically. The locally produced angiotensin derivatives probably also participate in Ang II – induced smooth muscle activity by interaction with AT2 or another receptor type, different from AT1. The possible local formation of active angiotensin fragments would alter significantly the development of the normal Ang II-induced contractile activity of the investigated organs.

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DO3. IN VITRO STUDY OF ROSEMARY OIL EFFECT ON SMOOTH AND STRIATED MUSCLE

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Abstract

Rosmarinus officinalis has been extensively used predominantly empirically since ancient times for maintaining a human health. The unfractionated oil extract from this herb is known as Rosemary oil (RO). It consists of many compounds with distinct biological activities which are studied in vivo and in vitro and many effects as antimicrobial, anti-inflammatory, antitumor and antioxidant are investigated. Effects of this oil on smooth muscle contractility are studied in a previous paper but keeping in mind that essential oils are predominantly used for manual massages the actions of RO on skeletal muscles are more important.

The aim of this study was to investigate the influence of Rosemary oil on striated muscles contraction provoked by electrical field stimulation and to compare with this on smooth muscles. We use abdominal transversal muscles strips isolated from guinea pigs. The twitch and tetanic muscles force were provoked by means of repeated multipulse electrical field stimulation (EFS), square-wave pulses of supramaximal intensity for 3s followed by a 7s pause. The concentration effect curve of the action of Rosemary oil (1,5.μM ÷1,5.mM) on this type electrical field stimulated - muscle activity was plotted on a graph. During our observation, we investigated the effect of Rosemary oil on contractile properties of abdominal muscles and calculated half maximal effective concentration (EC50). Our study revealed the myorelaxant activity of the essential oil on both type of muscle tissue after application of concentrations higher than 0,15 mM.

Key Words: Rosemary Oil, Electrical field stimulation, Striated and smooth muscle contractility

Introduction

The interest of the scientist to the herbal remedies has grown considerably, because of their harmless effect especially as therapeutic agents to reduce inflammation and pain. Rosemary oil is one of the most popular essential oils and has become popular over the years as its various medical application. According to the European Medicines Agency (EMA) from 2010, rosemary is a traditional herbal medical product [5]. Rosemary essential oil can be used for treating as an adjuvant in the relief of minor muscular and articular pain and in minor peripheral circulatory disorders [4]. Rosemary oil contains virtually only substances from the group of the terpenes: a-Pinen 12,1% (9,0%–14%), Campher 5,0% (2,5%–6,0%), b-Pinen 5% (4,0%–9,0%), b-Myrcen 1,5% (1,0%-2,%), Limonen 3,9% (1,5%-4,0%), Cineol 44,7% (38,0%- 55,0%), r-Cymen 1,4% (0,8%-2,5%), Campher 10,1% (5,0%-15,0%), Bornylacetat 1,0% (0,1%-1,5%), a-Terpineol 2,2% (1,0%-2,6%), Borneol 3,8% (1,5%-5,0%). The areas of application for rosemary oils are considered to be for dyspeptic complaints (4–6 g drug, to be taken internally), as an adjuvant for rheumatic diseases (external use, bath supplement, spirit, ointment), for circulatory complaints (external use), additionally to prompt wound healing, as a mild antiseptic and for use on the biliary tract and the small intestine because of its

spasmolytic effects [13]. The antibacterial, antifungal and anti-oxidant effects of RO have been described from many authors [2,12]. The oil also has antiseptic and antispasmodic qualities and it is therefore used in some respiratory diseases. Some rosemary constituents have analyzed in vitro and in vivo as a potential anticancer agent [7]. Its ability to reduce DNA damages and tumor formations determine the possible application as the chemo preventative agent [14]. Its anti-inflammatory and analgesic potential of RO was proven [16]. The smooth muscle cells have undergone relatively low differentiation and specialization during phylogeny and possess a variety of receptors specific to physiologically active compounds. That is why the smooth muscle model are widely used in medical and biological studies that deal with the mechanism of action of numerous pharmacological agents: drags, synthetic substances like the model products [15] or water-soluble fractions isolated from row biologically active compounds [1]. Smooth muscle cells are abundant in membrane receptors whose activation most often results in changes in the muscle tonus, namely muscle contraction or relaxation. The experiments with smooth muscles spontaneous activity are impeded by the fact that rosemary oil has different actions. Some authors induced contractions on muscles strips from ileum by KCl and demonstrated the involvement of calcium channels in this activity [17]. However, there is no scientific evidence concerning the RO effect on contractility of abdominal transversal muscles and mechanism of its action is unknown. This determines our interest to RO as a potential product for reduction of striated muscle contraction.

Methods

The impact of rosemary oil on spontaneous contractile activity (SCA) of the smooth-muscle strips (SMS) from a guinea pig stomach was examined. This model enables simultaneous registration of the spasmolytic effects of Rosemary oil on smooth muscles and comparison with electrical field indirect (nerve) and direct striated muscles stimulation.

Animals and tissue preparations

Ethics statement: All experiments (in vivo and in vitro) were approved by the Bulgarian Food Safety Agency and the Ethics Committee of the Medical University of Plovdiv, Bulgaria.

Fifteen male Guinea pigs (300÷350) g were kept under standard laboratory conditions (temperature 22±1°C, humidity 45% and 12-h light cycle). All in vitro experiments were approved by the Bulgarian Food Safety Agency.

Preparation of the smooth muscle fibres: The specimen of smooth muscles used for the experiments were taken from the stomach of male guinea pigs. The strips of stomach muscle are 12–14 mm in length and 1–2 mm wide and come from the corpus region of the stomach. The SMS were prepared in a circular direction, starting with the serosa, along the big curvature and as far as possible in the direction of the fibres.

Preparation of the striated muscle fibres: Male Guinea pigs were euthanized and transversus abdominis striated muscles (ASM) were isolated. Preparations were obtained while cutting the muscle tissue in strips (20.0 ± 1.5 mm length, 3.0 ± 0.5 mm width). The samples were immediately rinsed and cooled (4°C) preparation solution.

Measurement of the striated muscles electrical field stimulation (StM-EFS) and the spontaneous contractile activity (SCA) of the smooth muscles

The muscle strips were isometrically fixed in individual organ baths containing 15 mL modified Krebs' solution (KS) with temperature 35.5 ± 0.2 °C and constantly oxygenated with 95% O_2 and 5% CO_2. The preparations were connected to an isometric force transducer (TRI 201, LSi LETICA; Pnlab s.l., Barcelona, Spain). Preparations were allocated to the organ baths in a random manner and muscle tension (7 mN) was applied to achieve isometrical recording. They were allowed in an equilibration period of 20 minutes. For inducing contractile activity, we used electrical field stimulation (EFS) through platinum electrodes. The electrodes were connected to both sides of each strip and to an electronic stimulator (EFS-PZ03, C-optic, Bulgaria). Contractions similar to those induced by indirect (nerve) stimulation were achieved by a pulse with following parameters: repeated multipulse EFS, square-wave pulses of supramaximal intensity (80V) and 0.02 ms in duration were applied at a frequency of 5 Hz for 3s followed by a 7s pause (NS). Tetanic contractions were induced by a pulse with intensity 60V; duration (Timp) = 0.5 ms and frequency 50 Hz which determinate conditions, similar to direct (muscle) stimulation (MS). Shiina et al. (2010) and Su et al. (2012) conducted experiments with EFS and similar to our parameters. The mechanical muscles activity was recorded according to experimental protocol represented previously [8] with 500 ms interval of discretisation for SCA of SM registration and with 1 ms interval of discretisation for EFS of ASM.

Concentration-response curves for Rosemary oil

The normal contractile activity was recorded after the equilibration period when ASM was stimulated direct (60V; Timp=0.5ms and frequency 50 Hz) and indirect (80V; Timp=0.02ms and frequency 5 Hz). For indirect stimulation, RO in concentration 3 μM was added to the organ baths and the change in the contractile activity was recorded for a 5-min period. The strips were washed out with KS before adding a higher concentration. RO concentrations 15 μM; 30 μM; 45 μM; 90 μM; 120 μM; 150 μM; 300 μM; 600 μM and 900 μM were studied and a concentration - effect curve was obtained. For direct stimulation, RO was applied in the same concentration and with higher one 1,5mM. The cut-off time for each experiment was 45 minutes after muscle isolation. The concentration-response curve of Rosemary oil effects on CCA of SMS was calculated after application of concentrations of RO used for EFS and the lower two 0,3 μM and 1,5 μM.

Drugs and solutions

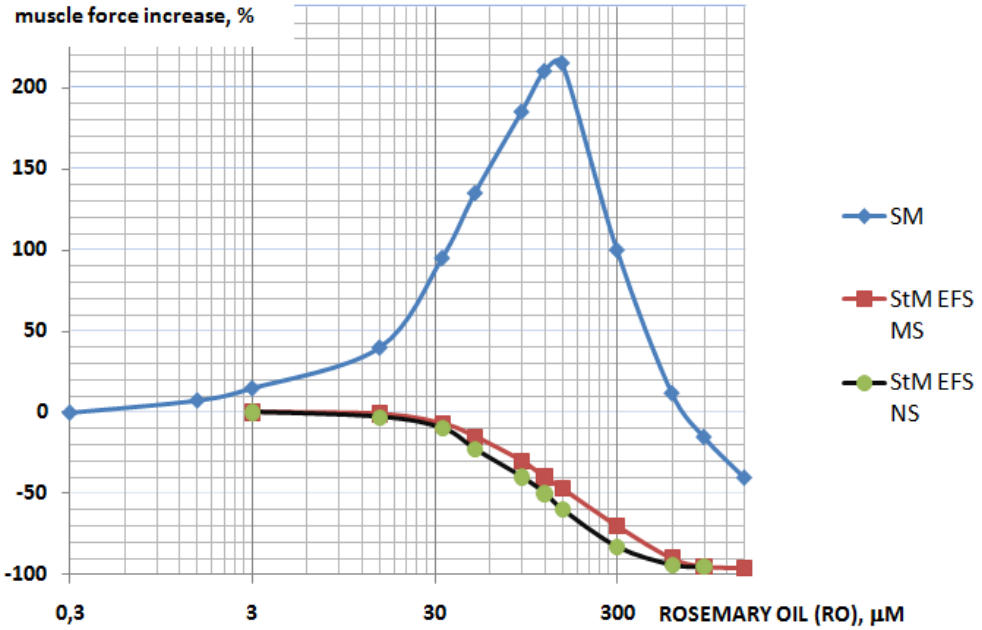
Rosemary oil Ph.Eur. (Ch. B. 296330, WE 06100393, Typ Tunesien, Dullberg comp. Hamburg, Germany. The preparation solution contains Na^+ (143 mmol/L); K^+ (5.84 mmol/L) and Ca^{2+} (3.7mmol/L).Composition of KS: Na^+ - 143 mmol/L; K^+ - 5.84 mmol/L; Ca^{2+} - 2.5 mmol/L; Mg^{2+} - 1.19 mmol/L; Cl^- - 133 mmol/L; HCO_3^- - 16.7 mmol/L; $H_2PO_4^-$ - 1.2 mmol/L and glucose - 11.5 mmol/L.

Statistics

Data are presented as MEAN and standard error of mean (SEM). Normal distribution was tested with One-sample Kolmogorov-Smirnov test. One-way analysis of variance (Kruskal-Wallis test) and Dunn's Multiple Comparison post hoc tests were used. The number of tested preparations is given as n. Results were considered significant at $P < 0,05$.

Results

We evaluated the effects of RO in concentrations of 3 µM (n=7; *P* >0.05); 15 µM (n=8; *P* >0.05); 30 µM (n=8; *P* >0.05); 45 µM (n=8; *P* <0.05); 90 µM (n=8; *P* <0.05); 120 µM (n=9; *P* <0.05); 150 µM (n=7; *P* <0.05); 300 µM (n=7; *P* <0.05); 600 µM (n=7; *P* <0.05); 900 µM (n=7; *P* <0.05) and 1,5 mM (n=7; *P* <0.05) on the muscle force, generated by ASM after StM EFS MS. The maximal muscle force after MS in absence of RO was taken as 100% (Fig.1.).



Kruskal-Wallis test			P<0.0001	
Dunn's Multiple Comparison Test			Difference in rank sum	P value	Summary
SM	vs	StM MS	26,90	P < 0.001	***
SM	vs	StM NS	29,20	P < 0.001	***
StM MS	vs	StM NS	2,300	P > 0.05	ns

Figure 1. top: Concentration-response curve of 3µM÷ 0,9mM RO on the generated muscle force of AMS after StM EFS-MS and StM EFS-NS, and representation of the effects of 0,3µM÷ 1,5mM RO on the spontaneous contractile activity of the smooth muscle strips of the guinea pig stomach. bottom Kruskal-Wallis test and Dunn's Multiple Comparison post hoc analysis.

The concentration-response curve of RO on the generated muscle force of AMS after StM EFS MS was calculated (n=7) with RO in concentrations of 3 µM (P>0.05); 15 µM (P>0.05); 30 µM (P >0.05); 45 µM (P <0.05); 90 µM (P <0.05); 120 µM (P <0.05); 150 µM (P <0.05); 300 µM (P <0.05); 600 µM (P <0.05) and 900 µM (P <0.05). Graphical representation of 0,3µM (P>0.05); 1,5 µM (P>0.05); 3 µM (P>0.05); 15 µM (P<0.05); 30 µM (P <0.05); 45 µM (P <0.05); 90 µM (P <0.05); 120 µM (P <0.05); 150 µM (P <0.05); 300 µM (P <0.05); 600 µM (P <0.05) and 900 µM (P <0.05). RO effects on SCA of SM was plotted by n=7.

The maximal muscle force of preparations was reduced with 50 % when 0,15 mM RO (EC50 =0,15 mM) after MS and with 0,10 mM RO after StM EFS-NS. We found no significant

difference in the duration of muscle twitches before and after treatment with RO (data not shown).

Single effect of 0,5 mM RO on the smooth muscle strips of the guinea pig stomach reveals time dependance and control calculation on 5min was 49,5% lower as the initial one. After 20min continuous application of the same concentration leads to 80% reduction of SCA of guinea pig SM strips (Fig. 2).

The application of EFS NS evokes a twitch muscle contraction. In the background of 0,10mM RO, applied for 5 min, the maximal muscle force (4.8 ± 0.7 mN) was reduced to 2.3 ± 0.4 mN (Fig. 3.- top) after 20 min the maximal muscle force was (1.6 ± 0.5 mN) (Fig. 3.- top). The application of stimuli with high frequency and long lasting duration of 0,5ms evokes a tetanic muscle contraction. In the background of 0,15 mM RO, applied for 5 min, the maximal muscle force (6.3 ± 0.5 mN) was reduced to 3.1 ± 0.8 mN. If 0,15 mM RO presences in the medium for 20 min we can detect only a little alteration of the normal muscular tonic activity. The muscle response to the electrical stimulus 500µs 60V 50Hz can be calculated as a 5%- rise of the tonic striated muscle force (Fig. 3.-bottom).

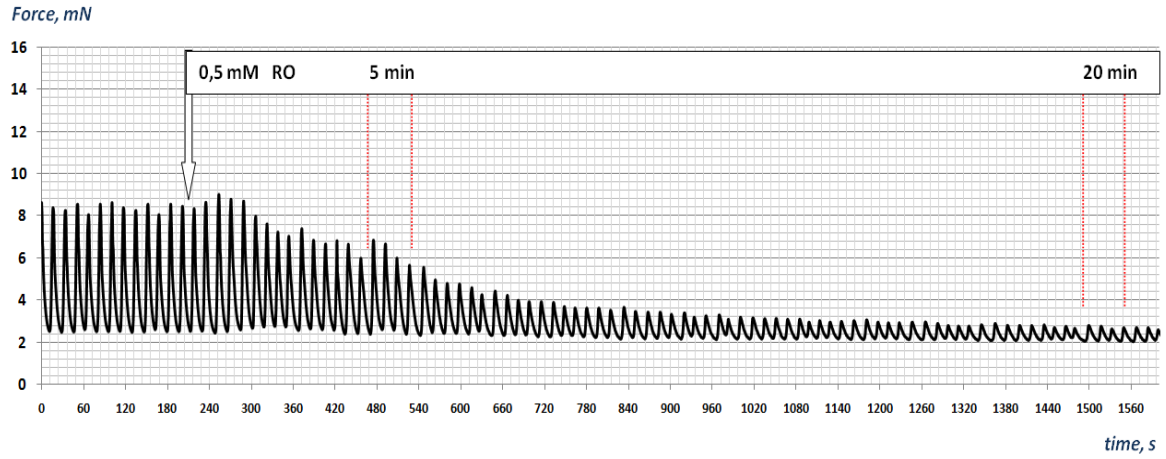


Figure 2. The record of mechanical activity of the smooth muscle strips of the guinea pig stomach after administration of 0,5mM RO in 210s.

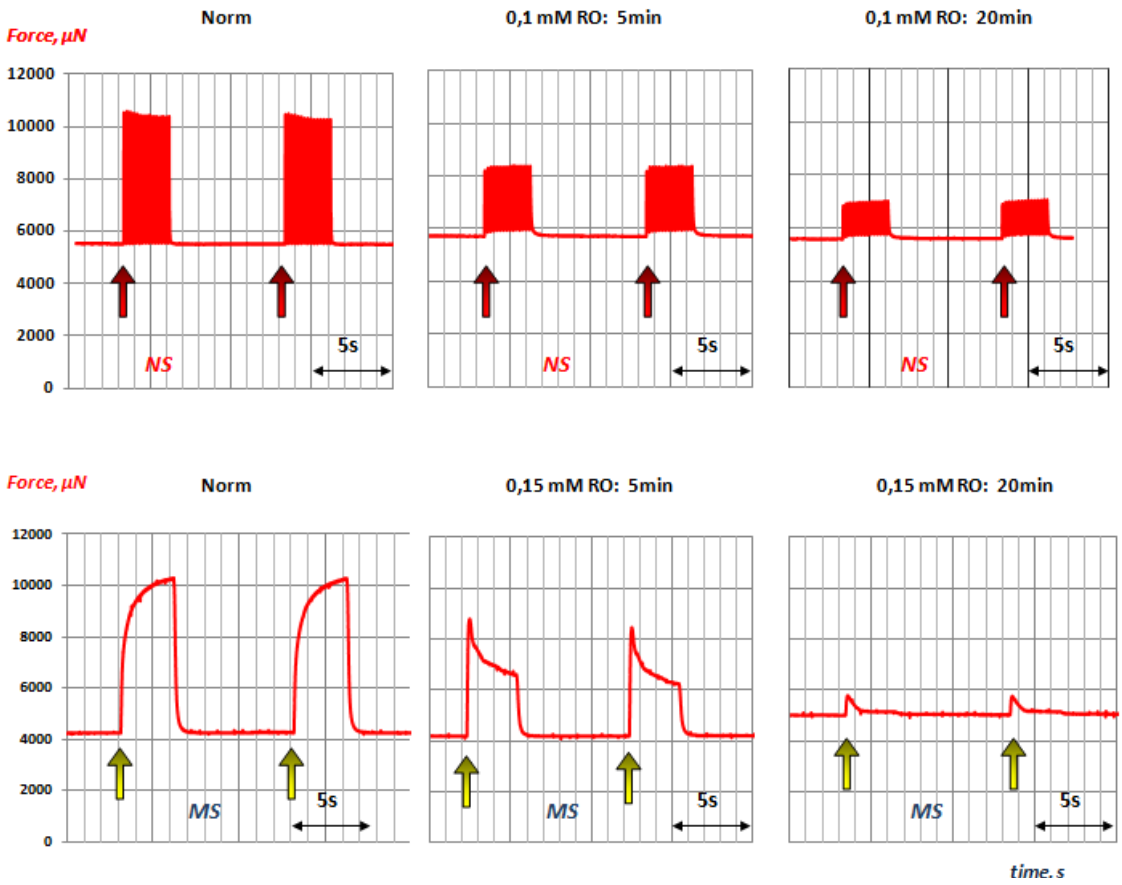


Figure 3 . top: Effects of 5 min and 20min 0,10 mM RO action on the maximal muscle force generated by AMS after NS. Muscle contractions (80V; 0.02ms; 5 Hz) before and after addition of RO to the organ baths. Arrows (↑) indicate the initial moment of NS. bottom: Effects of 0,15 mM RO of 5 min and 20min action on the maximal muscle force generated by AMS after MS (60V; 0.5ms; 50 Hz) before and after addition of RO to the organ baths. Arrows (↑) indicate the initial moment of MS.

Discussion

Due to the main application of essential oils in aromatherapy massage it will be important to prove their ability to penetrate through the skin and the existence of the local effect. But action on muscles is different and nonspecific[10]. The specific effects of the natural products are analyzed on different models in vitro – on cellular or tissues cultures. Cell cultures are informative for investigation of the effects on cellular level.[4] But to analyze the effect of some products on the whole organism is necessary to choose a subject, which is most close to the real subject. The chemical diversity of the essential oil is constituent determines the diversity of their ability to affect various biological structures, intramural nerves, membrane receptors or channels. RO is one of the most popular essential oils and has a wide array of health benefits. One reason for this can be seen in represented results, which reveal its ability to modulate muscle contraction of the both types of muscles (smooth and skeletal). The main advantage of our experimental model with striated muscles is a fully preserved nerve and muscle tissues and the most important preserved natural connection between them-

neuromuscular junction, which provides functional communication between them. The activation of nicotinic receptors by acetylcholine, followed by increased Na^+ and K^+ permeability is the main mechanism of skeletal muscle contractions. The muscle force generated depends mostly on the intracellular Ca^{2+} and the nicotinic receptors are one of the possible pathways of Ca^{2+} influx [3, 6]. The influx of Ca^{2+} is regulated by action potentials via transverse tubule L-type Ca^{2+} channels which also play a major role in the Ca^{2+} release from the sarcoplasmic reticulum. A single depolarizing stimulus leads to the opening of only a few channels [9] and a subsequent depolarization resulting in feedback connection, new acts of an opening of more channels, increased Ca^{2+} influx and muscle contraction. In this research for single stimulation is used right angle 80V impulse with a 0,02ms duration which results in twitch contraction and nerve and muscle stimulation together can be done by means of increasing of pulse duration up to 0,5ms. The motor-induced responses were studied in implementing the EFS on controls on such pre-treated with 0,3μM÷1,5mM RO. The maximal muscle force of preparations after NS was reduced with 50% when 0,10 mM RO was present in the medium. For tetanic EFS Rosemary oil EC50 is bigger 0,15mM, but two effects of electrical stimulation: direct and nerve are statistically undistinguished - Dunn's Multiple Comparison test P>0,05 (Fig. 1.). In our results, there are no differences between nerve and direct EFS of AMS after application of Rosemary oil and that fact leads to a conclusion about an existence of only one a direct muscle action of RO without any effect on the nerve structures. In smooth muscles experiments, EC50=0,5mM of RO is 5 times higher and one reason for that is a representation of many different receptors and channels in this type of tissue (Fig. 2).An application of RO in high concentrations on smooth muscles leads to spasmolytic effect which is reduced at low concentrations in vitro [11].

Our study revealed the time-dependent myorelaxant activity of RO on both types of muscle samples. It is known that a fundamental prerequisite for skeletal muscle relaxation is the reduction of intracellular Ca^{2+} concentration. This reduction can be done in different ways : an inhibition of transmembrane Ca^{2+} transport and Ca^{2+} release from intracellular calcium stores, activation of different types of Ca^{2+}pumps, repolarization, a release of various intramural mediators, other channels, second messengers, enzyme action and many others as well as the synchronized activation of several of the said processes [18]. The long lasting application of RO for skeletal (EC50=0,15mM, Fig. 3) and smooth (EC50=0,50mM, Fig.2) muscles describes the similar effects resulting in response amplitudes reduction and most likely possess the identical mechanism of action. An experimental program, in vitro, conducted with striated muscles diminishes a number of this muscle-relaxation agents to several ones: N-Ach receptors, Ca^{2+}channels, and hyperpolarization, but all connected with Ca2+transmembrane transport.

Conclusion

The present results suggest that Rosemary oil depresses force development, most probably by acting as a calcium influx reduction and can be used against cramps of skeletal muscle and for smooth muscle relaxation.

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DO4. INTERRELATIONS BETWEEN HEPCIDIN AND HOMEOSTASIS OF COPPER AND IRON

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Introduction

It is well established that copper and iron have significance for important physiological processes. With the development of scientific knowledge, there is increasing recognition of the importance of their optimal intake for normal erythropoiesis.
Copper (Cu) and iron (Fe) belong to the group of transition elements [3]. Both metals exhibit similar physico-chemical properties due to their ability to accept or donate electrons and to participate in electron transfer reactions [3,18]. Copper is a crucial for life trace element performing a number of physiological functions. Copper is included as a cofactor or allosteric component in enzymes involved in cellular respiration, defence against oxidative stress, iron metabolism, formation of connective tissue, synthesis and metabolism of neurotransmitters, melanin synthesis, nerve myelination [18,41,46,47]. In addition, as a component of copper-dependent transcription factors, copper also plays an important role in the regulation of gene expression [41,47,49].
Iron is the most abundant metal found in the human body. Over 65% of the body iron is included in the haemoglobin of circulating erythrocytes participating in oxygen transport and delivery [29]. Iron is also an integral component of haem-containing and non-haem-containing enzymes involved in electron transport, defence against free radical formation, phagocytosis, amino acid metabolism, DNA synthesis [3,36]. The role of iron as a cofactor is attributed to its redox active properties, similarly to copper [30]. However, the same physico-chemical properties of copper and iron that make them catalysts essential for the life, determine their toxic effects. Both metals (as Cu^{1+} and Fe^{2+}) are capable to participate in the Fenton reactions producing the most reactive hydroxyl radicals •OH [3,24,41]. Therefore, a strict regulation of copper and iron homeostasis is needed to meet daily requirements of these trace elements as well as to prevent their toxic effects.
During the recent years, numerous scientific advances in the field of copper and iron homeostasis have been gained. As the uptake of both metals as the defence against their toxic accumulation were found to be controlled by genetic mechanisms. Multiple points of intersections between the two metals appear to exist at different levels of their metabolism.

The objective of our review is to study the interrelations between homeostasis of copper and iron and to provide evidence for the role of hepcidin in connections between them.

Systemic copper homeostasis is achieved by the balance between intestinal copper absorption and copper excretion [3]. Copper absorption occurs primarily in the small intestine by an active saturable transport mechanism at lower levels of dietary copper and by passive diffusion at high levels. The major route of excretion is via the bile [41,42]. A population reference intake (PRI) has been established for the European population of 1.1 mg/day for adults [42]. Copper exerts its physiological effects when included in the structure of ceruloplasmin, a protein produced by the liver. Approximately 90% of plasma copper is in complex with ceruloplasmin [37,45]. Free ionic copper, similarly to iron, has toxic effects.

Intracellular transport and storage of copper are provided by the proteins chaperones and metallothioneins, thus preventing release and toxic accumulation of free copper ions in the cytosol [3,18,41,49].
Copper exhibits important interactions with iron at the level of three major processes in iron metabolism – intestinal iron absorption, iron mobilization from storage sites and iron utilization by the bone marrow.

Interactions between copper and iron at the level of intestinal absorption

Iron and copper are tightly intertwined during the process of absorption in the duodenum. Their absortion is provided by the activity of protein molecules, mainly transporters, at the apical and basolateral surfaces of intestinal epithelial cells.
Non-haem iron is absorbed in the duodenum as its reduced ferrous state (Fe^{2+}). Duodenal cytochrome b (Dcytb, Cybrd1) is the major ferric reductase in the duodenum, expressed on the apical membrane of duodenal enterocytes [26,30]. It was initially considered that Dcytb had only ferric reductase activity. Newer investigations confirm that in addition to the capability to reduce iron complexes, Dcytb also exhibits cupric reductase activity [26,50]. Reduction of iron and copper enables their uptake into enterocytes via divalent metal transporter 1 (DMT1) and copper transporter 1 (Ctr1), respectively. These data suggest that Dcytb plays an important physiological role in both iron and copper intestinal absorption.
Divalent metal transporter 1 is a protein expressed in the apical membrane of duodenal enterocytes [25]. DMT1 ensures the uptake of non-haem ferric iron after it has been reduced to ferrous form by Dcytb [24,30]. It was initially identified as iron transporter, also able to transport a broad range of divalent metal ions, including copper [16]. The issue of the role of DMT1 in copper transport still remains debatable. It has been demonstrated that DMT1 is able to transport Cu^{1+} [1]. Subsequent studies have found competitive inhibitions between iron and copper for their cellular uptake as well as influence of both metals on DMT1 expression and activity [2,4]. These interactions between iron and copper are potentially associated with reciprocal alterations in their absorption and bioavailability [4]. However, recent research has shown that DMT1 does not stimulate the transport of Cu^{1+} and Cu^{2+} [20].
Involvement of iron-related proteins in transport of copper suggests that physiological or pathological changes in the levels of these proteins could lead to modulations in metabolism of copper.
The process of cellular iron efflux from duodenal enterocytes depends on the basolateral iron transporter ferroportin (Fpn1, Ireg1). Ferroportin is a protein which transfers ferrous iron into the plasma. It is the solely known iron exporter in vertebrates. Ferroportin is expressed on the membranes of enterocytes, liver Kupffer cells and hepatocytes, splenic macrophages, and placental cells [12]. Cellular iron efflux from enterocytes is also proposed to depend on another membrane-bound protein, the multicopper ferroxidase hephaestin, anchored in the basolateral membrane [48]. The multicopper ferroxidases are a family of cuproenzymes involved in iron export from different tissues. These enzymes catalyze the oxidation of ferrous (Fe^{2+}) iron into the ferric (Fe^{3+}) state, which enables subsequent binding of iron to its plasma carrier, transferrin [8].
Hephaestin is also shown to be needed for the activity of ferroportin, and both proteins act in concert [9]. In an absence of a multicopper ferroxidase, ferroportin undergoes degradation, leading to decreased iron efflux into the circulation [10,23].
The recommended dietary intake (RDI) of iron has been estimated as 8-10 mg/day for adult males and 15-20 mg/day for women of reproductive age [43].

Interactions between copper and iron at the level of iron mobilization from storage sites

Iron metabolism is unique by the fact that most of the iron required daily has endogenous origin. It is supplied by the process of recycling senescent erythrocytes within the macrophages of liver and spleen. Another multicopper ferroxidase, ceruloplasmin (ferroxidase I), ensures the oxidation of ferrous iron after it has been transferred through the membrane by ferroportin [24]. Ceruloplasmin is thought to have an essential physiological role in iron mobilization from sites of its storage, namely reticuloendothelial cells and hepatocytes [17]. Ceruloplasmin is mainly expressed as a serum protein secreted by the liver, and, to a less extent, as a membrane-bound protein in astrocytes [19].
The multicopper ferroxidases are the best known links between iron and copper metabolism [39]. They play a central role in iron homeostasis for a wide range of species [45]. Decreased activity of the multicopper ferroxidases hephaestin and ceruloplasmin is considered to lead to disturbances of iron homeostasis [7,15,17,40,48]. A new member of the multicopper ferroxidase family has recently been identified, zyklopen, which is involved in placental iron transport from the mother to the fetus [8].

Interactions between copper and iron at the level of iron utilization by the bone marrow

The majority of iron transported in plasma is directed to the erythroblasts, where it is incorporated into the haem for haemoglobin formation [24]. Iron uptake by the erythroid precursors occurs via a process of receptor-mediated endocytosis. The endosomal DMT1 has been shown to mediate the transport into the cytosol of iron released from transferrin. Iron is subsequently delivered to mitochondria for heme synthesis [24,25,29,30].
Copper is tightly involved in iron utilization by the bone marrow [49]. Ferroxidase II is responsible for the oxidation of iron during erythropoiesis [51]. Ferrochelatase and cytochrome-C oxidase are copper-containing enzymes necessary for haem biosynthesis [35,51]. A feature of copper deficiency is the development of anaemia documented in animals and humans. It is characterized by low serum iron and accompanied by iron accumulation in the intestine, liver, and spleen [47,51]. Current view is that anaemia in copper deficiency is caused by impaired iron utilization or uptake by the bone marrow, rather than defective iron absorption and mobilization [21,28,37,38].

The role of hepcidin in iron homeostasis

Iron elimination occurs primarily by the exfoliation of epithelial cells [25]. In striking contrast to copper, there is no physiological mechanism for regulation of iron excretion [24]. Maintenance of iron homeostasis is achieved by the regulation of iron absorption, recycling, distribution, and storage [30]. Release of iron in plasma during its duodenal absorption, macrophageal recycling, maternofetal transfer, and storage in the liver is controlled by a small peptide hormone, hepcidin [32,34]. Hepcidin is a newly discovered peptide, which appears to have a central role in regulation of systemic iron homeostasis [22,32,36]. It is synthesized and secreted by the liver in response to inflammation and excess body iron levels [24,32]. Factors reducing hepcidin expression include anemia and hypoxia [33]. The mechanism of hepcidin action includes binding to its receptor ferroportin followed by internalization and lysosomal degradation of ferroportin [11,31]. Reduction in the number of iron exporters on the cell membrane leads to diminished iron release into plasma [22,32]. This results in decreased serum iron [44]. Hepcidin is considered to be the main regulator of iron plasma concentrations [24,32].

The role of hepcidin in connections between copper and iron homeostasis

Besides its direct effect on iron bioavailability by interaction with ferroportin, hepcidin also influences iron-transport proteins. Results from experimental and observational studies show that hepcidin inhibits expression and activity of Dcytb, DMT1, and Fpn1 [6,30,52]. As we previously described, iron-related proteins Dcytb and possibly DMT1 are involved in the transport of different from iron metals, such as copper. These findings suggest that physiological or pathological changes in hepcidin levels could lead to modulation of uptake and tissue distribution for both metals iron and copper.

Metals themselves could also modulate hepcidin expression at the transcriptional level. Metal responsive elements (MREs) are identified in the hepcidin promoter, which react to divalent metal ions, maximally to zinc, and to a lesser extent, copper. Copper increases hepcidin expression by an interaction between MREs in the hepcidin promoter and MRE-binding metal transcription factor 1 (MTF1) [5,24]. Therefore, hepcidin can be considered as a sensor of divalent metal ions [5]. Similar effects of zinc and copper have been observed on metallothionein transcription [5,47]. Metallothioneins (MTs) are the main intracellular copper binding proteins which have high affinity for copper and participate in its storage and protection against toxicity of free copper ions [3,49]. The functions of MTs in metal detoxification and protection against oxidative stress have been well demonstrated, thus raising the question on whether hepcidin is able to perform similar functions [5].

Hepcidin is a key hormone for iron homeostasis, but there is evidence that it also has the ability to bind divalent metal ions, including copper [27,45], zinc [45], and even iron [13,14]. The same amino acid residues determining the biological activity of hepcidin, namely destruction of ferroportin and hypoferremia, were found to be responsible for its copper binding properties [45]. These findings suggest a non-hormonal role of hepcidin in iron metabolism or potential conformational mechanism for binding divalent ions.

Conclusions

Improving our knowledge of physiological connections between hepcidin and homeostasis of copper and iron would be of considerable benefit to the clinical practice. These findings will gain insight into the molecular mechanisms of disturbances of copper and iron metabolism. In contemporary life, there is significant environmental and dietary exposition to various metals, and this requires taking into consideration potential interactions between copper and iron. Data would also provide the basis for programs of food fortification and optimal trace element supplementation in physiological and pathological conditions.

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DO5. APIPUNCTURE OR BEE VENOM IN ACUPUNCTURE- LITERATURE REVIEW

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Introduction

The venom of *Apis mellifera* has been used extensively in oriental medicine for many centuries and has become increasingly popular as a therapy in many nations, mostly in Korea. Bee venom acupuncture (BVA) is a kind of herbal acupuncture taking advantage of diluted bee venom instead of distilled herbal decoction . BVA simultaneously exerts pharmacological actions from the bioactive compounds isolated from bee venom and mechanical actions from the acupuncture stimulation. The extracted and processed venom, or a bee sting, is applied to the acupoints, according to the specific disease. BVA has been used to relieve various musculoskeletal conditions- neuralgia, arthralgia, cervical disc protrusion, progressive muscle atrophy, immune-related diseases -arthritis and rheumatism, MS, Parkinson's disease, central post-stroke pain, cancer associated pain.

Aim

To investigate the use of bee venom and acupuncture in treating different diseases.

Materials and methods

Literature searches on experimental studies and clinical trials of BVA were performed on the databases from PUBMED, EMBASE and others. During the search we used key terms 'bee venom', 'bee venom therapy', 'acupuncture' apipuncture, pain, cancer, arthritis, chonic pain.

Results

The active compounds of BV are few peptides- melittin, adolapin, and apamin, enzymes- phospholipase A_2, and amines. They have analgesic and anti-inflammatory effects, while analgesic effect induced by acupuncture strongly influences the psychological aspect of the pain. Acupuncture has been demonstrated to possess neurotrophic and neuroprotective effects while bioactive compounds of the bee venom suppress inflammation and have anti-cancer activity. BVA reduce pain in patients with musculoskeletal syndroms, central post-stroke pain, cancer associated pain.It is promising alternative therapy for the long-term treatment of diseases like rheumatoid arthritis, Parkinson's disease and MS.

Conclusion

BVA is kind of pharmacopuncture or herbal acupuncture which combines the effect of acupuncture and medicinal effect of bee venom injected in acupoint. A lots of findings suggested that BVA could be used with satisfactory results in many diseases thanks to his analgesic, anti-inflammatory and anti-cancer activity.

Key words. bee venom, bee venom acupuncture, apipuncture, pain, cancer, arthritis, alternative therapy

Session E.

Chairpersons:

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EO1. MODIFIED WITH 1,8-NAPHTHALIMIDE METALLODENDRIMERS AS ANTIMICROBIAL AGENTS

Ivo Grabchev[1], Desislava Staneva[2], Evgenia Vasileva-Tonkova[3], Paula Bosch[4]

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Dendrimers are a relatively new class of polymers of perfectly branched and well defined structure, possessing a good number of functional end reactive groups allowing modification of the dendrimers. In the last years, dendrimers are becoming more and more useful in the fields of biology and medicine due to their unique properties. That type of investigations is focused on the dendrimers as potential antimicrobial compounds or agents improving the antifungal or antibacterial activity of existing chemotherapeutics. Furthermore, many chemotherapeutics have been successfully incorporated into dendrimer nanoparticles or attached to their functional groups for improving the solubility and therapeutic efficacy. The synthesis and investigations of metal complexes with dendrimers as ligands being an approach to developing new drugs is a new research area in inorganic, pharmaceutical and medical chemistry gaining much interest.

This work describes the modification of poly(propylenamine) (PPA) dendrimers of different generations with 1,8-naphthalimide units as well as the formation of their Cu(II) and Zn(II) complexes. The chemical structures of dendrimers free of metal ions and the metallodendrimers were investigated spectroscopy spectroscopically and it was shown that the metal cations form complexes with the tertiary amines in the dendrimer core. Photophysical characteristics of the new compounds have been determined in organic solvents of different polarity and the results have shown that their functional properties depend strongly on the media polarity. *In vitro* antimicrobial screening of the metallodendrimers has revealed promising antimicrobial activities against some pathogenic Gram-positive and Gram-negative bacteria and antifungal activity against two yeasts. The minimum inhibitory concentrations (MICs) of the complexes against the test organisms were also determined. The results suggest that the new metal complexes could find application in designing new antimicrobial preparations to control the spread of infections.

PPA metallodendrimer

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EO2. ANTIMICROBIAL ACTIIVITY OF SYNERGISTIC COMBINATIONS OF BIOLOGICALLY ACTIVE COMPOUNDS

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The development of novel pharmaceuticals with wide spectrum of activity for application in human and veterinary medicine or for plant protection is necessary in connection with the increasing resistance of microorganisms to conventional antibiotics. We tried to implement an approach, using the combined impact of two groups of newly synthesized derivatives of naturally occurring compounds with antimicrobial properties in coadministration with rhamnolipid-biosurfactant. The antimicrobial potential of these combinations was assessed against model strains from different genera, able to cause damages to industrial manufacture, agriculture and human health.

The first group includes esters of thiosulfonic acid - compounds similar to allicin - the main phytoncide in *Allium sativa*. The second group of substances with 1,4-naphthoquinone structure are derivatives of lawsone, contained in the leaves of henna (*Lawsonia inermis*). The synthetic naphthoquinone analogues comprise different substituents, which probably determine the difference in their antimicrobial activity.

In this study was used rhamnolipid - biosurfactant with a proven permeabilizing effect on bacterial cell. The biosurfactant provoked changes in bacterial cell surface structures and this suggests facilitating the access of antimicrobials into bacterial cell.

The studies on the growth of model strains (*Pseudomonas aeruginosa, Bacillus subtilis* and *Alcaligenes faecalis*) were carried out to determine minimal inhibitory and minimal bactericidal concentrations of combinations - synthetic analogues/rhamnolipid-biosufactant. The results revealed that the presence of rhamnolipid - biosurfactant decreased significantly the minimal bactericidal concentration of thiosulfonates. The combining of naphthoquinone derivatives with rhamnolipid - biosurfactant affects only the minimal inhibitory concentration of naphthoquinones but bactericidal effect was not registered even at the highest tested concentrations.

The enhancement of antibacterial activity of inhibitors in the presence of biosurfactant significantly increased the therapeutic potential of these compounds. The application of combined preparations enables to modify the effect of the antimicrobial agents providing an perspective approach to overcome the drug resistance. The implementation of newly developed therapy may represent another productive antimicrobial strategy in the field of biomedicine.

EO3. MECHANISMS OF BACTERIAL COAGGREGATION

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In nature bacterial communities form biofilms under the influence of adverse factors of the environment associated with the lowering of the content of nutrients, fluctuation in temperature, concentration of oxygen, the presence of substances with antimicrobial activity. The first stage of biofilm formation is expressed in coadhesion (flocculation) of cells of one type or coaggregation of cell of species belonging to different, genetically distant genera. The essence of the process of the congregation is expressed in the formation of cell aggregates, which is due to the presence of specific molecules on the cell surface. They are characterized by protein and polysaccharide nature.

Once thought to occur exclusively between dental plaque bacteria, there are increasing reports of coaggregation between bacteria from other biofilm communities in several diverse habitats. A general role for coaggregation in the formation of multi-species biofilms is discussed.

Keywords: coaggregation, adhesions, receptors, adherence

EO4. COAGGREGATION BETWEEN *Bacillus subtilis* AND *Escherichia coli* K-12 STRAINS

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Biofilms are densely packed multicellular communities of microorganisms attached to a surface or interface. Bacteria seem to initiate biofilm formation in response to specific environmental cues, such as nutrient and oxygen availability. *Bacillus subtilis* is an industrially important bacterium exhibiting developmental stages. It forms rough biofilms at the air-liquid interface rather than on the surface of a solid phase in a liquid, due to the aerotaxis of the cells. On another hand, *Bacillus subtilis* has long served as a robust model organism to examine the molecular mechanisms of biofilm formation and a number of studies have revealed that this process is regulated by several integrated pathways. One mechanism involved in the development of bacterial biofilms with participation of *Bacillus subtilis* strains is coaggregation or interbacterial adherence that is affected by physicochemical conditions of the environment.

In this respect the aim of this study was to explore the physicochemical parameters that influence coaggregation between *Bacillus subtilis* and *Escherichia coli* K-12 strains**.** The effect of pH and temperature on the degree of coaggregation was assessed. Coaggregation occurred at a pH of 5-8, between 15 and 40 °C.

Keywords: coaggregation, pH, temperature, *Bacillus subtilis, Escherichia coli* K-12

EO5. METABOLIC SYNDROME AND CHANGES IN GUT MICROBIOTA

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The last few decades have seen a rapid expansion of the proportion of obese individuals worldwide. Metabolic syndrome and obesity are associated with alterations in the structure of the gut microbiota and its microbiome (gene content). Gut microbes can impact host metabolism via signaling pathways in the gut, with effects on inflammation, insulin resistance, and deposition of energy in fat stores. Restoration of the gut microbiota to a healthy state may improve the conditions associated with obesity and help maintain a healthy weight. Recent work has shown a shift in the representation of the dominant phyla of bacteria in the gut, both in humans and animal models. This review

summarizes the latest research into the association between microbial ecology and host adiposity and modulating of changes in gut microbiota by prebiotics, probiotics and synbiotics.

Two groups of beneficial bacteria are dominant in the human gut, the Bacteroidetes and the Firmicutes. Many autors show that the relative proportion of Bacteroidetes is decreased in obese people by comparison with lean people. This disorder is also observed in obese animals as revealed by animal studies. The identified differences are not homogeneous among the studies. Question remains as to whether changes in the intestinal microbial community are one of the environmental causes of metabolic syndrome and obesity or if they are a consequence of obesity, specifically of the unbalanced diet that often accompanies excessive weight gain. In the future, larger studies on the potential role of intestinal microbiota in human obesity should be conducted at species level using standardized analytical techniques and taking all of the possible confounding variables into account.

Session F.

Chairpersons:

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FO1. COMPOSITE MATERIAL COTTON FABRIC-HYDROGEL-NANOPARTICLES WITH POTENTIAL APPLICATION AS WOUND DRESSING

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Cotton fabric, modified with a hydrogel and containing different nanoparticles is suitable composite material for treatment of slow healing wounds with second infections. Different nanoparticles have shown good antibacterial efficacy. The hydrogel absorbs exudates from wounds and hydrate it. The textile material gives mechanical stability of the hydrogel and helps the treatment. The composite materials were obtained by surface initiate photopolymerization of acrylamide hydrogel. The modification of the cotton fabric and uniform distribution of the nanoparticles in the structure of the hydrogel have been analyzed with Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Fourier transform infrared (FT-IR) spectra, X-ray diffraction analysis (XRD), fluorescence and colorimetric analysis.
Antibacterial efficacy of the materials containing silver nanoparticles, zinc oxide nanoparticles and barium hexaferrite nanoparticles was evaluated against *Escherichia coli Acinetobacter johnsonii* and *Pseudomonas aeruginosa* known as important pathogens in clinical infections. The resulting composite material has potential to reduce the infection of wounds and the time necessary for their healing.

FO2. HYDROGEL WOUND DRESSINGS

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Hydrogel dressings contain 90% water and are usually clear or translucent, varying in viscosity and/or thickness. The high amount of water regulates the fluid exchange with the wound surface. By providing moisture to the wound, hydrogel dressings create a moist healing environment, which promotes granulation, epithelialization, and autolytic debridement. At the same time, the high water content of hydrogel dressings cools the wound, producing pain relief that can last up to 6 hours. Dressing-change discomfort is also reduced because hydrogels don't adhere to the wound surface.

Polyethylene glycol (PEG) refers to an oligomer or polymer of ethylene oxide with a molecular mass below 20 000 g/mol. PEG is used as an excipient in many pharmaceutical products as well as hydrogel dressing for wounds with different origin: diabetic skin ulcers, pressure ulcers, 1st & 2nd degree burns, post-surgical incisions, cuts, scrapes and abrasions, e.g. AMERIGEL® Hydrogel Wound Dressing.

Polyzwitterions (PZIs) are class of polymers known for their very low non-specific protein adsorption. In this respect PZIs are competing with PEG which is the current golden standard in the field. Still the potential of PZIs is to be explored in many different biomedical related applications as a competitor of PEG. More specifically, PZIs based hydrogel dressings are still not fully developed. In the literature very scarce information about PZI application in wound dressings is available.

Thus our interest arouse to synthesize and apply PZI based hydrogels crosslinked with PEG moieties bearing crosslinking agent as wound dressings thus taking advantage from all PEG and PZIs advantages.

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FO3. INNOVATIVE NEW MATERIALS FOR WOUND HEALING

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FO4. BRIEF OVERVIEW OF NEW MATERIALS FOR BONE IMPLANTS

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Abstract
Regeneration of large bone defects caused by trauma, infection, cancer (primary or metastatic) or inherent genetic disorders (such as osteogenbesis imperfects, osteopetrosis) is usually necessitates bone grafting materials. Autologous bone or autograft is still considered the clinical "gold standard" but its application is hindered due to limited bone supply and donor site morbidity. The allografts, which are transferred from other people, also have sirious disadvantages including foreign body immune reactions and risk of transmission of infections, lower quality compared to autologous grafts resulting in a slower rate of new bone tissue formation. That is why there is a growing need for new materials with optimal properties as close as possible to those of natural bone, that has to be substituted [8]. This mini review presents the main characteristics (advantages and disadvantages) of some materials for bone/dental implants used in current clinical practice.

Introduction

There are two major challenges facing modern biomedical sciences: i) to improve the treatment of the so called age-related diseases, among which are bone fiseases, and ii) to reduce the cost of health care to meet the needs of a growing percentage of elderly people [16].

The introduction of modern implants for the replacement of tooth and bone started in 1969 when was observed that a piece of titanium embedded in rabbit bone became firmly attached and difficult to remove [39]. Later, different groups of biomaterials have been developed. Each one of them has its own advantages and disadvantages and is suitable for specific applications in ortopedy and dental medicine.

Ceramics

The benefits of ceramics are well known. These materials have broad set of physico-chemical, mechanical and biological properties which can be modified by acting on their composition, porosity and surface texture to improve their characteristics making them more adequate for various biomedical applications. Bioceramics have traditionally been used for the repair of hard tissues, such as bone and teeth, mainly due to their suitable strength for load-bearing applications, wear resistance (especially alumina, zirconia and composites thereof) and, in some cases, bone-bonding ability (calcium orthophosphates and bioactive glasses) [3]. It is not surprising that in the last two decades the clinical use of ceramics has continuously increased in the world. The first material of this type used for medical purposes was alumina [28],[27]..

Aluminium oxide (Al_2O_3, Alumina) has been applied in clinic since 1971. Aong the most important properties of this materials are its inertness (biostability) with no evidence of

adverse *in vivo* reactions. Zirconia (ZrO_2) is also a bioinert material. These types of ceramic implants are not bioactive in that they do not promote the formation of bone. Aluminium oxide (Al_2O_3) dental implants osseointegrated well but was withdrawn from market because of its poor survival rate[1].

Zirconia (ZrO_2), was used for a long time as pigment for ceramics. The name "Zirconium" comes from Arabic word "Zargon" which means "golden in colour". ZrO_2 was accidentally identified by the German chemist Martin Heinrich Klaproth in 1789.
This biomaterial is characterized by superior biocompatibility, osseointegration and excellent physical properties such as high mechanical strength and fracture toughness that make zirconia a unique and stable material for use in high load situations. The advantages of Zirconia over other ceramic materials are due to the transformation toughening mechanisms operating in their microstructure that can be manifested in components made out of them. Today Zirconia is used mainly for total hip replacement (THR) but many efforts are focused to expant its application in other medical devices [35], [24], [7].
Some of the properties exhibited by zirconia (including aesthetics due to light transmission and its white color) make it particularly suitable for application in dental implantology [29], [2],[1]. Long-term clinical trials will clarify better the place and role of this material in this fireld.

Metals

Metals have been used as implants since more than 100 years when Lane first introduced metal plate for bone fracture fixation. Lane's plate was eventually abandoned owing to problems with corrosion [21]. Subsequently, Lambotte in 1909 [21] and then Sherman in 1912 [[34] introduced their versions of the internal fracture fixation plate [14].
Up to now, the three most used metals for implants are stainless steel, CoCr alloys and Ti alloys. Titanium was discovered in England by William Gregor in 1791. It was named by Martin Heinrich Klaproth for Titans of Greek mythology in 1795 [1].
Metals have been used in medicine because of their inertness and structural functions – they express an excellent combination of strength and ductility. Unfortunately, metals do not possess biofunctionalities like blood compatibility, bone conductivity and bioactivity. One of the main challenges in this field is connected with biocompatibility of metals. They can corrode easily in the body that can weakens the implant and cause harmful effects in tissues and organs. That is why these materials need surface modifications (for example coating with hydroxyapatite, biopolymers, etc) to improve their bone conductivity [20]; [12]; [22]; [31]. The capacity of such coatings to act as a carrier system for osteogenic agents has been suggested [22]. Biodegradable chitosan nanoparticle coatings on titanium for the delivery of Bone morphogenetic protein-2 (BMP-2) has been recently developed to enhance the osseointegration of endoprostheses after revision operations [30].
It has been recommendet to test the oxide film stability of new Ti alloys *in vitro* in advanced media (for instance including macrophages or bone cells) in order to simulate better *in vivo* conditions[11].

Metal objects may interfere with some medical imaging systems such as magnetic resonance imaging (MRI). In order to avoid this problem, metal biomaterials have to be non-magnetic and with high density. Their stiffness differs from natural bone and may cause stress-shielding and over-loading of bone [36]. Last, but not the least, metal biomatwerials have to be composed of nontoxic and allergy-free elements.

Bioactive glass

Bioactive glasses are a special group of synthetic silica-based bioactive materials that are able to bond to living tissues stimulating new tissue growth.. They were discovered in 1969 by Larry Hench.

Bioactive glasses are based on a random network of silica tetrahedra (containing Si–O–Si bonds) that can be modified by the addition of modifiers such as Ca, Na and P (which are bonded to the network via non-bridging oxygen bonds). They can degrade in the body at a rate matching that of bone formation, and through a combination of apatite crystallization on their surface and ion release they stimulate bone cell proliferation, which results in the formation of new bone. [15]; [17]; [18]; [6]; [17].

The rates of bioactivity and resorption for bioactive glasses are different and depend on their chemical compositions [37].

Because of their brittleness these materials are not suitable for all grafting applications, such as sites that are under cyclic loads [16].

The clinical implementation of bone implants could be hindered for various reasons such as: poor osseointegration; generation of wear debris; stress and imbalance between implant and surrounding tissues; infections. The average lifetime of the current bone biomaterials is less than 15 years [32]; [9].

The design and selection of biomaterilas depend on the intended medical application. However, in all cases these materials are expected to be biocompatible meaning that they have to be highly nontoxic and do not any inflammation or allergic reactions in the human body [9].

In addition, in the biomaterials bor bone implants should be able to make possible the realization of the following processes: Osteogenesis, Osteoconduction and Osteoinduction.

Osteogenesis – is the presence or recruitment of osteoblast precursors and growth factors (coming from the graft, recipient bed abd vasculature) that is necessary for bore regeneration [26]; [6]; [33].

Osteoconduction - is a function of a bone graft that provides a tridimensional scaffold for ingrowth of host capillaries and osteoprogenitor cells [10]; [33]. Material structure and design are critical for osteoconduction. High porosity levels are required for blood vessel ingrowth and bone matrix deposition. Pore size/shape and type of pore interconnections influences the biological behavious of the materials [25 [33].

Osteoinduction - The osteoblast precursors differentiate into mature osteoblasts under the influence of osteoinductors and synthesize new bone [33]. Some factors that stimulate osteoinduction are presented in Table 1.

Table 1. Factors stimulating osteoinduction

Factor	Function in osteoinduction
Platelet-derived growth factor (*PDGF*)	Stimulation of fibroblast and osteoblast proliferation
Fibroblast growth factor (FGF)	Stimulation of fibroblast and osteoblast proliferation
Insulin-like growth factor (IGF)	Effects on cell proliferation and extracellular matrix deposition
Bone morphogenetic proteins (BMPs) – especially BMP-2 and 7	Influnces mesenchymal stem cell (MSCs) differentiation and vascular proliferation; BMP2 controls the switch between bone and muscle differentiation by controlling miRNA expression
Transforming growth factor beta (TGF-β)	Acts on cellular proliferation, Matrix deposition, vascularization
Vascular Endothelial Growth Factor (VEGF)	Induces new blood vessel formation; has direct effects on osteoblasts through endothelial cell-based BMP production

[23]; [38]; [33]; [13]

Depending on their specific application, biomaterials must meet some additional requirements. For example materials for orthopaedic implants must possess enough toughness, elasticity, rigidity, strength and resistance to fracture. Wear resistance is important for materials used for total joint replacement. Dental restoration needs strong and rigid materials with good aesthetic properties

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FO5. BRUSHITE BONE CEMENTS BASED ON AMORPHOUS CALCIUM PHOSPHATE AND TARTARIC ACID

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Abstract: Double doped (with Mg^{2+} and Zn^{2+}) amorphous calcium phosphate (ACP) with molar ratios $(Ca^{2+}+Mg^{2+}+Zn^{2+})/P$ = 1.62, $Mg^{2+}/(Ca^{2+}+Mg^{2+}+Zn^{2+})$ = 0.09 and $Zn^{2+}/(Ca^{2+}+Mg^{2+}+ Zn^{2+})$ = 0.03 was used as a cement solid precursor. Tartaric acid, 18% solution, was used as a liquid phase for the bone cements preparation. The effects of $MgCl_2$ and MgO solid modificators and of Na_2HPO_4 liquid modificator on the cements phase composition, setting times and morphology were investigated. It was found that the manipulation leads to partial dissolution of ACP and subsequent crystallization of a mixture of dicalcium phosphate dihydrate and calcium tartrate tetrahydrate in all cases. The presence of magnesium salt increases the complexation ability of the tartaric acid and crystallization of magnesium tartrate also expected but not detected. Na_2HPO_4 leads not only to sodium tartrates crystallization but also to liberation of H_3PO_4 in the systems that could provoke a crystallization of monocalcium phosphate monohydrate also.

Introduction

Calcium phosphate biomaterials as ceramics, cements or injectable systems have gained acceptance in dental and orthopaedic applications, such as, repair of bone defects, tooth root replacements, coatings on orthopaedic and dental implants [1,2] etc. Calcium phosphate based cements (CPCs) that harden inside bone defects are preferable bone grafts because of their good suitability for injection application using non-invasive surgery, good osteoconductive and partly osteoinductive properties, excellent biocompatibility and bioactivity and low price [3]. At present, there are two types of CPCs depending on the end-product of the reaction: apatite (AP) cements and dicalcium phosphate dihydrate (DCPD) or brushite cements [3-5]. The last have raised special interest because they are resorbed *in vivo* much faster than apatite ones [6-8] due to brushite metastability under physiological conditions. Moreover brushite based cements possess shorter setting times [3] than apatite ones. The major disadvantage of brushite cements is their low mechanical strength. Different carboxylic acids, such as citric, or glycolic have been used to improve the mechanical properties of brushite cements [9–10].

In recent years, ionic incorporations in calcium phosphate biomaterials, such as magnesium, (Mg), strontium (Sr) and zinc (Zn), have been the subject of great interest owing to the crucial role of these ions in the biological processes and as well changing physico-chemical properties of the materials [11-12].

The aim of this study was to prepare brushite type cements on the base of double doped with Mg^{2+} and Zn^{2+} ions amorphous calcium phosphate (ACP) and tartaric acid and to investigate the influence of solid modificators $MgCl_2$ and MgO and of the liquid modificastor Na_2HPO_4 on the cement phase composition, setting times and morphology.

Experiments

Preparation of cements

Double doped (with Mg^{2+} and Zn^{2+}) amorphous calcium phosphate (ACP) was used as a solid precursor and tartaric acid, 18 % solution, was used as a liquid phase for bone cements preparation. Modificators $MgCl_2.6H_2O$, MgO and Na_2HPO_4 were also used. The solid and liquid phases in a constant solid to liquid ratio of 2.85 g/ml were mixed and homogenized for 15 minutes to form a plastic mass. The latter was molded in rubber-molds with diameter of 10 mm and height of 5 mm for measurements of the initial and final setting times and subsequently dried in air for 24 h for X-ray and SEM studies. Three series cements were prepared:

Series A, non-modified: Starting from pure precursors: (1) (Mg ,Zn)-ACP; and (2) tartaric acid, 18 % solution.

SeriesB, magnesium salts modified: Starting from: (1) magnesium modified (Mg, Zn)-ACP precursor; and (2) tartaric acid, 18 % solution. 5% solid mixture of $MgCl_2.6H_2O$ and MgO in a molar ratio 1:5 (of sorel cement) was used.

Series C, phosphate salt modified: Starting from: (1) (Mg, Zn)-ACP; and (2) Na_2HPO_4 modified tartaric acid, 18 % solution. The molar ratio $Ca_3(PO_4)_2$ (in the solid phase) : Na_2HPO_4 was 1:1.

Characterization of cements

X-ray diffraction analysis - The phase composition of the cements was determined on D 500 (Germany) apparatus for XRD analysis, applying CuKα radiation obtained with the monochromator of the secondary beam, within the 2θ range of 10-60^o, with a step of 0.02^{o}2θ and counting time of 30s/step.

SEM images - The dried cylindrical cement samples were broken perpendicularly to the height of the cylinder and the surface of the rings was sputter-coated with gold. Their morphology and microstructure were observed using scanning electron microscope JEOL JSM-5510 equipment.

Mechanical characterization - the initial and final setting times of the cement samples prepared in the rubber-molds were determined by the Vicat needle method [13].

Results and Discussion

Double doped (with Mg^{2+} and Zn^{2+}) XRD amorphous calcium phosphate (ACP) was prepared applying two steps method: (i) continuous precipitation in the medium of simulated body fluid at pH 8; and (ii) calcination at 400^{o}C. As obtained cements solid precursor was with molar ratios $(Ca^{2+}+Mg^{2+}+Zn^{2+})/P$ = 1.62, $Mg^{2+}/(Ca^{2+}+Mg^{2+}+Zn^{2+})$ = 0.09 and $Zn^{2+}/(Ca^{2+}+Mg^{2+}+ Zn^{2+})$ = 0.03 and a specific surface area of 26 m^2/g. The liquid phase for cement preparation was tartaric acid, 18 % solution. The ratio solid to liquid phases was kept constant at all experiments and the modificators were used and varied.

The brushite cements setting reaction [14] consists of few stages: (i) dissolution of the solid precursor; (ii) formation of a super-saturated suspension; (iii) nucleation; and (iv) crystal

growth. In our all experiments the phase transformation/crystallization processes occurs in an acid media (pH 1.3). The liquid phase dissolves initial (Mg, Zn)-ACP partially or fully and less soluble salts stable in this conditions, like brushite ($CaHPO_4.2H_2O$) and calcium tartrate tetrahydrate ($CaC_4H_4O_6.4H_2O$), crystallize. It is known that Mg^{2+} ions have a strong inhibitory effect on the brushite crystals growth [15] but we have not registered it (Table 1). The results show that the presence of $MgCl_2.6H_2O$ and MgO modificators in the solid phase (Series B) does not change visible the formation features of the cements (Table 1) while Na_2HPO_4 modificator of the liquid phase (Series C) decreases two times the initial time and three times the final setting time.

Table 1. Formation features of the obtained cements

Cement	**Initial setting time, min**	**Final setting time, min**
Series A, non-modified	36	60
Series B, magnesium salts modified	30	60
Series C, phosphate salt modified	15	17

The XRD analysis of the dried cements (Fig. 1) reveals the presence only of dicalcium phosphate dihydrate (DCPD, brushite) and calcium tartrat tetrahydrate in the three series. The presence of magnesium salts (Series B) increases the complexation ability of the tartaric acid and crystallization of magnesium tartrate also could be expected. Magnesium in a large quantity creates opportunities for crystalization of low soluble magnesium salts like newberyite ($MgHPO_4.3H_2O$) [16]. We failed to detect the effect of magnesium modificators probably due to the low detection limit of the apparatus (2-3%). While the XRD spectra of the cements of Series A and B are almost equal, the spectra of Series C show small differences. In this last experiment the presence of Na_2HPO_4 in the tartaric acid solution leads not only to sodium tartrates complexation and crystallization but also to a liberation of H_3PO_4 in the systems which could provoke a quick crystallization of calcium phosphates, even of less hydrates, e.g. monocalcium phosphate monohydrate (MCPM), that could not be registered but effect on the whole spectra.

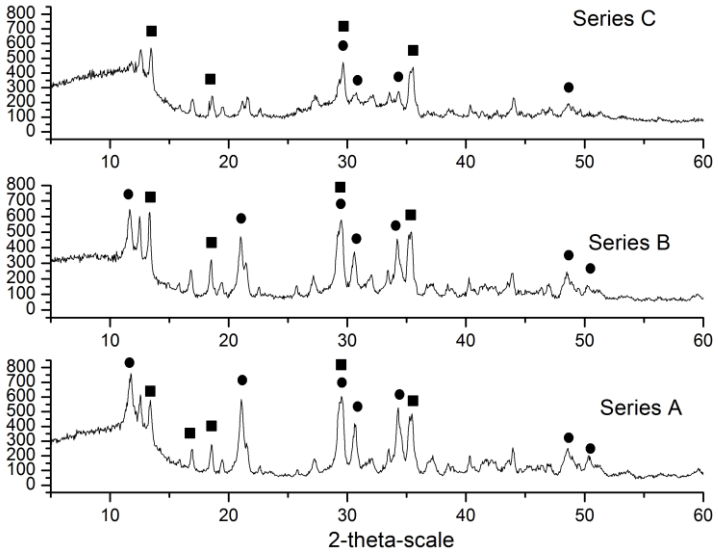

Figure 1. XRD analysis of dried cements: (●) – brushite (DCPD), (■) – calcium tartrate tetrahydrate

The above assumtions are confirmed by the SEM studies. Different prysmatic crystals, typical for DCPD form the cement structures of Series A and B, while plastered zones typical for quick spontaneous mass crystallization, and zones with a higher porosity were found in the sample of cement Series C (Fig.2).

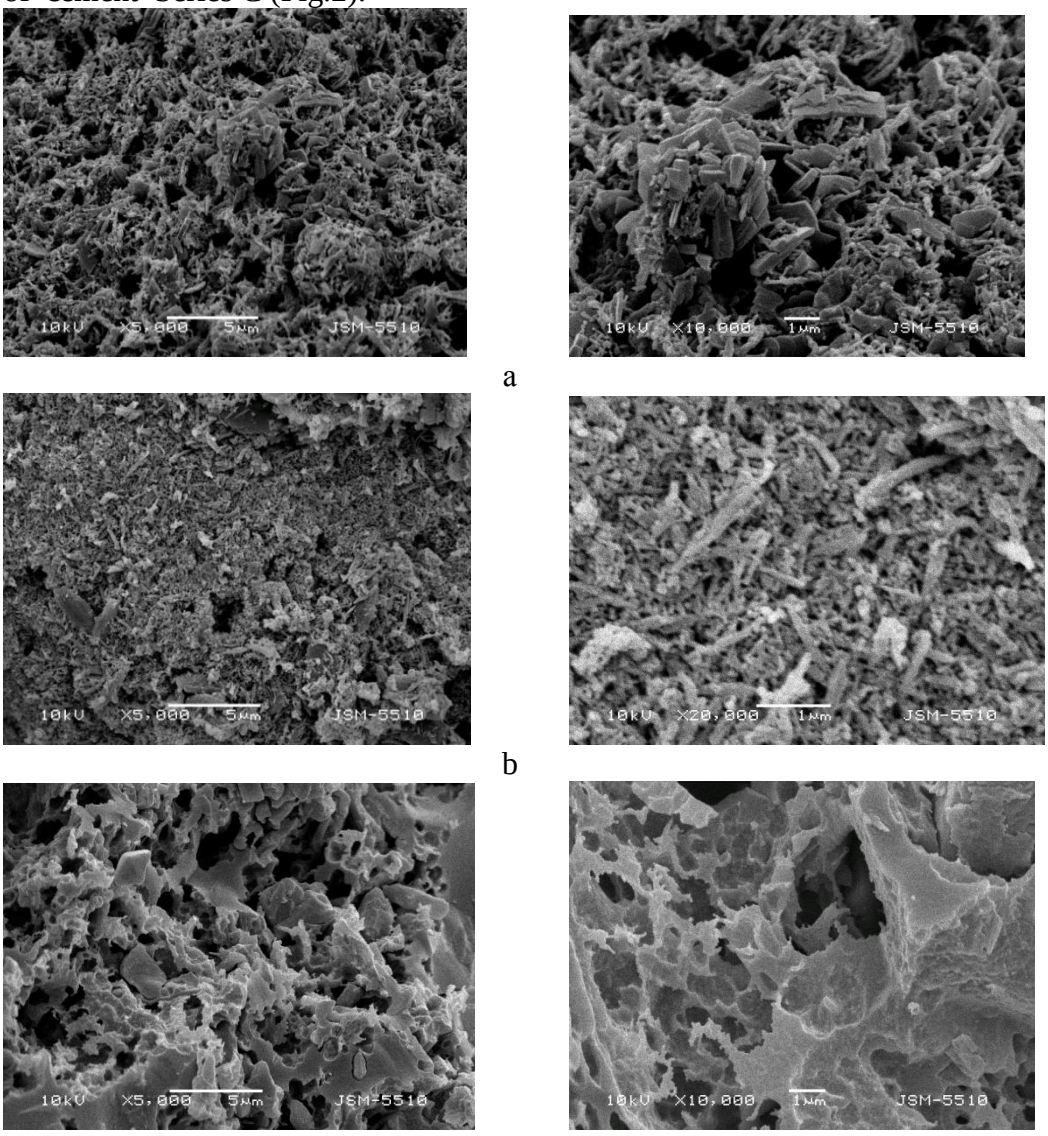

Figure 2. SEM images of: cement Series A (a); cement Series B (b); and cement Series C (c);

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FO6. ANTIOXIDANT STATUS AND HISTOLOGICAL STUDIES AFTER IMPLANTATION OF MODIFIED HYDROXYAPATITE IN RAT CALVARIA

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ABSTRACT

The study was carried out on the biochemical indices (oxidant/ antioxidant status) and histological response after implantation of hybrid material of chitosan/nano hydroxyapatite in rat calvarial defects. Three groups of rats were used in the experiment: **1st group – control** – healthy animals, **2nd group** – rats received critical size skull defect with no scaffold implantation and **3rd group** – animals received a critical size skull defect and hydroxyapatite implants.

Biochemical and histological studies were done 3 months after the implantation. Serum was analyzed for free radical index contents MDA, SOD, GPx and GSH. Quantitative tissue response towards the implant was histologically investigated. MDA level was higher in group 2 compared to the rest groups. GSH content was the highest in group 1. GPx is the lowest in group 3. There were no differences in SOD activity among the groups. No signs of inflammation were noted from the scaffold 2 months after the implantation. Evidence was provided in our study for good biocompatibility of the newly biomaterial.

Key words: calvaria**,** hydroxyapatite implant, MDA, SOD, GPx, GSH

INTRODUCTION

During the last decades a variety of biomaterials have been used for the fabrication of orthopedic and dental implants. They serve as matrices for tissue formation and thus should fill multiple roles including mechanical strength, biodegradability and biocompatibility. Several attempts have been aimed to modify implant composition and morphology to optimize implant-to-bone contact and improve integration [6]. The development of a fully synthetic, readily available and osteogenic bone substitute as an adjunct to autologous tissue grafts is strongly encouraged and considered as a great milestone in the clinical field [8].

The aim of the present study was to carry out a comprehensive safety evaluation of a newly developed modified hydroxyapatite implantation. For this reason serum oxidative/ antioxidant status: malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) were investigated as well as histological studies were done in rats with calvarial defects. MDA is the biomarker of lipid peroxidation and the enzymes SOD and GPx are the primary step of the defense mechanism in the antioxidant system against oxidative stress [2]. The second line of defense includes the non-enzymatic radical scavenger GSH, which scavenges residual free radicals resulting from oxidative metabolism and escaping decomposition by the antioxidant enzymes [3].

MATERIAL AND METHODS

Used bone implant material

In this experimental study for bone defect reconstruction was used as bone graft substitute material a new hybrid material prepared by mixing powder of nano- hydroxyapatite and electrospinning fibers of poly(lactic acid) PLLP 3 % solution of chitosan-in citric acid.

The composition of the proposed, hybrid material was inspired from the accepted idea that the final group for bone graft substitutes as smart materials are polymer-based materials and degradable polymer is ideally used in bone tissue engineering.

Animal model. Eight-week old Wistar male rats weighed approximately 350 g were used in the experiments. The rats were allocated to three experimental groups. The rats from group 1 are healthy animals. Animals from the group 2 received a critical size skull defect (CSD) with no scaffold implantation. The rats from group 3 received a critical size skull defect (CSD) and hydroxyapatite implants.

General anesthesia was given. To create a CSD in the skull the head was shaved and cleaned with an antiseptic. A lateral longitudinal incision over the head was made under aseptic conditions. The skull cortex was drilled and a calvarial bone defect 1.8 mm wide and 6 mm long was created. The biomaterial was implanted into the defect zone and their position was checked. The wound was then closed with continuous subcutaneous stickes. Animals had free access to food and water and were monitored daily in the postoperative period for any complications or abnormal behavior.

After 3 months the animals were sacrificed with over dose of pentobarbital. Blood was collected from the abdominal aorta in collection tubes for serum.

Histology. Immediately after death, the head was cut off at the atlanto-occipital joint, and immersed in 10% neutral buffered formalin for a week. Then the mandible and all surrounding soft tissue were removed and the remaining cranium was cut at two transversal segments 3-4 mm wide at the calvarias implant levels using an Axis Diamond Disc for dental use (Fig. 1). The obtained transversal segments were immersed in 10% formic acid for demineralization which lasted at least three weeks at 37°C. The acid solution was changed every 24 hours. The demineralization process was monitored daily, testing the specimens with a needle. Finally the specimens were each submitted to a neutralization process with PBS pH 7.2 (3 changes in 24 hour intervals) and dehydrated in ethanol. Than the materials were processed in chloroform (3 changes in 24 hour intervals until the implants were totally dissolve), embedded in paraffin, cut at 6-8 µm sections and stained with hematoxylin-eosin according to the standard histological technique. The histological evaluation of tissue response against the implants was carried out of Leica DM 5000B microscope.

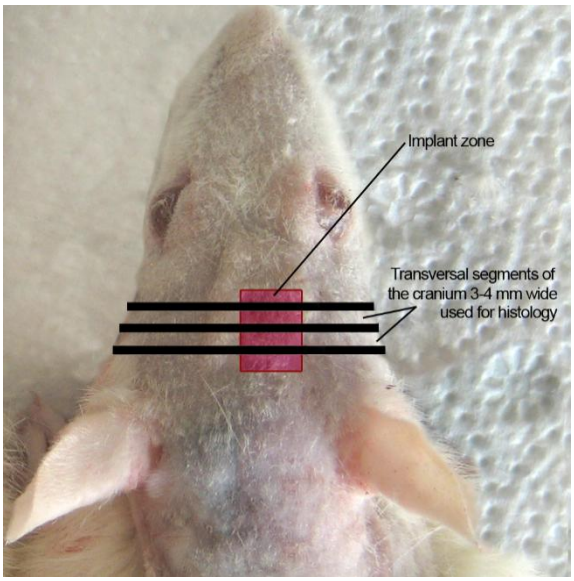

Fig.1. Schematic rat head with a calvarial defect covered with implant. The image shows the implant levels where two transversal segments 3-4 mm wide were obtained for histology.

Serum biochemistry. Serum concentrations of malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity were measured by colorimetric assay kits (Cayman Biomol GmbH, Hamburg, Germany).

RESULTS AND DISCUSSION

Animals

Mortality and clinical signs. No behavior changes or visible signs of physical impairment were observed during 2 months postoperative period. Macroscopic analysis of the implant sites demonstrated comparable scar formation and subsequent healing processes in all experimental groups.

Histology

By means of histology it was shown that the implant was totally dissolved following continuous processing in chloroform and it place in transversal sections appeared as empty zone surrounding by a thin fibrous capsule which covered the calvarial defect. Foreign body reaction around the implant was no visible (Fig. 2).

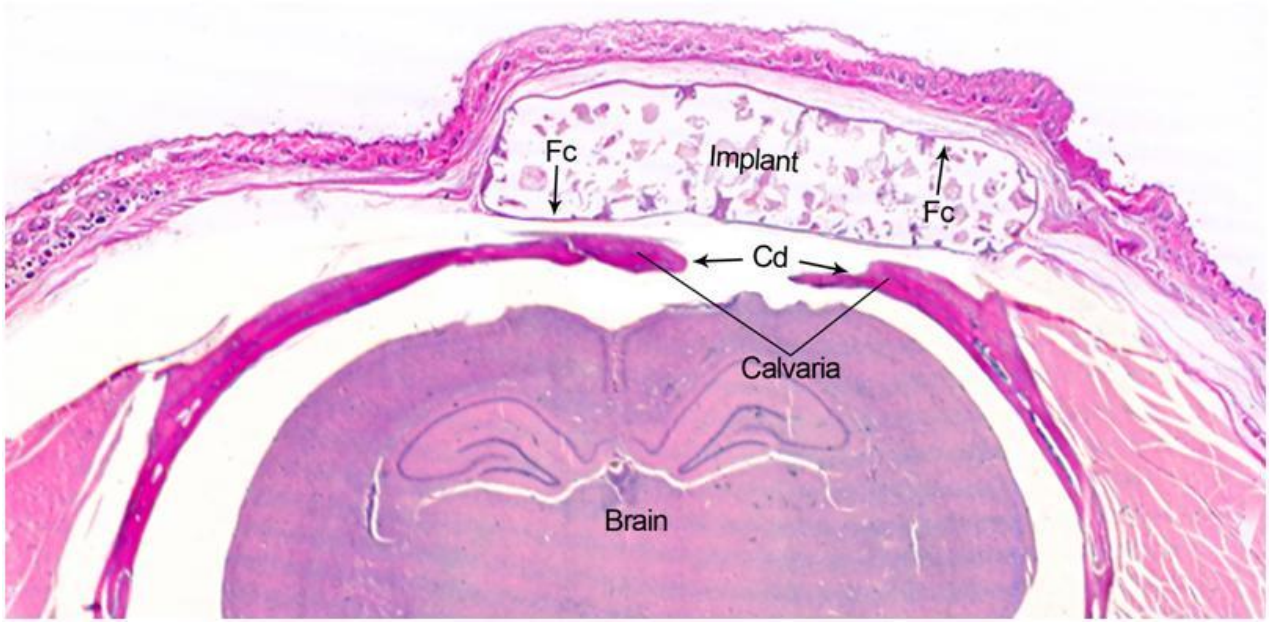

Fig. 2. Cross section through the head of a rat with an implant covering calvarial defect (Cd) on the right parietal bone. Well-formed thin fibrous capsule (Fc) around the implant.

Serum biochemistry

The biochemical indices of antioxidant/oxidant status MDA, SOD, GSH and GPx were measured as indicators of free radical burden (Table 1).

Table 1. Oxidant/ antioxidant parameters

	MDA μmol/L	GSH μmol/L	SOD U/mg protein	GPx U/mg protein
healthy control	22,46±3,44	7,25±1,22	0,23±0,06	7,38±1,9
calvarial defects with no implementation	28,4±1,1	6,11±0,1	0,21±0,045	8,16±1,53
defects with implants	26,84±3,68	5,26±0,99	0,23±0,08	5,78±1,58

MDA serum levels indicated the lipid peroxidation induced by reactive oxygen species (ROS). MDA level was increased in group 2 (with defects without any implants). It was the highest in group 2 than that in groups 1 (control) and 3 (defects with implants).

GSH level was the highest in group 1 than that in groups 2 and 3. In group 3 GSH was reduced significantly in comparison to the control level.

There were no differences in SOD activity among the all three groups.

GPx was lower in group 3 than that in group 1 and group 2.

This study was performed to evaluate the tissue reaction to newly synthesized implants. The new biomaterial was well tolerated by the host organism. It didn't evoke adverse reactions such as long-term reactions. Histological examination demonstrated that the new material supported bone formation. The lack of visible inflammatory complication at the implantation site, body temperature and a histological examination provided no signs of a systemic inflammatory reaction.

Essential alterations in the MDA level as well as in the GSH level and antioxidant enzyme GPx were observed at the end of the experiment. These findings showed a development of antioxidant imbalance in the hosts with calvaria defects with or without implantation. Created calvaria defects enhance ROS production which leads to significant increase of lipid peroxidation. ROS may play a critical role in the adverse effect of the organisms with implants on the biological performance of implants. This has not been well investigated previously. It is one of the main manifestations of oxidative damage. The development of oxidative stress in rats with implantation is demonstrated by increased of MDA, alterations in the activity of antioxidant enzymes and reduced glutathione.

Oxidative stress plays a very important role in the complications of the implants. This is in line with available reports in this field [7, 10]. Free radicals reactive oxygen species (ROS) are normally present in the body in small numbers. Normal levels of free radical can be beneficial to the body while, excessive free radicals formation causes damage to the cells and tissues. The body naturally protects itself against oxidants by forming antioxidant compounds. Antioxidants play an important role in scavenging oxidants and consequently preventing cell damage [5]. Inflammatory response is part of a general pattern of recovery and wound healing that leads to eventual acceptance of foreign material placed in the body [1]. This pattern of events typically leads to fibrotic encapsulation of the implant. Prolonged inflammatory responses can have the consequence of more intense reactions requiring extrusion of the implant [9, 4].

CONCLUSION AND FUTURE WORK

In conclusion the present experimental study confirms that the processes of calvaria implantation with the new synthesized material correlate well with oxidative stress status witch can be assed using enzymatic and non-enzymatic biomarkers. We suggest that oxidative stress is caused of tissue pathophysiology induced by applied implants. Studies are needed of

the coadministration of antioxidants as an approach to ameliorate implant-induced oxidant damage.

The biological efficacy of the newly implant is well expressed in a calvarial defect rat model. Our study gave essential information for the design of novel implanted biomaterial.

The histological examinations performed clearly indications that the implant used in the present study successfully could be applied for covering of small skullcap defects. Indicative for that were the lack of foreign body reaction and inflammation around the implant, as well as, the good integration of implant with the surrounding tissues.

No signs of inflammation were noted independently from the scaffold 3 months after implantation. We provide an evidence in our study for good biocompatibility of the newly biomaterial. Moreover, the suitability of them for other kinds of bone defects has to be proven in further experimental studies.

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FO7. PHOSPHORILATED CHITOSAN: SYNTHESIS AND BIOMEDICAL APPLICATIONS

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Chitosan is a natural polymer which is non-toxic, biocompatible and biodegradable. Its biomedical applications are defined besides by the above mentioned properties also by its antibacterial properties. The chemical modification of chitosan aims to generate new functions of this material and thus to further widen up its applications.
Phosphorylated chitosan (P-chitosan) has modifies properties as compared to the neat chitosan, e.g. improved solubility, less thermal stability and less crystallinity.
Our interest towards the phosphorylated chitosan arouse because of its affinity to interact with potassium ions due to the phosphate groups availability. In this way the phosphorylated chitosan could play the role of a polymer scaffold for biomineralization thus resulting into hybride inorganic organic materials as bone and dental materials.
In this presentation the main synthetic routes for preparation of phosphorilated chitosan are outlined along with its biomedical applications reported so far.

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FO8. MEDICAL ADVANCEMENTS IN THE TREATMENT OF DIABETES MELLITUS TYPE 1

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Abstract

Type 1 diabetes (T1D) a chronic condition characterized by abnormally high blood sugar levels, due to the destruction of the insulin-producing beta cells in the pancreas. Because of the pathophysiology of the disease, T1D patients have to undergo insulin therapy to stay alive. However, the therapy does not offer a permanent cure – it merely delays the onset of complications such as diabetic retinopathy and neuropathy. This problem has driven scientists to find new ways of combating the disease. This article provides a comprehensive review of some alternative methods for the treatment of type 1 diabetes, starting from pancreas transplantation and continuing with the ideas of islet encapsulation and bioartificial pancreas.

Overview of Diabetes type I
Type 1 diabetes is mostly associated with deficiency or lack of insulin, owing to an autoimmune response (1) against the beta cells and resulting in their destruction. T1D is typically diagnosed during childhood, although there have been several cases of people developing it later in life. The resulting lack of insulin leads to hyperglycemia. Common symptoms include polydipsia, polyuria, polyphagia and rapid weight loss (2). The low levels of insulin stimulate the process of lipolysis, leading to an acute condition known as

ketoacidosis. Moreover, the generalized cell atrophy leads to long-term complications such as neurapthy, nephropathy, retinopathy and cardiovascular disease.
It is widely believed that T1D is caused due to genetic and environmental factors. The risk of a child developing type 1 diabetes is about 10% if the father has it, about 10% if a sibling has it, about 1-4% if the mother has it. However, it has been reported that the genetic factors may not have such a significant role. For example, studies involving identical(monozygotic) twins, have shown that if one twin has T1D, the other has less than 50% chance of developing it (3). However, these studies suggest that there are environmental factors at play. Viral infections such as Coxsackievirus B, herpes, rubella, cytomegalovirus and at last but not least enteroviruses have been known to trigger an autoimmune response against the beta cells (4). Other less common causes of type 1 diabetes include injury to the pancreas from toxins, trauma, or after the surgical removal of the pancreas, whereas the patients are deprived of the endocrine function of the pancreas (5).
Type 1 diabetes must be treated with insulin. This involves injecting insulin subcutaneously for it to get absorbed into the bloodstream where it can then access all the cells of the body that require it. Insulin cannot be taken orally because the digestive fluids of the stomach would destroy the insulin before it could work. However, insulin therapy is not a permanent solution. Long-term symptoms such as neuropathy and retinopathy are simply delayed (1, 2).
Type 1 diabetes has historically been most prevalent in populations of European origin, but is becoming more frequent in other ethnic groups. Within Europe the highest rates of childhood diabetes are found in Scandinavia and north-west Europe, with an incidence range from 57.4 cases/100,000 per year in Finland to 3.9/100,000 in Macedonia for children aged 0–14 years. The incidence of type 1 diabetes remains relatively low in populations of non-European descent around the world (6).

Pancreas transplantation.
The purpose of pancreas transplantation is to ameliorate type I diabetes and remove the need of exogenous insulin intake (2). The first successful pancreas transplantation in conjunction with a simultaneous kidney transplantation was performed by W.D. Kelly, MD, and Richard Lillehei, MD, from the University of Minnesota in 1966. Until 1990, the procedure was considered experimental. Now it is a widely accepted therapeutic modality, with virtually all insurance carriers covering the procedure. The pancreas comes from a deceased organ donor. However, select cases of living-donor pancreas transplantations have been performed (1).
According to the International Pancreas Transplantation registry, life expectancy for pancreas recipients 1 year following the operation is 86% (1). However, the technique constantly undergoes improvement, achieving technological advance and more precise postsurgical treatment (nutrition, immunosuppression etc.). Dr. Occhipinti at the university of Pisa presented her institution's 10-year follow-up data for the 17 male and 17 female pancreas-transplant recipients. Mean age at transplant was 37 years and mean duration of type 1 diabetes was 24 years. All underwent pancreas transplant alone, using the portal-enteric drainage approach. Patient survival at 10 years was 97%. Only one patient survived for 5 years posttransplant as a result of stroke (7).
An alternative of whole pancreas transplantation could be merely pancreatic islet transplantation (PIT). Seven consecutive patients with type 1 diabetes underwent islet transplantation in conjunction with a glucocorticoid-free immunosuppressive regimen. Pancreases were removed from brain-dead donors and stored and chilled at the University of Wisconsin, where pancreatic islet cells were isolated and prepared. In all seven patients, exogenous insulin therapy quickly became unnecessary once sufficient numbers of islets were transplanted (8).

Although pancreas transplantation or PIT almost thoroughly enhance the quality of life of patients with type 1 diabetes, it should not be considered an eventual treatment of the disease due to the constant intake of immunosuppressants and the limited endurance of the transplanted tissue.

Islet Encapsulation

The potential of using encapsulated islet cells for treatment of T1D has been extensively studied by scientists. Cell encapsulation is a method of enveloping living cells inside a semipermeable membrane. The membrane provides an inward flow of nutrients and oxygen as well as outward flow of waste products and therapeutic proteins. Moreover, the encapsulation technique protects the cells from the immune system, thus circumventing graft rejection.

Capsules are either made from biologic polymers (alginate, collagen, chitosan etc.) or synthetic agents (12, 13). However, there are several consideration that must be taken into account before using microcapsules in clinical applications. These include biocompatibility and longevity of the implant, mechanical strength and durability, membrane permeability and preservation of cellular function (9).

Generally, cell encapsulation devices fall into two categories: fairly rigid preformed or flexible. Fairly rigid preformed devices are made of synthetic polymers which invoke a specific response from the host such as neovascularization around the implant (10). Flexible devices often strive to reduce response from the host by avoiding the immune system (11).

Lim and Sun are widely recognized as the first group to produce groundbreaking results involving the encapsulation of islets. In their studies, islet cells were encapsulated in alginate microcapsules and transplanted onto recipient rats. The encapsulated cells survived for 3 weeks, compared to the naked islet transplants that survived for only 6-8 days (14).

Few researchers have also been able to proceed to clinical trials. Soon-Shiong et al. were the first to do so. The islet capsules were implanted into the peritoneum of a patient. Initially, the patient's insulin requirements were reduced by 1-2 units per day and in the ninth month he was able to discontinue all exogenous insulin.

Another study performed by Elliot et al. which involved the usage of alginate-encapsulated porcine islet transplantation reported a long-term survival (9 years) of the encapsulated cells. Moreover, the patient's daily insulin dose was reduced by 30% and C-peptide remained detectable for over a year. There was also no sign of porcine viral infections following the transplant (16).

Although encapsulation protects against large cells such as antibodies and T-cells, small proinflammatory cytokines such as interleukin-1 and anti-tumor necrosis factor-a can freely diffuse through the membrane. These cytokines are thought to cause graft injury and failure in islet transplants (17). To resolve the problem, many researchers have begun using coencapsulating antirejection molecules which are supposed to provide immune suppression at a local level, while avoiding systemic toxicity (18). Studies using this method have yielded promising results. For example, Su et al. showed that islet cells encapsulated with an IL-1 inhibitor maintained a 60% greater viability than controls when placed in solution with proinflammatory cytokines (19). Another study by Bunger et al. showed that islets coencapsulated with dexamethasone and implanted into mice exhibited less local tissue fibrosis compared to the capsules, which did not contain dexamethasone, resulting in greater graft survival (20).

Despite the success of cell encapsulation, studies have demonstrated that encapsulated islet grafts have limited graft survival due to neovascularization complications and inadequate oxygen supply. (21, 22, 23)

Bioartificial pancreas

The problems associated with neovascularization, inadequate oxygen supply and issues concerning the immune system have compelled scientists to find new ways of active oxygen supply to islet cells inside an immunoisolating device.

The Israeli company Beta-O2 Technologies have managed to create a subcutaneous implantable bioartificial pancreas. The device has been proved to overcome the problem with inadequate oxygen supply to the graft, while providing protection of the donor cells from the immune system (27).

The Beta-O2 artificial pancreas is essentially a macrocapsule that consists of an islet module and a gas chamber, which is separate from the module. The cells in the module are implanted in an alginate hydrogel structure which provides an adequate and fully-isolated environment in which the cells can function. Furthermore, the hydrogels provides protection against the body's immune system (24).

Since the islet cells consume large amounts of oxygen in order to function properly, oxygen needs to be injected through one of the two ports implanted under the skin. The procedure is very user friendly and there is little possibility of failure (26).

The pre-clinical trials showed a number of positive results. Firstly, the cells in the device were able to use around 65% of the oxygen supplied through refueling. Furthermore, the device was able to maintain normoglycemia in diabetic rats throughout the entire implantation period. Removal of the device resulted in an increase of glucose levels. Visual and histological examination of retrieved devices demonstrated a physiological reaction involving the formation of a fibrotic pocket around the device without an inflammatory response. The fibrotic pocket showed signs of neovascularization around the device which would create short diffusing distances for insulin and glucose. Moreover, immunohistological analysis of the islet cells showed no signs of dysfunction (24).

The first clinical trial was performed in 2012 when a 63-year-old German patient with type- 1 diabetes was transplanted with the Beta –O2 device. The patient was observed for 10 months at the Technische Universität in Dresden, Germany. The device showed persistent graft function, along with regulated insulin secretion and preservation of islet morphology and function without any immunosuppressive therapy (25).

Furthermore, eight patients are expected to be enrolled in a two-year investigation which will evaluate the safety and efficacy of implanting the device. So far, only four patients have been implanted with the device. The first patient in the study was implanted in October 2014. The study is being conducted at Uppsala University Hospital in Sweden (25).

Conclusion

Type 1 diabetes is a serious health problem affecting millions of people worldwide. In order to combat the disease, many treatment options have been developed such as insulin therapy, pancreas transplantation and cell encapsulation. The field of cell encapsulation stands out as having the most potential in treating T1D patients. Although there have been vast accomplishments in this particular field, it is still far from seeing clinical applications. The results have been difficult to replicate and researchers have not yet established a clear protocol on the engineering, processing and implantation of these capsulated cells. Nevertheless, researchers are actively looking for new ways of engineering the ideal cell capsule. Continuous improvement in islet encapsulation techniques could transform modern medicine and provide physicians with viable alternatives for the treatments of T1D.

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FP1. VITAMIN A AND WOUND HEALING

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FP2. VITAMIN C AND WOUND HEALING

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FP4. VITAMIN K AND WOUND HEALING

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FP5. SILVER AND WOUND HEALING

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FP6. ZN AND WOUND HEALING

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FP7. HONEY AND WOUND HEALING

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FP8. AMORPHOUS CALCIUM PHOSPHATE AND VIABILITY AND PROLIFERATION OF MOUSE EMBRYONAL FIBROBLASTS.

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FP9. IN VITRO BIOCOMPATIBILITY ASSESSMENT OF NEW MATERIALS FOR BONE IMPLANTS: SOME FACTORS THAT CAN INFLUENCE CELL VIABILITY AND GROWTH.

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Session G.

Chairpersons:

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GO1. AN EPIDEMIOLOGICAL STUDY OF ACUTE POISONING BY PHARMACEUTICAL AGENTS WITH FATAL OUTCOME

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The aim of the study was to present an analysis of acute poisoning by pharmaceutical agents with fatal outcome. *Methods:* The records of the Toxicology Clinic, Department for adults, UMHATEM "N.I.Pirogov" were reviewed retrospectively for all poisonings during a 5 year period - from January 1, 2009 to December 31, 2013. The variables analyzed were gender, age and type of medicines. *Results:* A total of 9194 patients have been hospitalized in the Toxicology Clinic for the studied period, and 75 patients were with fatal outcome. Deaths caused by pharmaceutical agents were twenty-one (28%), deaths caused by other agents were fifty-four (72%). The patients with fatal exogenous intoxication by medicines were between the ages of 31 and 88. Male mortality was higher: eleven males (52.4 %) and ten females (47.6 %). All of the cases were intentional. Most deaths occur in the age over 70 years (71 - 88 years - 42.9%. The most often implicated groups of pharmaceutical agents were benzodiazepines, antihypertensives, antidepressants, neuroleptics and analgetics. *Conclusion:* The analysis of the data of the fatal poisonings by medicines revealed that for the studied period of time, lethality is a stable indicator with little variations throughout the years.

Key words: acute poisonings, pharmaceutical agents, fatal outcome

GO2. ПИЛОТНО ПРОУЧВАНЕ СРЕД ПАЦИЕНТИ НА ПЕРОРАЛНА АНТИКОАГУЛАНТНА ТЕРАПИЯ ОТНОСНО ТЯХНАТА ИНФОРМИРАНОСТ ЗА БЕЗОПАСНО И ЕФИКАСНО ПРОВЕЖДАНЕ НА ЛЕЧЕНИЕТО

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Въведение: Информираността на пациентите е от особено значение за безопасното и ефикасно прилагане на антикоагулантна терапия. Проучване от 2005 показва, че повечето анкетирани пациенти имат ограничени познания за тяхното кардиологично заболяване и ползата от антикоагулантната терапия. 2003 година учени посочват, че познанията на пациентите относно значението на антикоагулантната терапия, кореспондира на качеството на терапията и трябва да се окаже повече внимание на образованието на пациентите в тази насока. Цел на това проучване е да се установи нивото на информираност на пациентите свързана с тяхната терапия и да съобрази ефективността на даденото лечение спрямо информираността на пациентите. Метод: провеждане на анкета от тринадесет въпроса. Резултати:Общо 39 пациента анкетирани. „Знаете ли защо приемате тези лексарства?“ 22 отговарят „да“ и 17 с „не“. „Знаете ли какви са нежеланите ефекти на лекарствата, които приемате?“ - „не“ отговарят 28. Едва 9 от анкетираните знаят стойностите, в който трябва да се поддържа INR. Изводи: Според проведеното проучване установяваме, че 2/3 от анкетираните пациенти не са добре информирани относно нежеланите лекарствени реакции при антикоагулантната терапия. Това може да доведе до сериозни усложнения. Необходимо е осъществяването на активна обратна връзка от пациента към лекаря, за да се намали процента на неинформирани пациенти и да се подобри качеството на лечебния процес. Предоставянето на информационни материали на пациентите относно същността на терапията допринася за по-ефективна терапия. Проблема с липсата на информираност на пацинтите е съществен и е необходимо взимането на трайни мерки, с цел подобряването на качеството на терапията и намаляването на усложненията от нея.

GO3. IN VIVO ANTITUMOR EFFECT OF THE NOVEL ALKYLPHOSPHOCHOLINE ERUFOSINE APPLIED ALONE OR IN COMBINATION WITH DOXORUBICIN AGAINST GRAFFI MYELOID TUMOR IN HAMSTERS

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Abstract
Erufosine belongs to the group of alkylphosphocholines (APCs), which are novel synthetic membrane-targeting anticancer agents. APCs have attracted the scientific interest not only with their pronounced antineoplastic properties, but also with the ability to increase the efficacy of chemotherapy and radiotherapy *in vitro* and in animal experiments.
In the present study, the *in vivo* antitumor effect of erufosine applied alone or in combination with the conventional chemotherapeutic drug doxorubicin was assessed in hamsters with experimental Graffi myeloid tumor. The results demonstrate the protective antitumor effect of erufosine and doxorubicin, expressed by reduction of the transplantability, tumor growth inhibition, decreased mortality and extension of the mean survival time. These effects were most clearly pronounced in the experimental groups with a combined treatment. The presented results suggest that the combined application of doxorubicin and erufosine could be a promising antitumor treatment strategy. Further studies are needed to clarify the biological and therapeutic effects of these substances using different experimental conditions. The results obtained may be used for the purposes of the medical oncotherapeutic practice.
Key words: erufosine, alkylphosphocholines, Doxorubicin, Graffi myeloid tumor

Introduction

The control of the tumor is the main goal of the medical oncology that most often could be achieved by the implementation of complex therapy. Chemotherapy is one of the methods successfully used as a part of the anticancer treatment. Despite the undoubted achievements in the treatment of oncological diseases in a global and national scale, conventional chemotherapy has several disadvantages such as non-selectivity (it affects not only tumor cells but also normal); toxicity (myelosuppression, alopecia, nausea, vomiting, general intoxication, immunosuppression, cardiotoxicity, nephrotoxicity, hepatotoxicity, etc.); narrow therapeutic index; multidrug resistance of cancer cells to the cytostatic drugs. These limiting factors of the oncotherapy necessitate the conduction of further research for the development and characterization of new antitumor agents and novel therapeutic regimens, which can be applied in the clinical practice.
Alkylphosphocholines (APCs) are novel class of synthetic phospholipid analogs with pronounced antineoplastic activity [8, 10, 17]. In contrast to the conventional chemotherapeutic agents, which affect mainly the genetic apparatus of the tumor cells APCs primarily interfere lipid metabolism and modulate lipid-dependent signal transduction [3, 17]. Most important for their antitumor effect are the inhibition of phosphatidylcholine synthesis and the modulation of specific signaling processes such as the proapoptotic stress-activated protein kinase (SAPK)/c-Jun N-terminal protein kinases (JNK) pathway, the prosurvival

PI3K/Akt/mTOR pathway, and the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway [10, 12, 14, 17].
Erufosine is the first alkylphosphocholine suitable for intravenous application because it does not induce hemolytic and myelotoxic effects and even stimulates normal hematopoiesis [1, 2]. Significant antiproliferative activity of erufosine was established in different human malignant cell lines [5, 11, 12, 13] as well as in various *in vivo* tumor models [4, 7]. Moreover, experimental data indicate the ability of erufosine to increase the efficacy of chemo- and radiotherapy in experimental conditions [5, 6, 7, 14]. These findings together with the lack of myelosuppression, which is the major side effect of conventional cytostatic drugs, make the erufosine a promising combination partner for complex chemotherapy of malignant neoplasms.
The aim of the present study was to assess the *in vivo* antitumor activity of erufosine, applied alone or in combination with doxorubicin against Graffi myeloid tumor in hamsters.

Materials and methods

Experimental animals

Syrian Golden hamsters of both male and female sexes, 2–4 months old, weighing approximately 100 g were purchased from a breeding base Oncology Center, Sofia. The animals were kept under standard conditions in individual plastic cages with free access to food and water. All experiments were conducted in accordance to the ethical standards of the institutional and national guidelines for care and use of laboratory animals.

Experimental tumor model

Graffi myeloid tumor (GMT) was primary induced by the Graffi murine leukemia virus in new-born hamsters [9] and maintained monthly *in vivo* by subcutaneous transplantation of viable tumor cells (2×10^6/mL PBS) in the interscapular area of hamsters [16]. The tumor usually occurs between 7 and 15 days after the inoculation of the tumor cells as a solid formation, which progressively increases in size and causes death of experimental animals between 25^{th} and 30^{th} day after the transplantation. The GMT is 100% transplantable and spontaneous regression in this experimental tumor model was not observed. For the present experiment $2x10^4$ viable Graffi tumor cells/0.5 ml PBS per animal were transplanted subcutaneously (s. c.) on hamsters.

Antineoplastic agents

- ***Erufosine (EPC_3)*** was kindly provided by Prof. H. Eibl (MPI for Biophysical Chemistry, Gottingen, Germany). EPC_3 was dissolved in PBS and administered subcutaneously (s. c.) twice a week for 4 weeks. The single dose of EPC_3 was 30 μM/kg body weight (1.5 mg/100g b.w), which correspond to the IC_{50} value established in previous *in vitro* experiments.
- ***Doxorubicin hydrochloride (Dox)*** was purchased from Sigma-Aldrich and dissolved in PBS. Dox was applied s. c. twice a week for 4 weeks. The single dose of Dox was 0.1 μM/kg b. w. (0.00058 mg/100g b. w.), which correspond to ½ IC_{50} value established in previous *in vitro* experiments**.**

Experimental design

For the examination of the protective effect of erufosine applied alone or in combination with Doxorubicin, two schemes of administration of the antitumor compounds were used - starting simultaneously with the tumor transplantation or staring after the transplantation, when the tumors reach about 10 mm in diameter (11^{th} day). The experimental animals were

transplanted s. c. with 2.10^4 viable tumor cells and were separated in several experimental groups as fowolls:

- ***Group 1*** – tumor-bearing hamsters (TBH) with EPC_3 treatment (1.5 mg/100g b.w) starting simultaneously with the tumor transplantation (day 0).
- ***Group 2*** - TBH with EPC_3 treatment (1.5 mg/100g b.w) starting after the appearance of tumors with diameter about 10 mm (11^{th} day post transplantation)
- ***Group 3*** - TBH with combined treatment with EPC_3 (1.5 mg/100g b.w) and Dox (0.00058 mg/100g b.w) starting simultaneously with the tumor transplantation (day 0).
- ***Group 4*** - TBH with combined treatment with EPC_3 (1.5 mg/100g b.w) and Dox (0.00058 mg/100g b.w)) starting after the appearance of tumors with diameter about 10 mm (11^{th} day post transplantation)
- ***Group 5*** – TBH without any treatment (untreated control).
- ***Group 6*** - TBH with Dox treatment (0.00058 mg/100g b.w) starting simultaneously with the tumor transplantation (day 0).

Assessment of the in vivo antitumor activity

The protective effect of the antineoplastic agents was assessed by examination of the following biometric parameters of the tumor growth:

- ***Tumor transplantability (TT)***

Tumor transplantability (%) was determined for each experimental group on days 8, 11, 15 and 18 as a ratio between the number of tumor-bearing hamsters and the number of all hamsters in the group.

- ***Tumor volume (TV)***

The tumor size was measured for each animal using a caliper and the tumor volume was calculated by the formula: $V = AB^2/2$, where A - major axis; B - minor axis
The change in the TV was followed for 30 days after the tumor transplantation.

- ***Tumor lethality (TL).***

Lethality (%) was followed in dynamics between the 20^{th} and 50^{th} day after the tumor transplantation.

- ***Mean survival time (MST)***

At the end of experiments, the mean survival time was calculated for each group.

Statistical analysis

All data are presented as mean arithmetic value ± standard deviation. Significance testing was performed by GraphPad Prism software package using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. Values of $*p<0.05$, $**p<0.01$ and $***p<0.001$ were considered statistically significant.

Results and discussion

The *in vivo* antitumor effect of erufosine applied alone or in combination with doxorubicin was studied in hamsters transplanted with Graffi myeloid tumor. The biometric parameters transplantability, tumor volume, lethality and mean survival time were used as markers for assessment of the antitumor activity of the tested substances.
The effect of treatment with erufosine and doxorubicin on the transplantabilty of Graffi tumor was followed for 20 days after the tumor transplantation (Fig. 1).

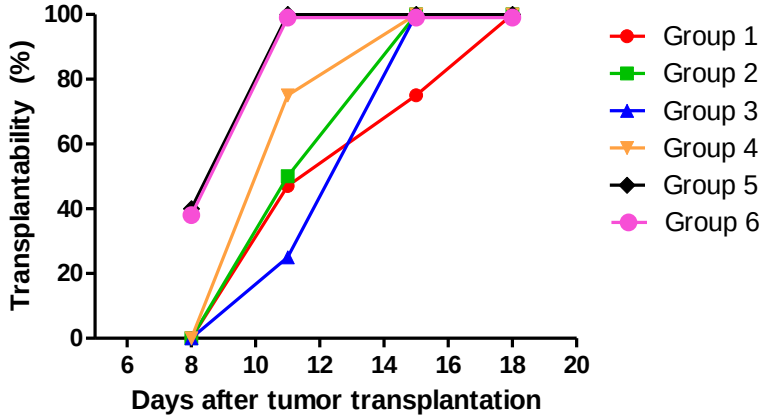

Figure 1. Transplantability of Graffi tumor in hamsters treated with erufosine (EPC_3) and Doxorubicin (Dox). Experimental groups: Group 1 – tumor-bearing hamsters (TBH) treated with EPC_3 from day 0; Group 2 – TBH treated with EPC_3 from day 11; Group 3 – TBH treated with EPC_3 and Dox from day 0; Group 4 – TBH treated with EPC_3 and Dox from day 11; Group 5 – TBH, untreated control; Group 6 – TBH treated with Dox from day 0.

The highest percentage of hamsters with palpable tumors was observed in groups 5 and 6. In both groups, on day 8 and day 11 tumors were established in 40% and 100% of the experimental animals, respectively. In the experimental groups treated with the EPC_3 alone or in combination with doxorubicin, starting on day 0 or day 11 the transplantability was lower compared to the controls (groups 5 and 6). On the 8^{th} day of the study, tumors were not found by palpation (transplantabilty 0%) in groups 1, 2, 3 and 4. In these experimental groups palpable tumors appeared on 11^{th} day, with the lowest percentage in group 3 (25%), followed by groups 1 and 2 with 50% and group 4 with 75%. On the 15^{th} day, the transplantability observed in groups 2, 3 and 4 reached 100%. In group 1, palpable tumors were observed in 100 % of the experimental animals on day 18 of the experiment. The highest protection was established in the groups with treatment, starting simultaneously with the transplantation of the tumor cells (groups 1 and 3) (Fig.1).

The influence of the different schemes of experimental therapy on the tumor volume in Graffi myeloid tumor-bearing hamsters was monitored for 30 days. The mean tumor volume in the untreated control group of hamsters with Graffi myeloid tumor (group 5), showed a rapid growth (Fig.2).

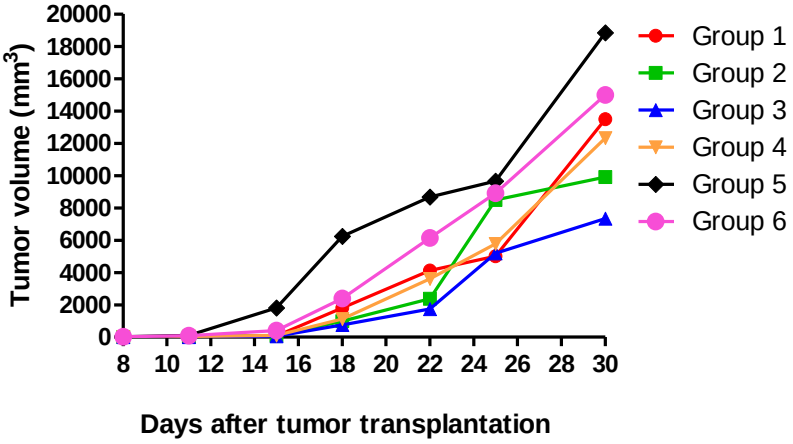

Figure 2. Tumor volume of Graffi tumor-bearing hamsters (TBH) treated with erufosine (EPC_3) and Doxorubicin (Dox). Experimental groups: as noted in Fig.1.

As evident from the data presented at Fig. 2, the rate of the increase in the tumor volume in all experimental groups was noticeably reduced compared to the untreated TBH (group 5) and to the TBH treated with doxorubicin (group 6). The mean volume of the tumors in the hamsters treated with a combination of erufosine and doxorubicin was significantly lower compared to the untreated control (100% inhibition of the tumor growth on day 8 and day 15). Graphs are shifted to the right from that of the controls. The optimal effect was established in group 3 - tumor-bearing hamsters treated with a combination of the two drugs, starting on day 0. The tumor volume in this group was the smallest in all stages of the study (up to the 30th day).
The lethality of the tumor-bearing hamsters treated with the anticancer agents was found to decrease for all groups between the 25th and 40th day of treatment as compared to the control (Fig. 3).

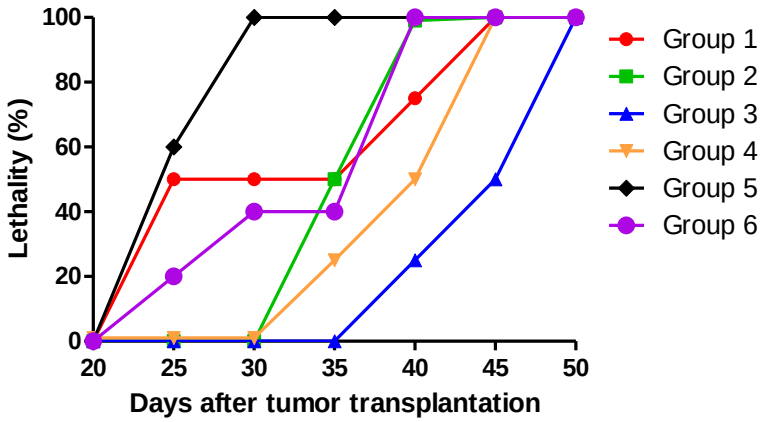

Figure 3. Lethality of Graffi tumor-bearing hamsters (TBH) treated with erufosine (EPC_3) and doxorubicin (Dox). Experimental groups: as noted in Fig.1.

The most significant decrease of the lethality was observed in hamsters treated with EPC_3 and Dox in combination (Fig. 3). As can be seen from the graph, the lowest mortality was found for the animals treated with both agents starting from day 0 (group 3). In this experimental group, 100% mortality was established on the 50th day after the beginning of the experiment.
It was found that the s. c. application of erufosine on Graffi myeloid tumor-bearing hamsters, starting from day 0 and day 11 (groups 1 and 2) and the administration of doxorubicin starting from day 0 (group 6) resulted in a non-significant prolongation of the mean survival time (31.75 ± 5.6 days, 33.75 ± 1.3 days and 33 ± 2.4 days, respectively) (Fig. 4).

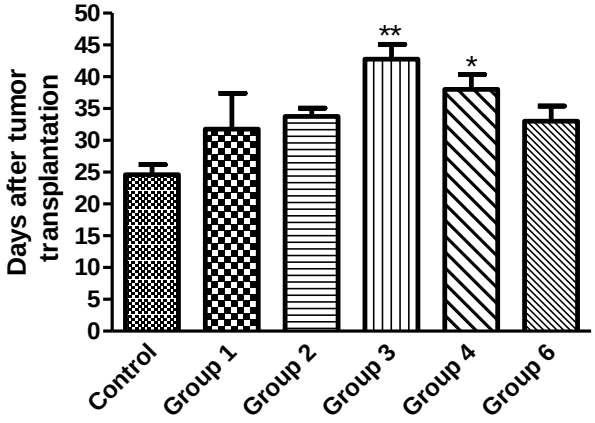

Figure 4. Mean survival time (MST) of Graffi tumor-bearing hamsters (TBH) treated with erufosine (EPC_3) and Doxorubicin (Dox). Experimental groups: as noted in Fig.1.

Statistically significant increase of the mean survival time was found in the experimental groups with the combined treatment with erufosine and doxorubicin starting on day 0 and day 11 (groups 3 and 4). It was found that 75% of the hamsters from group 3 and 50% of the hamsters from group 4 survive more than 40 days (42.75 ± 4.7 days and 38 ± 4.6 days, respectively), while the mean survival time of the untreated control TBH was 24.8 ± 3.5 days. (Fig. 4).

The results are in accordance with previous *in vitro* investigations showing pronounced antitumor effect of erufosine on various hematopoietic malignances [5, 13]. In the present study, the optimal protective effect was observed in experimental groups treated with combination of erufosine and doxorubicin. The later compound have found a wide use in the clinical practice as single or multiagent therapy against a range of hematological malignances as well as various types of solid tumors [15]. The mode of action of doxorubicin include intercalation between the base pairs of DNA and inhibition of topoisomerase enzymes [15]. In contrast, erufosine act mainly on the cellular membranes. The combination of two different mechanisms of action affecting distinct molecular targets in the tumor cells could be an explanation for the higher effectivity of the simultaneous administration of both anticancer agents. Since erufosine have been found to stimulate the normal hematopoiesis [1], it could also be supposed that this alkylphosphocholine ameliorate the myelosupressive effect of doxorubicin and thus increase the overall efficiency of the anticancer therapy.

Conclusion

The results of the present study demonstrate the protective antitumor effect of erufosine and doxorubicin administered s. c. twice a week for 4 weeks, starting from day 0 or day 11 in hamsters with experimental Graffi myeloid tumor. Applied experimental antitumor therapy resulted in decrease of the biometric parameters transplantability, tumor volume and mortality as well as in extension of the mean survival time. These effects were most clearly expressed in the experimental animals treated with the combination of both anticancer agents.

Based on the present results, it could be suggested that erufosine is a promising antitumor agent and its application as a part of complex chemotherapy may contribute to increase of the effectivity and reduction of the adverse side effects of the conventional cytostatic drugs.

Acknowledgement

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GO4. IS SUGAR ACTUALLY TOXIC? CUTTING OUT SUGAR DRASTICALLY IMPROVES OVERALL HEALTH

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Sugars are used by the industry to enhance the attractiveness of foods and drinks. These added sugars, or "free" sugars, are not easily identified in food or drink labels. Certain manufactured foods and drinks with "safe" names, such as dried fruit and fruit juice, still contain free sugars and can be confusing. Guidance states that daily consumption of free sugars should be less than 10% of total energy intake (no more than 5% in the UK). However, it is found that both tooth decay and obesity are associated with consumption of free sugars in large quantities and at inappropriate times.

Sugar contributes to metabolic syndrome and is the strongest evidence to date that the negative effects of sugar are not because of calories or obesity. Government and health organisations worldwide have recently reviewed the evidence on the role of dietary sugars in relation to health outcomes. Hence, it is timely to review current intakes of dietary sugars with respect to this guidance and as a benchmark for future surveillance.

Most countries reported intakes of total sugars, with fewer reporting intakes of added sugars. No country reported intakes of free sugars. Intakes of added sugars were higher in school-aged children and adolescents (up to 19% of total energy) compared to younger children or adults.

Actual data about nutritional status type of children and adolescents from the city of Sofia and Smolyan region (Bulgaria), investigated in the period 2012-2015, shows that the frequency of the overweight and obesity increases significantly (30.0%) with comparison analogical data of over nutrition (15.0%) in students, investigated in 1998-2002.

Sugar is making children sick and it's not just because of the extra pounds it adds to their waists — it's also due to the way it breaks down in the body. Sugar calories are the worst, because they turn to fat in the liver, driving insulin resistance, and driving risk for diabetes, heart, and liver disease. "A calorie is not a calorie", the researchers explain the common misconception that all calories are created equal. Further research into the dietary patterns contributing to added sugars intake in children and adolescents is warranted. It would also be beneficial to policy guidance if future dietary surveys employed a uniform way of expressing sugars that is feasible to measure and has public health significance.

Keywords: sugar consumption, dietary surveys, overweight, obesity

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GO5. ASSESMENT OF THE HEAVY METAL POLLUTION LONG-TERM EFFECTS ON POPULATION GENETIC STRUCTURE OF COMMON DANDELION *TARAXACUM OFFICINALE*

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Pollution with heavy metals exposes plant populations on chronic environmental stress. Despite the numerous studies, the long-term effect of heavy metal toxicity on the population genetic structure remains elusive. Two apparently opposite genetic treats can be expected due to the growth of plants on polluted soil. On the one hand, pollution can select adapted genotypes, decreasing genetic variations and thus trigger population extinction. On the other hand, stress may increase mutability and directional selection of newly arisen genotypes, which can lead to speciation and evolution. The explanation for these controversial events may come from the different species and methods used in the studies. A step toward resolving this issue is finding a proper model for population genetic studies of plant put under prolonged environmental pollution stress.

In the current study we have examined the usefulness of apomictic *Taraxacum officinale* as a model plant for assessing population genetic alterations due to heavy metal pollution. Sampling sites include two areas polluted with heavy metals - one around "Kremikovtzi" and the other close to "Stomana – Pernik" metal plants, and two clean control regions around the villages of Lokorsko and Bosnek. Molecular genetic analysis included two hyper variable loci of *Taraxacum*'s genome: the intergenic spacer of ribosomal DNA and the microsatellite marker - MSTA64.

Our results revealed quantitative and qualitative differences between genotypes of the examined populations. Namely strong distinction was observed between populations from polluted and rural areas. Finally, our study confirms the reliability of apomictic *Taraxacum* populations as a superior model for evaluating the influence of heavy metal pollution on the population genetic structure.

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GO6. GENOTOXIC POTENTIAL OF SOIL SAMPLES COLLECTED IN THE REGION OF KCM PLOVDIV

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Abstract

The aim of this study was to set up fast and cheap tests with good resolution for early revealing the toxic /genotoxic/ mutagenic potential of anthropogenically contaminated soil samples collected in the area of KCM Plovdiv.

Material and methods:*Chlamydomonas reinhardtii* strain 137 C+ was used as a model organism. A set of test systems with different resolution were used for research toxic / genotoxic, mutagenic and DNA damage potential of extracts of five soil samples collected from different plots situated nearby KCM Plovdiv. The statistical assessment of the results was performed using t-test ,one way ANOVA two way ANOVA (GraphPad).

Results: Data show that the level of DSBs induced after the treatment with soil extracts for 72 hours was higher than that in control and samples treated for 2 hrs. The significantly higher levels of HSP70B were induced after the treatment with samples 1 and 5 for 72 h. This result is analogous to results obtained by the tests of genotoxicity: spot test, growth rate and the percentage of inhibition.

Introduction

Heavy metal pollution is a serious problem in many countries in Europe, including Bulgaria. In our country, soils contamination with heavy metals and metalloids represents 0.7% of the agricultural lands and could results in disruption of soil functions, pollution of surface and underground water, decreasing the yield of agricultural production and in some cases to the disturbance of plants genome. On the other hand soil pollution could lead to negative consequences in the ecosystems and food chain beings direct social implications posing risks to public health .

The problem for the development of plant bio-indicators and biomarker test systems for early diagnostics of the degree of anthropogenic load and for environmental risk assessment is particularly relevant on global and national scale. It is directly related to the strategies for conservation of bio-diversity, genome and plant populations protection in contaminated areas. Till now, no rapid, highly sensitive and informative eukaryotic cellular /bioindicators and subcellular /biomarkers for the detection and characterization of the genotoxic and mutagenic potential of low doses of anthropogenic factors inducing oxidative stress are available. According to [9] the requirements of "good" test-systems include: rapid, sensitive, and relatively inexpensive methods with good resolution and capabilities to extrapolate results to higher eukaryotes. Previously it was shown by us that unicellular green algae *Chlamydomonas reinhardtii* could be used as a robust model system for plants.

The aim of this study was to set up fast and cheap tests with good resolution for early revealing the toxic /genotoxic/ mutagenic potential of anthropogenically contaminated soil samples collected in the area of KCM Plovdiv.

Materials and methods

Strain *C. reinhardtii*: WT137 C+ was cultivated on liquid and solid TAP medium under standard conditions in a growth chamber Phytotron GC 400 at optimal conditions– continuous light of 5000–5500 lx and t = 23 ◦C ±0.1 ◦C [4]

Sampling: Five soil samples collected from different plots situated nearby KCM Plovdiv were kindly provided by Institute of Soil Science, Agrotechnologies and Plant Protection "N. Pushkarov" Heavy metals contents in soil samples were measured by ICP-AES. Extraction was performed with 0.01M $CaCl_2$ solution for 48h. In this experiments 5–7 days old cell suspensions at the end of the exponential and the beginning of the stationary phase of growth we used. Cells were harvested and after that treated for 72 hours under continuous light on a shaker at a cell density of 1×10^6 cells/ml [4] The toxic and genotoxic potential of the soil extracts were analyzed by several methods:

Genotoxicity screening: spot-test [7] is a rapid, inexpensive and informative test for the presence of bioavailable xenobiotics in natural samples. The intensity of spots is an indicator for the presence of xenobiotics with genotoxic/toxic potential. Preliminary data obtained from this test outline the next steps the examination.

Growth rate (ISO 8692:2004) - for testing the inhibitory effect of test samples 72 hours after the treatment with soil extracts. This method provides information on how the population could overcome the harmfull action the treatment [7].

Percentage of inhibition (ISO 8692: 2004) – for the revealing the cells growth inhibitory effects of samples at 72 h treatment; cells were counted 72 hours after impact.

Clonal assay: the toxic/ genotoxic potential of the soil samples was assessed by the colony forming ability. Cell survival was determined by counting colonies visible to naked eye after 7–10 days growth in the light in TAP medium.The fraction of surviving colonies (SF) was calculated [4]:

$$SF = \frac{\%\ \text{surviving macro colonies in treated sample}}{\%\ \text{surviving macro colonies in control sample}}$$

Survival fraction (SF) was calculated on the basis of colony forming ability in treated cells vs plating efficiency (PE) in the control samples (). This test allows to analyze to what extent the damages, caused by extracts are reversible. This test gives us information whether the toxic/genotoxic effects, registered at the 72-nd hour, may be eliminated from the cells after removing the distorting impact.

Mutations: The mutagenic potential of both extracts was assessed by the "visible" mutant colonies assay, based on changes in size, morphology and pigmentation of surviving colonies [4]. This assay is informative for the type of induced genetic damage – low-size mutants, which are smaller than 1/3 of the average colony size (impaired cell division), pigment mutants (gene point mutations), mosaic mutants (long-lived micro-chromosomal aberrations), morphological mutants (altered cell wall structure and composition).

The percent of induced mutations was calculated as follows:

% induced mutations = % mutations in the treated sample − % spontaneous mutantions in the control sample

The mutagenic activity of the extracts was evaluated by the mutagenic index (MI):

$$MI = \frac{\%\ \text{of mutations in treated sample}}{\%\ \text{of spontaneous mutations in the control sample}}$$

When MI is < 2.5 – no mutagenic action is observed;

MI = 2.5 – 10 – shows a weak mutagenic action;

MI > 10 – shows a strong mutagenic action [8].

Constant Field Gel Electrophoresis:-According to the requirements for the implementation of "best practices [6] in ecotoxicology, it is necessary to use direct tests analyzing the DNA damaging potential of xenobiotics. To that end, the quantity of DSBs

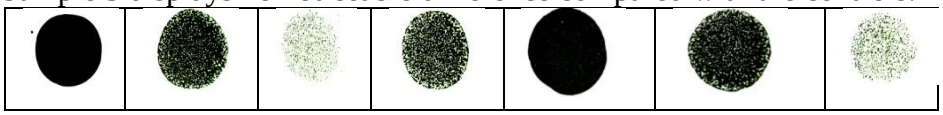
induced after 2 and 72 hours treatment with soil extracts from the region of Plovdiv by KCM CFGE, was defined. The procedure was previously described in detail [1].

Heat shock proteins: The level of heat shock proteins (HSPs) was determined by gel–electrophoresis and Western blotting [2]

Statistics: Experiments were repeated at least three times using independently grown algal cultures. Student's t-test for statistical assessment of results was used. One-way ANOVA was performed to compare differences among types of soil extracts (GraphPad Prism 5software). Two-way ANOVA were applied to determine how a response was affected by the types of soil extracts and treatment time (GraphPad Prism 5 software).

Results and discussion:

Fig 1 demonstrates the most pronounced genotoxic potential of soil samples 1 and 5 .Sample 3 displays no noticeable difference compared with the controls.



K_{TAP} K_{SG} sample 1 sample 2 sample 3 sample 4 sample5

Fig.1 Genotoxic potential of soil samples from the area of KCM Plovdiv

Fig.2 shows the percentage of inhibition of algae cells at 72 hours of the treatment (ISO 8692: 2004). Experimental data obtained is statistically significant ($p < 0.001$). The highest percentage of inhibition was observed after treatment with soil extracts 1 and 5 (72.60% and 64.38%), which corresponds well with the data presented on fig.1 and fig.2.

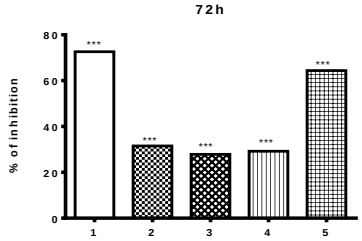


Fig.2. Percentage of inhibition of algae cells counted 72 hours

The kinetics of growth rate was determined according to ISO 8692: 2004 at 24, 48 and 72 hours during the treatment. . Genotoxic potential of soil extracts 2, 3 and 4 were considerably less. Two-way ANOVA analysis revealed well expressed relationship between treatment time and the level of contamination ($p < 0.001$). Our data show toxic/genotoxic potential of samples 1 and 5 - statistically significant decrease of growth rate of cells was observed.

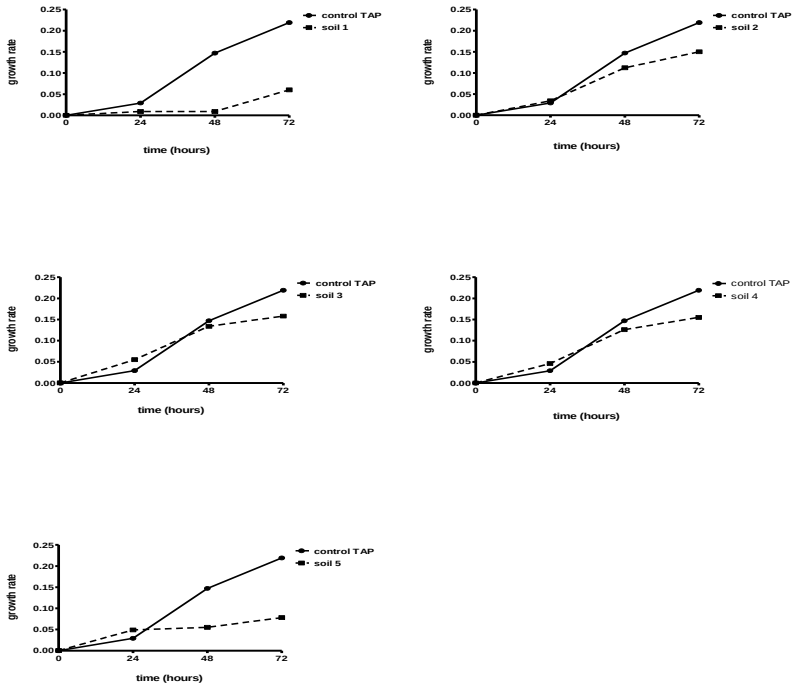


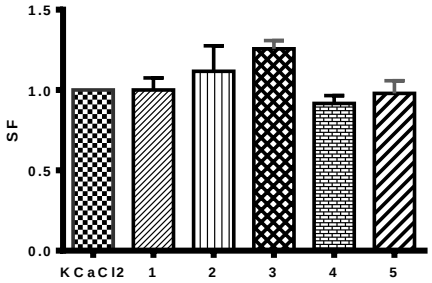
Fig.3 Kinetics of growth rate determined at 24, 48 and 72 hours, ISO 8692: 2004

Survival fraction data (SF), presented in Fig. 4(a) demonstrate that cells cultivated 72 h in contaminated samples after the plating in a fresh medium can overcome "harmful" effect of samples. In some cases minor stimulating capacity is revealed - samples 2 and 3.Colony forming ability test is very informative concerning long term consequences as a result of chronic contamination and is a good basis for the revealing mutagenic capacity of samples.

Mutagenic index (MI) was calculated using data obtained by the test of "visible" mutations. [8].

Data on fig.4 (b) show that soil extracts don't exhibit statistically significant mutagenic activity. Only the soil extract of sample 4 has MI=4.49 which indicates minor mutagenic potential. Two main types of mutations dominate - size and pigment that indicates the mechanism of cell division and the photosynthetic pigments have been affected.

a)



b)

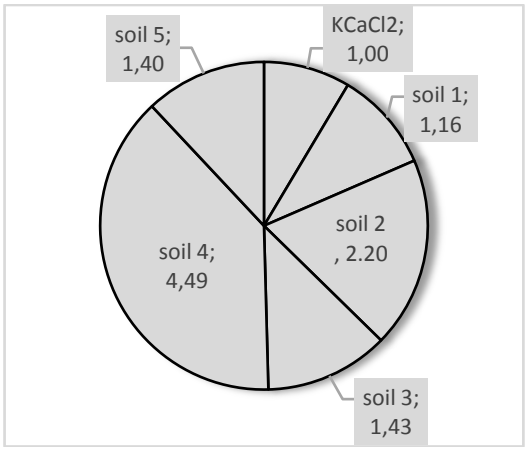


Fig.4 Survival fraction a) and mutagenic index (MI) b)

Effect of soils extracts on DNA.

The requirements to apply "good practices" (WHO, 1989) in ecotoxicology make it necessary to use direct tests for analyzing DNA-damaging potential of xenobiotics. For this purpose the quantity of DSBs, induced after 2 and 72 hours of incubation with soil extracts was measured. Data on fig. 6 show that the level of DSBs induced after the treatment with soil extracts for 72 hours was higher than that in control and samples treated for 2 hrs. It should be mentioned that samples collected at different plots could increase the level of DSBs slightly and in a similar way. Results presented here are interesting because it is well known that unrepaired DSBs could be fatal for cells. The data obtained were statistically significant: *(*57, 46%; $p<0,001$) - (18, 44 %;

$p< 0,001$), (22,39; $p<0,001$) and illustrate simultaneously the impact of the soil extracts and the treatment time - Two-way ANOVA.

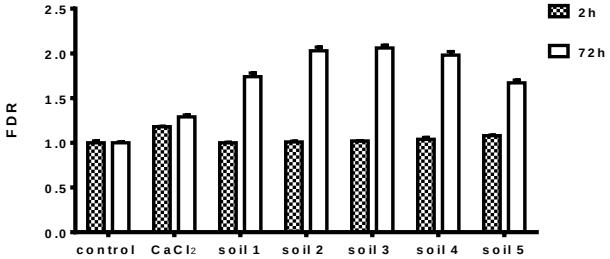


Fig.6 Induction of DSBs after the treatment with soil samples for 2 and 72 hours

Induction of HSP70B

Previously, it was shown by us that HSP70B could be recommended as an early warning marker for oxidative stress [3]. Because the such reason the level of heat shock proteins (HSP70B) was determined after the treatment with soil extracts for 2 and 72 hours. The highest induction of HSP70B was observed after the treatment with soil extracts 2 and 4 for 2 hours. The same two samples also demonstrated a statistically significant increasing the level of DSBs .Quite different results were obtained when cells were cultivated for 72 hrs in contaminated samples. The significantly higher levels of HSP70B were induced after the treatment with samples 1 and 5 for 72 h.

It could be important to point out that even the fact that HSP70B values induced after the treatment for 2 hours are about two fold higher than those obtained after the treatment for 72 hours, but a full match between the tested criteria is obtained by analysis of data at 72 hour

impact. This result is analogous to results obtained by the tests of genotoxicity: spot test, growth rate and the percentage of inhibition.

Treatment time	K_{CaCl2}	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
2 hours	3190.99	2599.40	7097.65	3701.70	6620.44	3433.55
72hours	1830.01	4798.76	2907.07	2582.36	3154.64	4124.83

Tabl.1. Induction of HSP70B after the treatment with soil extracts for 2 and 72 hours.

Conclusions:
Our data show that soil extracts from the different sampling sites near KCM Plovdiv possess more like cell growth inhibitory effect than DNA damaging and / or mutagenic potential;

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GO07. HONEY BEES AND THEIR PRODUCTS AS INDICATORS OF ENVIRONMENTAL POLLUTION WITH PESTICIDES

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Abstract

In the present work a literature review of the experiments that explore the using of honey bee and their products as bio-indicator of environmental pollution with pesticides is presented. In all cases of pesticides implementation certain amounts of them have been always accumulated in the bees and their products. Bees in this respect, which are in a constant contact with the atmosphere, plants, waters and soils are the object of numerous ecological studies aimed at establishing their role in the detection of polluted areas. A great attention is also being paid to the honey bee products which also yield information about the state of the surroundings inhabited by the bees. Honey is most often tested for pollutants followed by the bee body, pollen, wax, propolis and faecal masses of the bees. The studies on the royal jelly, nectar honey and honeydew are in single numbers. According to the researchers the pollutants accumulate in the bees and their products to different extents. Pesticides have been established in the bodies of honey bees in larger quantities with

relevance to the honey. Most of the authors show that bee honey is a suitable tool for monitoring the pollution with pesticides.
Key words: bee products**,** bio-indicators, honey bees, honey, pesticides, pollution

Introduction

Environment is the factor together with the genetic background of the human influencing to a greater extent his health and quality of life. In the recent years as a result of the activity of man (harmful emissions of burnt fuel, deforestation, the deposition of chemical, radioactive and biological refuse, etc.) serious deviations from the normal parameters recording the state of the environment have been observed. The environmental changes on their part have their effect on man directly or indirectly. For example, the contamination of soil and water with heavy metals, pesticides and chemicals leads to their accumulation in the plants and animals inhabiting the polluted areas. The human as an end consumer accumulates these pollutants, a part of which are deposited in the bones, teeth and stay there till the end of life having harmed his health in the meantime.

The danger of negative changes in the environment makes the scientists look for novel, more efficient methods for monitoring of the environment for early detection of pollutants. Along side with the confirmed and widely used methods attempts are being made recently for the testing different biological species (plants, insects, fish and other small animals) as bio-indicators of changes [4, 10, 14, 16, 19]. Honey bees, due to their morphological features, and also bee products are regarded as good indicators of environmental pollution by toxic substances, be these heavy metals, radioactive elements, or persistent organic pollutants such as pesticides. Bees can carry back to the hive many contaminants deposited on utilitarian plants. The pesticides used in agriculture (especially in spring and summer when farming activities reach their peak) may not only be the cause of the large-scale mortality of bees, but can also get into bee products [2, 3].

It is well-known that at the time of active pasture bees gather pollen and nectar in the radius of seven square kilometers [20]. Together with the nectar, propolis and pollen they introduce a number of chemical and physical pollutants in the hive, which pass into the honey and affect the production, health and life of the bees [6, 18].

According to Girotti et al. [13] honeybees can be applied, coupled to suitable immunoassays, to the determination of azinphos-methyl and thiram pesticides.

The pollutants which exert influence on the bees and the bee products can be of different nature. Such are for example the contaminations which man willingly or unwillingly produces in his everyday activities (burnt gases, heavy metals and metalloids, radionucleoides and other toxic emissions), pesticides used against different harmful agents in agriculture, the veterinary-medical preparation used in the struggle against viral, bacterial and parasitoses in the bees, etc. Bogdanov [5] has divided these pollutants into two main groups – those related to the activity of the bee-keeper and the other caused by the state of the surroundings from which the bees gather nectar and pollen. He has included in the environmental group of pollutants the heavy metals, radioactive isotopes, organic contaminators, pesticides (insecticides, fungicides, herbicides and bactericides), pathogenic bacteria and genetically modified organisms. In the group of pollutants driven into the hive by the beekeeper Bogdanov [5] mainly relates the acaricidic remedies used in the anti-parasite struggle as well as the chemical remedies for fighting bacterial infections which also leave traces in the honey, pollen, wax, propolis and royal jelly.

The bee acts as a detector of environmental pollution in two ways, as it signals either via high mortality rates the presence of toxic molecules, or via the residues in honey, pollen, and larvae the presence of heavy metals, fungicides and herbicides that are harmless to it. Bee monitoring also contributes to the ecological impact statement by culminating in the charting

of environmental health maps, which include such data as mortality rates, apicide number, type and risk-level of molecules detected, and so forth [7].

The aim of the present work is to provide a literature review of the experiments that explore the using of bee and their products as bio-indicator of environmental pollution with pesticides.

Results

According to some authors the utilization of different types of pesticides in agriculture can be established by detecting traces of them in the bees and bee products.

Monitoring of pesticide residues in honey, wax, and bees helps to assess the potential risk of these products to consumer health and gives information on the pesticide treatments that have been used on the field crops surrounding the hives [11].

According to Porrini et al. [17] in many cases, pollution caused by abuse or by erroneous application of pesticides could not be proven without the help of honey bees. This group of authors has carried out a monitoring of pesticides with honey bees. This work has been applied in some areas surrounding Bologna. Each monitoring station has been consisted of two beehives equipped with collection cages for dead bees. Once a week, families have been checked and the number of dead bees has been recorded. When the mortality rate has exceeded the critical threshold (250 bees/week/station), laboratory analyses have been carried out. The authors have indicated periods of major bee poisoning risk, and identified the most frequently used pesticides (also those that are prohibited) and the crops treated. These studies with honey bees have revealed the type of plant protection management applied to the area under investigation and allowed to prove the application of molecules not permitted under certain circumstances or even forbidden.

Samples of honeybees from 14 beehive monitoring stations located in 3 townships in the province of Bologna have been aiming to evaluate the concentration of 32 organophosphorus pesticides and 5 carbamates [12]. The most contaminated samples have been from Granarolo Emilia where cereals (wheat, sorghum, and corn), sugar beets, and potatoes have been the main agriculture products. Thirty-five pesticides have been detected, with organophosphorus being the most abundant ones. Malathion has been detected in 58% of the samples (mean level 0.360 mg/kg) followed by fenithrothion in 53% of the samples (mean level 0.544 mg/kg) and pirimiphos methyl in 48% of the samples (mean level 0.006 mg/kg). Temporal trends have showed that the maximum detection frequency has occurred in late spring and has been associated with the use of treatment products and less rainfall. The results have demonstrated the feasibility of using honeybees for assessing pesticide exposure in agriculture settings.

A field survey has been performed on French apiaries to monitor weakness of honey bee colonies [8]. Five colonies have been randomly selected in each apiary, leading to a total of 125 studied honey bee colonies. For 3 yr colonies have been visited four times per year: after winter, before summer, during summer, and before winter. Pollen loads from traps have been collected at each visit. Multiresidue analyses have been performed in pollen to search residues of 36 different molecules. Specific analyses have been conducted to search fipronil and metabolites and also imidacloprid and metabolites. Residues of 19 searched compounds have been found in samples. Contamination by pesticides has ranged from 50 to 0%. Coumaphos and tau-fluvalinate residues have been the most concentrated of all residues (mean concentrations - 925.0 and 487.2μg/kg, respectively). Fipronil and metabolite contents have been superior to the limit of detection in 16 samples. Residues of fipronil have been found in 10 samples. Nine samples have contained the sulfone compound, and three samples have contained the desulfinyl compound. Residues of imidacloprid and 6-chloronicotinic acid have been found in 69% of samples. Imidacloprid contents have been quantified in 11

samples with values ranging from 1.1 to 5.7μg/kg. 6-Chloronicotinic acid content has been superior to the limit of quantification in 28 samples with values ranging from 0.6 to 9.3μg/kg.

The frequency of occurrence and relative concentration of 44 pesticides in apicultural (*Apis mellifera*) matrices collected from five French locations (24 apiaries) have been assessed from 2002 to 2005 by Chauzat et al. [9]. The number and nature of the pesticides investigated have varied with the matrices examined—living honeybees, pollen loads, honey, and beeswax. Pollen loads and beeswax have had the highest frequency of pesticide occurrence among the apiary matrices examined, whereas honey samples have had the lowest. The imidacloprid group and the fipronil group have been detected in sufficient amounts in all matrices to allow statistical comparisons. Some seasonal variation has been shown when residues have been identified in pollen loads. On the basis of their results (highest frequency of presence) and practical aspects (easy to collect, matrix with no turnover, unlike with bees that are naturally renewed), the authors have concluded that pollen loads are the best matrix for assessing the presence of pesticide residues in the environment in given conditions.

Naccari et al. [15] have evaluated the presence of insecticides (organochlorines, organophosphates, pyrethrins and pyrethroids) in carob, chestnut and eucalyptus honey samples from Sicily and have carried out a risk assessment to dietary intake of these contaminants. Their results have shown that the concentrations of all pesticides have been under the LOD (< 0.01 mg kg-1).

The pollution of six agricultural areas of Greece by insecticides used in crop protection has been investigated utilizing, as a bio-indicator, bee honey produced in those areas [1]. Honey samples collected randomly from apiaries located in those areas have been analyzed for pesticide residues with a multianalytical method, able to determine simultaneously up to 10 organophosphorous insecticides from the same honey extract. Findings concerning the acaricide coumaphos have been also included, even though it is not used in crop protection. The above areas have been cultivated in large extent with citrus trees or cotton or sunflower crops, which are good forages for honeybees. The main pests of those crops have been insects; hence, insecticides are used on a large scale for crop protection. The most contaminated samples have originated from citrus groves; 16 out of 19 have had pesticide residues: 4 samples have had chlorfenvinphos (21.05%), 10 have had chlorpyrifos (52.63%) and 2 - phorate (10.53%). Out of 17 samples from cotton fields, residues have been found in 8, phorate in 6 (35.29%), chlorfenvinphos in 1 (5.88%), and chlorpyrifos in 1 (5.88%). Out of nine samples from fields of sunflower, four have had phorate residues (44.44%). In brief, from the 50 analyzed samples, residues of chlorfenvinphos have been detected in 5 samples (10%), residues of chlorpyrifos in 11 samples (22%), and residues of phorate in 12 samples (24%). Their levels have ranged between 0.70 and 0.89 microg/kg. Coumaphos residues have ranged from 0.10 up to 4.80 microg/kg and have been derived exclusively from beehives treated with Perizin (the commercial formulation of coumaphos) for *Varroa* control. This study has indicated that in agricultural areas with developed apiculture, useful information about the occurrence and the distribution of pesticide residues due to crop protection treatments can be derived from the analysis of randomly collected honey samples, used as bio-indicators. It also has shown that, very often, the chemicals used by apiculturists inside the hives in order to control disease are the main pollutants of the produced honey.

Discussion

In the summing-up and analysis of the literature data it is great number of the studies on the bees and their products as bio-indicators of the environmental pollutions has been carried out on the contaminations with pesticides.

It has become clear from the literature references made that in all cases of pesticide implementation certain amounts of them are always accumulated in the bees and their products.

Honey is most often tested for pollutants followed by the bee body, pollen, wax, propolis and faecal masses of the bees. The studies on the royal jelly, nectar honey and honeydew are in single numbers.

As a whole the authors quoted in the present study think that bees and their products represent suitable bio-indicators of environmental pollutions. According to the studies of those of them, however who have compared the role of the different bee products as bio-indicators [20] we have arrived at the conclusion that the pollutants accumulate in the bees and their products to different extents. Their assertion can be confirmed by the results of Tonelli et al. [20], according to which bee honey as compared to pollen and bees has only yielded tips for its possible use as bio-indicator.

Conclusion

In conclusion it can be stated that the present literature review undisputedly shows that bees and their products are a suitable model for bio-monitoring environmental pollution of different nature which renders them a promising subject for future studies in this field.

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GO8. ОТРАВЯНЕ С ТЕЖКИ МЕТАЛИ

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GO9. ANTIOXIDANTS AS NOVEL POTENTIAL THERAPY AGAINST MEHG-INDUCED NEUROTOXICITY

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GO10. ВЛИЯНИЕТО НА УОЗ ПЕСТИЦИДИ ВЪРХУ ЗДРАВЕТО НА ЖИВИТЕ ОРГАНИЗМИ. ОЦЕНКА, АНАЛИЗ И УПРАВЛЕНИЕ НА РИСКА ОТ ТЯХНОТО ЗАМЪРСЯВАНЕ

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In assessing the risk of contamination with residues of POPs pesticides and distribution of crop protection products in the environment addresses all elements of the environment, including flora and fauna. Analysis of pesticide risk in this case is done with purpose, environmental and food safety and the impact on human health and animals. Risk management of persistent organic pollutants are carried out through an integrated approach in the management of the process "Environment - Health"

Key words: risk assessment, risk analysis, risk management, pollution, POPs pesticides, environment, integrated approach, health, human, animals, soils

ВЪВЕДЕНИЕ

По-голяма част от човечеството е сериозно загрижена от продължаващото изпускане на устойчиви органични замърсители в околната среда. Тези химически вещества се пренасят през международните граници далеч от техните източници и се задържат в околната среда, биоакумулират чрез хранителната верига и поставят в риск човешкото здраве и околната среда. Следователно е необходимо да се предприемат спешни действия за ограничаване на тези замърсители. Освен това, осъзнаването от реалната опасност за необратимите поражения върху живите организми от употребата на опасни химични вещества, наложи синхронизираното действие на световните институции и организации за предприемането на адекватни мерки за защита, контрол и управление и поправката и приемането на редица нормативни документи.

В настоящият доклад се спирам само на някои аспекти от въздействието на УОЗ пестициди върху здравето на човека и животните, с цел да се направи анализ и оценка на риска и се приложи, правилен подход на управление на производството, употребата, вносът и износът им, като се използват най-добрите налични практики и техники и алтернативни методи. Направен е обстоен преглед на УОЗ-пестициди, като се акцентира основно върху тези по Стокхолмската конвенция и по-конкретно, методически са описани свойствата, употребата и въздействието им върху здравето на човека и живите организми. Приложен е хармонизиран подход при изискването, събирането, обработката на данни, методите за оценка и програмните продукти.

ИЗЛОЖЕНИЕ

Обезпокоена съм от факта, че въпреки въведените официални забрани от повече от три десетилетия насам, се откриват и регистрират остатъчни количества от устойчиви органични замърсители - пестициди по Стокхолмската конвенция в почвите. Това е един от най-лесните начини за попадането им в растителните, животинските организми и в човека. Задържането им в концентрации и количества над пределно допустимите норми, крие осъзнат и неосъзнат риск за околната среда и човека.

Твърдения, че пестицидите могат да бъдат опасни за здравето на човека са в основата за употреба на органични храни. Нивото на тези остатъчни количества е определено в европейските и правителствените стандарти за безопасност на храните (ISO 31000 – управление на рисковете), като са описани допустимите безопасни нива за дневната консумация от тези храни от възрастни и деца. Допустимите нива са изчислени на

базата на модели за отчитане на риска, които се прилагат от производителите на пестициди. Извършва се обстоен токсикологичен анализ и анализ на остатъчните вещества, с цел тестване на пестицидите преди тяхната регистрация и пускането им на пазара. По такъв начин може да се предотврати евентуалното повишаване съдържанието на остатъци в първичното селскостопанско производство и в хранителната верига (”от полето до масата”).

При оценка на риска от замърсяване с остатъчни количества устойчиви органични замърсители пестициди и разпространението на ПРЗ в околната среда се разглеждат всички елементи на заобикалящата среда, включително флората и фауната. Анализ на риска от пестициди в случая се извършва с цел, опазване на околната среда и безопасност на храните и въздействието върху здравето на човека и животните. За да се осигури високо ниво на защита и да се предотврати вредното въздействие на устойчивите органични замърсители, е необходимо тясно сътрудничество и координирани усилия от страна на органите, отговорни за изпълнение на политиките в областта на околната среда, здравеопазването, енергетиката, промишлеността, селското стопанство и транспорта. Подход за управление на риска, при който, ако съществува вероятност дадена политика или действие да може да предизвика вреда за обществеността или на околната среда, и ако все още не съществува научен консенсус по въпроса, въпросната политика или действие да не се прилага. Страните трябва да разработят планове за изпълнение на задълженията си по всяка от тях и определят национална фокусна точка, за да се улесни обменът на информация.

1. Управление на риска от устойчиви органични замърсители

Управление на риска от устойчиви органични замърсители се извършва чрез интегриран подход в управлението на процесите „околна среда – здраве”(фиг.1)

Фиг. 1. Интегриран подход в управлението на процесите “околна среда – здраве”

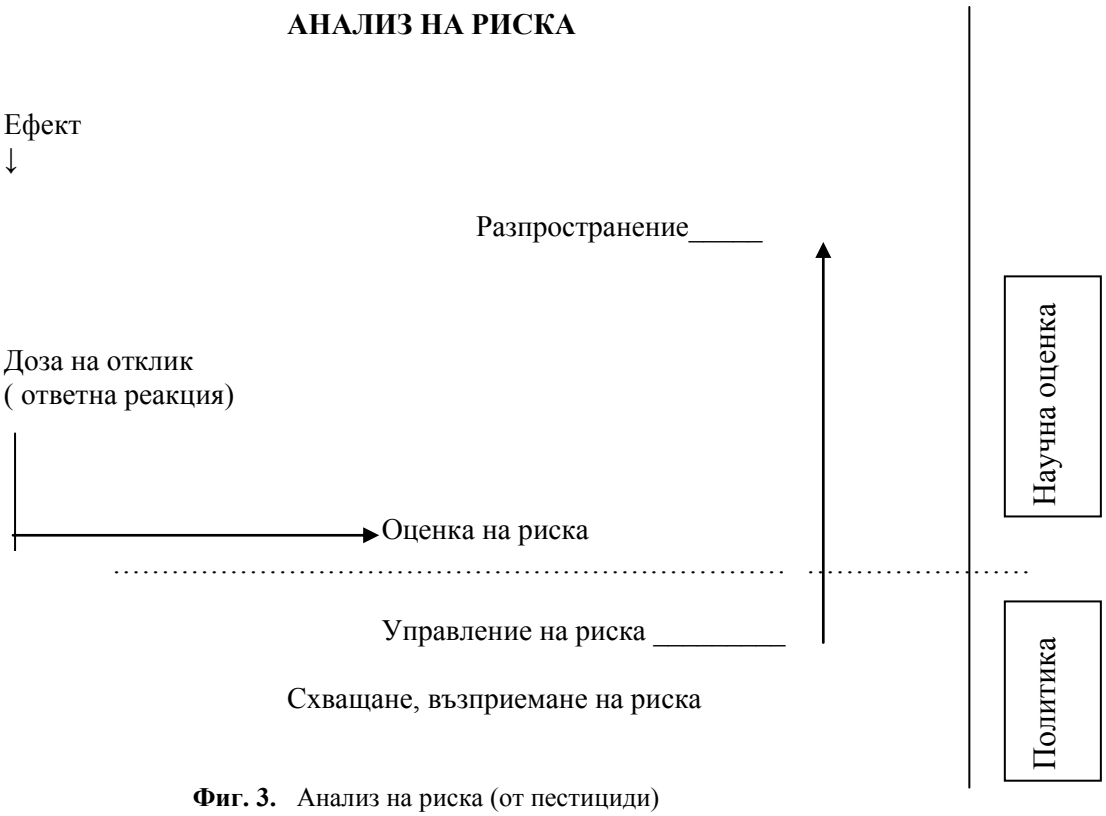
2. Оценка на риска от замърсяване с устойчиви органични замърсители
Рискът е нежелано събитие. Той се изразява с математически модел (произведение от експозиция по опасност) (фиг.2).

ОЦЕНКА НА РИСКА

Фиг. 2. Принципна схема на отразяване на оценка на риск

При оценката на риска, често използувана е линейната екстраполация (фиг.3).

3. Анализ на риска от замърсяване с устойчиви органични замърсители



Фиг. 3. Анализ на риска (от пестициди)

4. Устойчиви органични замърсители пестициди по Стокхолмската конвенция. Конвенцията обхваща вече 23 приоритетни УОЗ, произведени както преднамерено, така и непреднамерено (например от източници като инсинератори за отпадъци и др.). Те са: алдрин, хлордан, хлордекон, дихлородифенилтрихлоретан (DDT), диелдрин, ендрин, хептахлор, хексабромодифенил, хексабромоциклододекан, хексабромдифенилен етер и хептабромодифенилен етер, хексахлоробензен (HCB), алфа хексахлорциклохексан, бета хексахлорциклохексан, линдан, мирекс, перфлуорооктан сулфонова киселина, нейните соли и перфлуорооктан сулфонил флуорид,

полихлорирани дибензо-р-диоксини (PCDD), полихлорирани дибензофурани (PCDF), полихлоробифенили (PCB), технически ендосулфан и сродните му изомери, тетрабромодифенилен етер и пентабромодифенилен етер и токсафен.
Конвенцията предвижда прекратяване на производството, употребата, вноса и износа на забранените УОЗ. Тя е подписана на 22 май 2001 г. и е в сила у нас от 20.03.2005 г.
Целта е да се сведе до минимум и където е възможно, да се премахне непреднамереното производство и изпускания на УОЗ, използвайки заместващи материали, продукти и процеси.
Програмата по околна среда UNEP на ООН, извършва глобална оценка върху влиянието на първите 12 опасни химични вещества и препарати, приети под общото наименование устойчиви органични замърсители (УОЗ). В тази група на УОЗ - пестициди са включени: алдрин, диелдрин, ендрин, мирекс, токсафен, хептахлор, хексахлорбензен, хлордан, ДДТ, полихлорирани бифенили PCB, диоксини и фурани.

5. Свойства и употреба на устойчивите органични замърсители пестициди.

Химическата трайност (персистентност) на пестицидите в околната среда е относителна величина, която силно се влияе от условията в околната среда. Трябва да бъдат отчитани физическите и физико-химичните фактори (светлина, температура, ултравиолетова радиация, pH) и чисто химичните процеси на хидролиза, окисление и др., а също така и възможностите на различните микроорганизми да метаболизират пестицидите и техните разградни продукти.

Пестицидите имат различна устойчивост към разлагане, тъй като техните органични форми са въглероден източник за хранене на почвените микроорганизми.Това предпоставя възникването на остатъчни количества от тях в почвите с различна трайност във времето.Устойчивите форми се наричат персистентни и техен най-ярък представител е инсектицидът ДДТ с трайност десетки години. Подобни са и други хлорорганични пестициди. Хербицидите се отличават с по-малка трайност, между които традиционни представители са хербицидите симазин и атразин. Проблем за околната среда в резултат от използването на пестициди е т. нар. “постоянно органично замърсяване” (presistent organic polluation POPs).

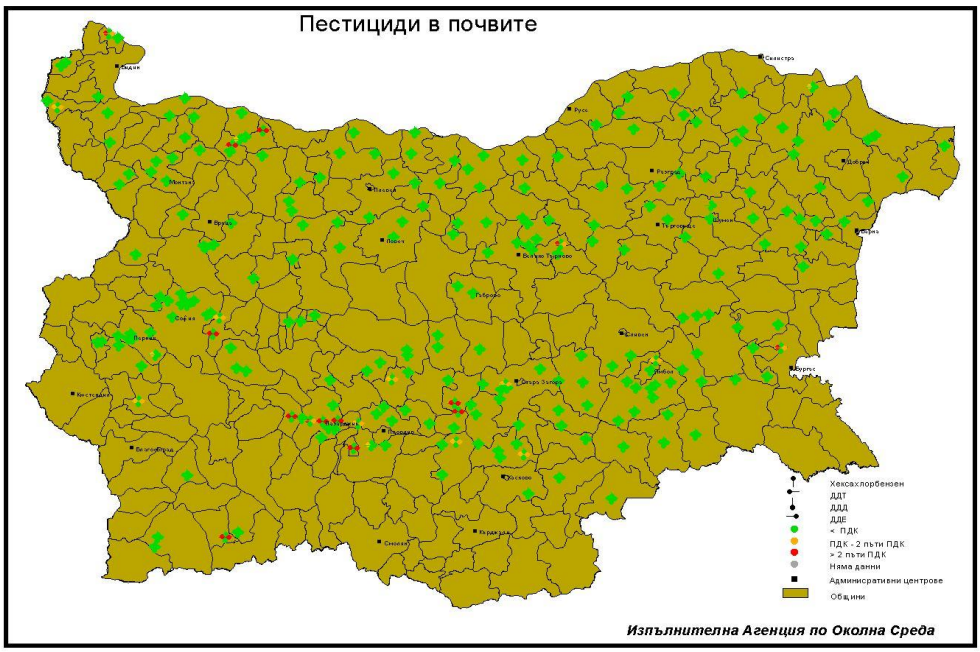
Химични средства за унищожаване на различни вредни организми определят диференциацията на пестицидите по назначение: инсектициди - за насекоми; бактерициди - за бактерии; фунгицнди - за гъби; хербициди-за плевелни растения и т.н.
Оценка на риска от остатъчни количества устойчиви органични замърсители УОЗ пестициди по Стокхолмската конвенция на територията на Република България се извършва на базата на:

- Мониторинг на остатъчни количества от пестициди в почвите
- Мониторинг на остатъчни количества от пестициди в земеделската продукция
- Мониторинг на съдържанието на химични и биологични замърсители в поливните води
- Мониторинг на остатъчни количества от пестициди във или върху третираните с ПРЗ, храни и фуражи
- Мониторинг на съдържанието на химични и биологични замърсители
- Мониторинг на болести, неприятели и плевели
- Мониторинг за въздействието на ГМО за прехвърляне на устойчивостта на болести, неприятели и плевели върху заобикалящата растителност
- Мониторинг за поведение на разпространение, подвижност и устойчивост в почвата, водата и въздуха(период на разграждане, на разпадане до метаболитни форми, промените в pH и в общата концентрация на активното вещество в почвата, карантинен срок, токсикация, екотоксикация, възможноста да влезе в контакт с подпочвените води)

• Оценка на потенциалния или реалния риск от постъпване на остатъчни вещества по хранителен или друг път, например данни, отнасящи се до контрола на остатъчните пестициди, които се съдържат в разпространени в търговската мрежа продукти, или данни отнасящи се до риска за замърсяване чрез почвата, водата, въздуха и др.

• Изграждане на Географски информационни системи

Наблюдението и контролът върху състоянието на земите и почвите у нас се осъществява от Националната система за екологичен мониторинг



Фиг.4. Замърсявания на почвите с пестициди на територията на Р България
Източник: Изпълнителна агенция по околна среда и води (ИАОСВ), 2000 г.

В резултат на картографски отразените замърсявания на почвите с устойчиви органични замърсители пестициди, можем да обобщим, че в областите, където има промишлени обекти за производство на тежки и цветни метали и оранжерийно производство, се наблюдава натрупване на най-големи остатъчни количества устойчиви органични замърсители пестициди в почвите.

При оценка на риска от замърсяване на почвите с устойчиви органични замърсители пестициди се вземат предвид изискванията на редица нормативни документи.

Страните разработват планове за изпълнение на задълженията си по Конвенцията. Всяка от тях определя национален приоритет, с цел по-лесен обмен на информация.

6. Критерии за оценка на риска от пестицидите с оглед опазването на човешкото здраве и околната среда

Рискът от пестицидите върху човешкото здраве и околната среда се проявява като:-остра токсичност: орална, дермална, инхалаторна;

- Дразнене на кожата на очите;
- Сенсибилизация
- Тератогенно действие
- Мутагенно действие
- Канцерогенно действие
- Устойчивост в почвата
- Подвижност в почвата

- Биокумулация

Набазата на тези критерии пестицидите в България се класифицират на три категории за употреба:

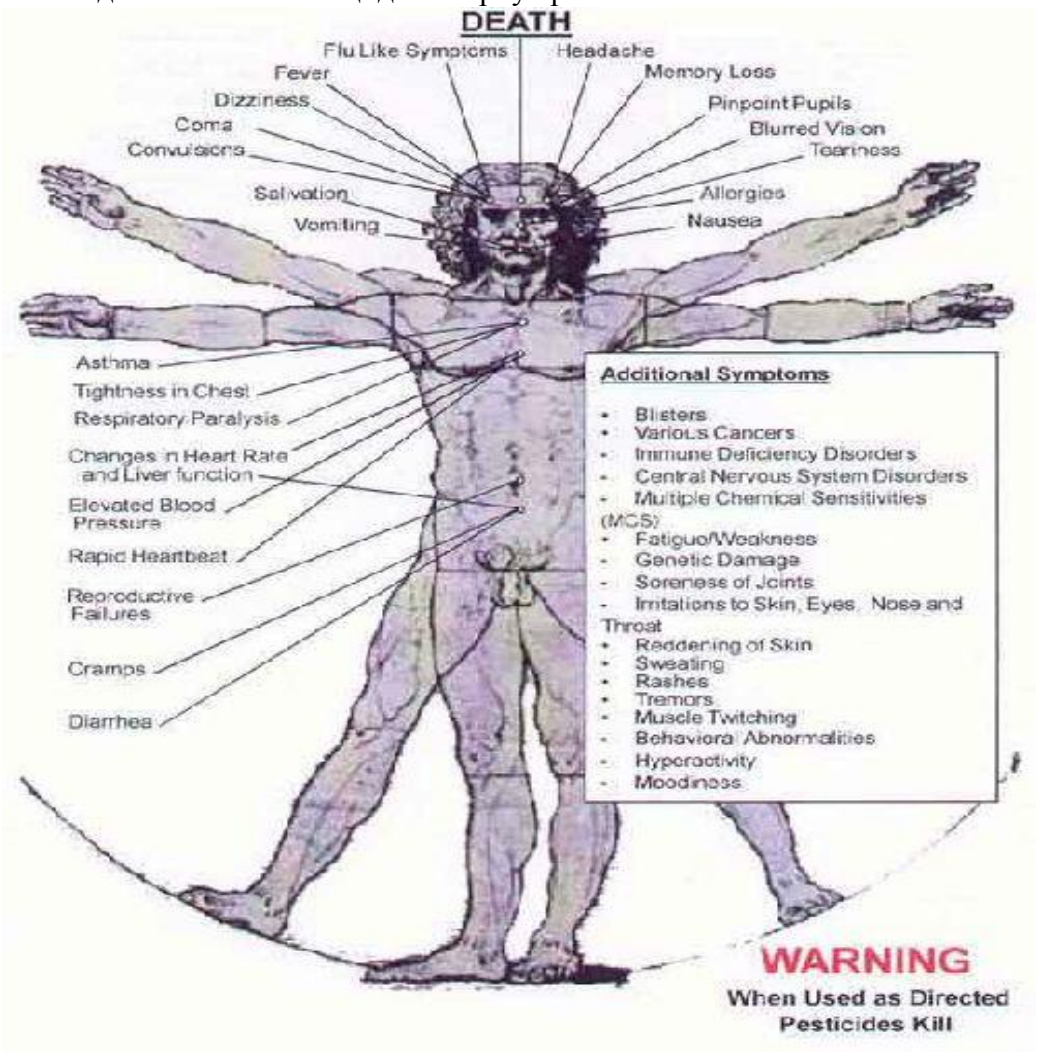
I категория – пестициди, които могат да се прилагат само от квалифицирани специалисти по растителна защита;

II категория – пестициди, които могат да се прилагат от лица с документ, че са обучени за безопасна работа с пестициди;

III категория – пестициди, които могат да се прилагат от всички лица, навършили 18 годишна възраст.

Постоянното органично замърсяване отравя останалите организми и до голяма степен увеличава шансовете за увреждане на ендокринната система, причинява рак, безплодие и генетични мутации при човека. Въпреки това тези "дълготрайни хронични ефекти" са все още недостатъчно изследвани **(**фиг. 5).

Въздействие на пестицидите върху органите на човешкото тяло



Фиг. 5. Въздействие на пестицидите върху органите на човешкото тяло
Източник: Национален план за действие по околна среда и здраве

Връзката между нивото и продължителността на действието на устойчивите хлорорганични пестициди върху здравния статус на човека е предмет на много проучвания (Таблица 1.).

Таблица 1. Класификация и здравен риск за УОЗ пестициди

Пестицид	ЛД50 (mg/kg b.m.) WHO[1]	Клас опасност WHO[1]	Категория канцерогенност JARC[2]	Вредни ефекти
Алдрин	98	I b	3	Имунотоксичен, увреждане на черния дроб и мъжката репродуктивна функция
Диелдрин	37	I b	3	Имунотоксичен, увреждане на черния дроб и мъжката репродуктивна функция
ДДТ и метаболити	113	II	2B	Имунотоксичен, увреждане на естрогенната и ендокринната системи,
Ендрин	7	Ib	3	Алергични реакции, токсичен хепатит, увреждане на централната и периферната нервна система
Хептахлор	100	II	2B	Увреждане на репродуктивната функция и ендокринната система
Хексахлорбензен	> 10 000	Ia	2B	Негативен ефект върху нервната, ендокринната и репродуктивната системи, порфирия при хора.
Токсафен (камфехлор)	80	II	2B	Увреждане на централната и периферната нервна система
Хлордан	460	II	2B	Увреждане на ендокринната и репродуктивната системи
Мирекс	306		2B	Тератогенен

[1] WHO – Класификация на Световната здравна организация на пестицидите
[2] JARC - Категория канцерогенност по Международната агенция за изследване на рака

Според Световната здравна организация (WHO), класификацията на на пестицидите се представя в следните класове:

Ia – извънредно опасен;
Ib – силно опасен;
II -умерено опасен;
III – слабо опасен.

Категориите за канцерогенност по Международната агенция за изследване на рака (JARC[2]) са:

Категория 1 – доказан канцероген за човека;
Категория 2А – възможен канцероген за човека,
Категория 2В – вероятен канцероген за човека,
Категория 3 – не се класифицира като канцероген за човека.

*Данните са отчетени преди влизането в сила на новите стандарти

Околната среда е факторът, който наред с генетиката на човека влияе върху неговото здраве и качество на живот. През последните години в резултат на човешката дейност (отделяне на вредни емисии от изгорели горива, изсичане на горите, изхвърляне на химически, радиоактивни и биологични отпадъци и др.) се наблюдават сериозни отклонения от нормалните показатели, отчитащи състоянието на околната среда. Промените в околната среда от своя страна рефлектират пряко или косвено върху човека. Например изхвърлянето на тежки метали и химикали в почвата и водите води до натрупването им в растенията и животните, които обитават замърсените райони. Човекът като краен консуматор акумулира тези замърсители, част от които (например някои тежки метали) се отлагат в костите, зъбите и остават там до края на живота, като при това увреждат здравето му. Постъпване на токсини от околната среда (тежки метали или други индустриални агенти), предизвикват състояния и заболявания, негативно въздействащи върху сперматогенезата. В резултат от вредното им въздействие се увеличава и стерилитета на човешкия организъм (Стоянов,1999). Наличието на антитела или абнормална морфология на сперматозоидите могат да предотвратят оплождането на яйцеклетката. През последните години се оформи едно ново направление в репродуктивната медицина и биология – изучаване влиянието на факторите на околната среда.

Устойчивите органични замърсители заедно с алкохолът, никотинът, лекарствените вещества, температурата, травмите, лъчевите увреждания, тежките метали, стресът и други, оказват отрицателно влияние на качеството на сперматозоидите и нормалното протичане на сперматогенезата (Coutts et al., 2007; Kobayashi et al., 201)

В световен мащаб са проведени значителен брой изследвания за съдържание на УОЗ в майчино мляко с цел определяне експозицията на кърмачетата и свързания с това риск. Възрастта на майките, броят на кърмачетата и хранителните навици са критични параметри за определяне замърсяването на майчиното мляко с УОЗ и натрупването им в човешкия организъм. Р България е сред страните с най-ниски установени нива на PCB (под 5 pg TEQ/g fat) и на сумарното съдържание на трите индикатора (под 40 ng/g fat) в майчино мляко.

В Р България няма данни за регистрирани случаи с остри и хронични интоксикации при употреба на устойчиви хлорорганични пестициди и не са изследвани.

В рамките на разработвания от 19 европейски страни международен проект "WHO-coordinated Exposure Study on the Levels of PCBs, PCDDs and PCDFs in Human Milk, Organohalogen Compounds, 2003" в РБългария е извършено проучване на съдържанието на устойчиви хлорорганични пестициди в майчино мляко от 30 здрави жени, разпределени по 10 от три района на страната (Банкя -екологично чист и два (София и Благоевград -в различна степен екологично замърсени).

Предварителните резултати показват, че в майчиното мляко в екологично чистия район (Банкя) отсъстват ендрин, токсафен и мирекс. Установява се наличие на хексахлорбензен (0.012 mg/kg lipids), хлордан (0.018 mg/kg lipids), хептахлор (0.013 mg/kg lipids), диелдрин/алдрин (0.004 mg/kg lipids) и ∑ DDT (0.499 mg/kg lipids), представена от pp· - DDE (0.452 mg/kg lipids), op′-DDT (0.003 mg/kg lipids) и pp′-DDT (0.044 mg/kg lipids)(Таблица 2.)

Таблица 2. Концентрации в околната среда на избраните POPs в България

Химикали	Концентрации							
	Подземни води		Почви		Въздух		Храни	
	Стандарт	Статус	Стандарт	Статус	Стандарт	Статус	Стандарт	Статус
DDT (Сума)	ET=0,01 µg/l PT=0,1 µg/l	Годишен справочни kconc.,µg/l 1998-0,1171; 1999-0,4463 2000-0,0058 2001-0,0003 2002-0,0034	PC=0,3 mg/kg DS MAC=1,5 mg/kg DS CLI=4 mg/kg DS; US EPA AHR-2-3ppm	Годишен справочник. conc., µg/kg 1997-714,7 1998-318,8 1999-113,6 2000-120,1 2001-316,4	EC standard 20 pg/m3	Провинциални области= 1-22.10-6 ng/m3 Others-1 = 2,3.10-6 ng/m3	MACмесо =100 µg/kg MACкартофи =100 µg/kg MAC пшеница =200 µg/kg MACмляко =1000 µg/kg fat	Месо: 1987-4328µg/kg 1989-1100 µg/k 1991: Картофи92 µg/ Пшеница17 µg/ 1995: Мляко– to 387
Диоксини и фурани	SAE = 0,5 ng I-TEQ/l Отпад.води MAC PAH питейна вода = 0,10 µg/l	Няма мониторинг	BL_{PAH} = 0,15 mg/kg PC_{PAH} = 0,4 mg/kg MAC_{PAH} = 4 mg/kg	Няма мониторинг	SAEDW = 0,1 ng I-TEQ/m3	Няма мониторинг	EC Препоръчителни нива за храни и фураж	Няма монитори

Източник: www.moew.government.bg;Здраве,Статистичен годишник , 2003
n.d. – не е открит
Екологичен праг–ЕП; Праг на замърсяване- ПЗ
Защитна концентрация–ЗК; Максимално допустима концентрация– МДК; Ниво на концентрация за намеса–НКН; Суха почва- СП
Приемлив здравен риск–ПЗР

Стандарт за допустими емисии от изгаряне на битови отпадъци– SAEDW
Фоново ниво на ароматни въглеводороди (PAH)- BLPAH

От проведените изследвания се констатира, че от всички хранителни вещества майчиното мляко е най-наситено с хлорорганични съединения.Чрез кърменето организмът на майката усилено се самоочиства. Положителното действие на кърмачето за майката трябва да се преценява с риска за детето, особено в по-късните фази от живота.Това обяснява фактът, че концентрацията на тези съединения при кърмачето, е 75 пъти по-висока от при възрастния човек.

Поради широката замърсеност на околната среда и фуражите с инсектициди, практически е невъзможно да се получат хранителни продукти от животински произход, свободни от пестициди и по-специално от хлорорганични съединения. За конаминиране на човешкия организъм с пестициди, тези продукти имат около 90% участие, близо 4/5 от ДДТ и 2/3 от диелдрина, които попадат с храната в организма, произхождат от месото, яйцата, мазнините и маслото.
Съгласно регламента REACH веществата, нарушаващи функционирането на ендокринната система, могат да се идентифицират като особено опасни, заедно с химичните вещества, за които е известно, че предизвикват рак, мутации и са токсични за репродуктивните способности. Целта е употребата им да се намали и в последствие да бъдат заместени с по-безопасни алтернативи.
Много са причините за възникване на риск за здравето: замърсената околна среда, повишеният радиационен фон, съвременният начин на живот с високи нива на стрес и негативни емоции, нездравословният начин на хранене, нелекувани хронични възпалителни процеси. Излагането на пестициди и инсектициди и др. химикали също има връзка с формирането на възли и проява на автоимунен тиреоиден процес. Наскорощно проучване на СНА за икономическата стойност на обезщетенията за избягване на избрани нежелани резултати за здравето на човека поради излагане на химикали. Тази информация може да се използва в социално-икономическия анализ, при оценката на здравните и екологични въздействия на химикали.

7. Анализ на риска от съдръжнието на УОЗ в продуктите от растителен и животински произход.
Установена е тясна връзка между съдържанието на ДДТ във фуража и отделянето му с млякото и натрупването в сиренето. Съгласно българските и други международни не се допуска за храна мляко, съдържащо малки количества фосфоорганични препарати. С най-ниско съдържание на фонови остатъци от алфа и бета НСН е измерено в овчето сирене произведено в Пазарджишки окръг (59,1,7 мкг/кг за 1984 г.), следвано от Пловдивски (73,+/-3,9мкг/кг), Старозагорски (80+/-3,1 мкг/кг), Хасковски (82+/-2,6мкг/кг), Ямболски (89+/- 3,1 мкг/кг), Бургаски (135+/-3,0мкг/кг), а най-високо в Плевенски окръг (254+/-0,5 мкг/кг).

Предложените изследавния са от особено значение за профилактиката на репродуктивните заболявания и ще предоставят нови данни за усъвършенстване оценката и управлението на риска от ЕД. Установено е, че ДДТ причинява и родилни аномалии при хората и животните.
Съдържанието на остатъци от хербициди в млякото и захарта се движи в границите от 0,001 до 0,025 мг/кг. Тези граници представляват хигиенен риск за консуматорите. Определените остатъци от атразин зависят тясно от неговата трайност в почвата и относително високата му химическа стабилност.

Установено е, че всички хлорорганични пестициди при попадане в организма се натрупват в тлъстините и в органите, съдържащи липиди, и то в следния низходящ ред: алдрин – диалдрин, хексахлоран/технически НСН/ - ДДТ – хлордан –ендрин –

хептахлор ---токсафен, т.е. най-силно кумулативно действие притежава алдринът, а най-слабо токсично –фенът. В тлъстините на домашните животни кумулират повече алфа – изомерът на НСН, отколкото гама – изомерът /линдинър/.

При заклани животни след отравяне с фосфорганични съединения, тлъстините са с неприятна миризма и вкус. Месото от телетата и овцете, прасетата и кокошките, отровени с фосфоорганични препарати, е с по-кисела реакция, отколкото месото добито от другите животни. Най-ефикасен метод за намаляване на съдържанието на НСН е варенето на месото с отвеждане на образувалите се пари. Основната причина за намаляване на ДДТ в месото при варене е свързано с редукционното му дехлориране до ДДД. Установено е, че след приемане на хлорорганични пестициди с храната се понижава биологичната стойност на месото. А това се дължи на въздействието им върху метаболизма на мазнините и на витамин А в черния дроб.

От почвата устойчивите хлорорганични пестициди попадат във водоемите, реките и моретата. Те кумулират в организма на рибите.

Чрез прашеца в пчелния мед могат да попаднат остатъци от пестициди.

По данни от изследвания в 22 страни, средните стойности на хлорорганичните пестициди в мастната тъкан на хората са : ДДТ (всички изомери) - от 1,75 до 30 мг/кг; НСН (всички изомери) от 0,16 до 2,43 мг/кг; диелдрин –от 0,046 до 0,68 мг/кг; хептахлорепоксид – от 0,0085 до 0,19 мг/кг. Чрез дневната дажба у нас до 1990 г. в организма на човека постъпват :35,6мг ДДТ и 22,4 мг линдан (гама – НСН), което е под допустимата дневна норма на тези пестициди. За човек със средно тегло 65 кг се допуска 325 мг ДДТ и 650 мг линдан.

Широката употреба на различни видове пестициди в растениевъдството е предпоставка за тяхното разпространение в биосферата. Попадането на пестицидите в животните директно от стратосферата е сравнително рядко и се осъществява чрез натрупването им естествено във веригата фуражи – животно.

Третираната с пестициди растителност, служи за изхранване на селскостопанските животни във вид на сочен, груб или концентриран фураж, е основен преностител на пестицидите в животинския организъм.

Установено е, че е възможно замърсяване при обработка на животните, с цел обезпаразитяване, особено, когато се използват пестициди, имащи голяма резорбтивна способност и добра разтворимост в мазнини, като хлорорганичните съединения и др. След еднократна обработка на говеда с 0,25% разтвор от ДДТ остатъци от пестициди са открити в околобъбречната тъкан в продължение на около 2-3 месеца, и то в количества по-големи от 20 мг/кг.

Дезинфекцирането на животинските ферми и на помещенията в месопраработвателните комбинати с пестициди, също може да доведе до замърсяване на месото.

Рискът от опасността от пестицидите в много от плодовете и зеленчуците (ябълки, чушки, череши, грозде, нектарини, праскови, сливи, картофи, малини, спанак и ягоди) съдържат остатъчни количества от пестициди, дори след като бъдат измити и обелени.

Мониторинг за съдържание на остатъчни количества от пестициди в листни зеленчуци (фиг. 9) и домати (фиг.10.)

фиг. 9. **фиг.10.**

Източник: Отчетен доклад на ЦЛКПТМТ на МЗГ за 2004 г.

8. Анализ на риска от наличието на УОЗ пестициди в екосистемата

Освен рисковете за здравето на човека, употребата на пестицидите причинява вреда на цялата екосистема. Успоредно с унищожаването на вредителите, пестицидите нанасят вреда на някои хищници или паразити, които се хранят с тях. Обикновено техните популации се възстановяват по-трудно и по-дълго. Например третирането на комарите временно преустановява размножаването на популациите им, но причинява дълготрайни вреди на други видове.

Един от най-ранно установените проблеми от използването на пестицидите е, че вредителите много бързо стават резистентни към химикалите. При третирането им не всички от тях биват унищожени. Някои от тях, имащи по-различна генетика оцеляват и така благодарение на естествената селекция вредителите могат да станат резистентни. Незнаещи как да се справят с този проблем, фермерите увеличават количеството на използваните пестициди.

Затова методът за използването на алтернативи на пестицидите е едно решение за управление на риска и е познат като "Интегрирано управление на пестицидите" (Integrated Pest Management - IPM). Този метод е широко използван в САЩ. Старите и вредни пестициди са заместени от ново поколение пестициди. Много от тези пестициди съдържат биологични и ботанически деривати. Инженерите разработват постоянно нови пестициди, а земеделските производители са насърчавани при използването на алтернативни продукти и прилагането на методи за намаляване използването на химически пестициди.

9. Анализ на риска от отпадъци, съдържащи УОЗ пестициди

Агрохимичните опасни отпадъци (код 02 01 08), състоящи се от залежали УОЗ пестициди представляват едва 1.14% от всички залежали и негодни за употреба пестициди в България (Таблица 3).

Таблица 3. Опасни отпадъци, състоящи се от залежали УОЗ пестициди през 2010 г. в България

		Единица	Количество
УОЗ пестицид		**kg**	**6 547**
Хептахлор в 2 ББ куба		**kg**	**50 312**
Линдан, общо - в 61 ББ куба - в 4 склада		**kg**	**104 045 99 575 4 470**
ОБЩО УОЗ пестициди			**160 904**

Източник: www.moew.government.bg;, 2013

Към 31 декември 2011 г. в страната са идентифицирани отпадъци от следните залежали УОЗ пестициди – хептахлор (6 547 кг), DDT (50 312 кг) и линдан (104 045 кг), съхранявани в складове и ББ-кубове.

Райони с потенциал за образуване на УОЗ в емисии

Райони с потенциал за образуване на емисии PCDD, PCDF, PCB, HCB и PAH в атмосферния въздух са промишлените центрове, където са разположени повечето от големите топлоелектрически централи, работещи на лигнитни въглища и мазут, промишлени предприятия от металургията, и големите градове, където минават основните пътни и ж.п. артерии на страната. През 2010 г. не са регистрирани нови нива на замърсяване на почвите с УОЗ.

Получените резултати от мониторинга показват, че на този етап извършваните земеделски дейности не водят до нови замърсявания на почвите. Този факт се дължи от една страна на намаленото потребление на торове и пестициди, както и на провежданите програми за еколосъобразно земеделие и биологично производство.

Резултатите от почвения мониторинг за периода 2007 г – 2010 г. показват, че почвите от земеделските земи са в много добро екологично състояние и не са установени замърсявания с УОЗ пестициди над МДК.

В периода 2005 г - 2010 г. измерените съдържания на PCB са под границата на откриване.

10. Управление на риска и бъдещо производство и употреба на прогнозни емисии на УОЗ пестициди

Производство: УОЗ пестициди, в т.ч. и новите УОЗ не са произвеждани в България и не се предвижда бъдещо производство;

Внос и употреба: Вносът, пускането на пазара и употребата им са забранени в България;

Износ: Износът на УОЗ пестициди е забранен, освен ако той не е за екологосъобразно обезвреждане. Следните УОЗ пестицидите подлежат на процедурата за уведомление за износ (PIC процедура), забрани и строги ограничения: алдрин; хлордан; хлордекон; DDT; диелдрин; ендосулфан; HCH изомери; линдан; хептахлор; хексахлорбензен HCB и токсафен.

Прогнозни стойности за емисиите на УОЗ (PCDD/PCDF, PCB, HCB, PAH, PeCB) до 2020 г.

Разработена е стратегия за ограничаване на емисиите от определени замърсители в атмосферния въздух, в т.ч. и УОЗ, чиито прагове и времеви хоризонти са регламен - тирани от международните ангажименти на България. Стратегията включва емисиите на следните УОЗ в атмосферния въздух : DIOX/F, HCB, PAH и PCB.
В последните години Р България провежда политика на активна подкрепа на международни инициативи, свързани с глобалното подобряване на екологичното състояние на планетата Земя. С усилията, които полага за изпълнение на поети по силата на различни конвенции, спогодби и протоколи задължения тя дава своя принос в тази насока, независимо от ограничените възможности от териториален, демографски и икономически характер. Устойчивото развитие е една основна цел.
В България съществуват редица програми за мониторинг на различни замърсители в компонентите на околната среда, включително и УОЗ, като част от Националната система за мониторинг на околната среда.
НСМОС е създадена и функционира в съответствие с чл.1, т.7 от ЗООС. Системата осигурява своевременна и достоверна информация за състоянието на компонентите на околната среда и факторите, въздействащи върху нея, въз основа на която се правят анализи, оценки и прогнози за обосноваване на дейностите по опазване и защита на околната среда от вредни въздействия.
Национална информационна система за докладване по ЕРИПЗ
Разработена е и функционира Национална информационна система за докладване по ЕРИПЗ, в съответствие с изискванията на Регламент № 166/2006 и ЗООС. Системата осигурява докладване през интернет от операторите, верифициране и потвърждаване на докладите от РИОСВ и изготвяне на докладите до ЕК от ИАОС. Част от информационната система е и публичният регистър, осигуряващ възможност за извършване на справки от данните в системата. Контролно-информационна система за състоянието на отпадъчни води. Информационна система за забранени и залежали пестициди.
Информационна система за локални почвени замърсявания със залежали УОЗ пестициди
Информационна система за индустриално замърсяване на почвите с PCB и PAH. Информационна система за опазване на земните недра.
Информационна система за отпадъци.
От 2011 г. БАБХ поема контролът на храните по цялата хранителна верига и обединява 3 мониторингови програми в Национална програма за мониторинг на остатъци от пестициди и други вредни вещества в и върху храни от растителен и животински произход (НПМКО).

ЗАКЛЮЧЕНИЕ

В заключение може да се направи следната обща оценка на риска при замърсяване на почвата с устойчиви органични замърсители пестициди – източници на вреди съществуват в част от дейностите, но рискът е приемлив и съществуват средства за неговото ограничаване и предотвратяване. Анализ на риска в случая се извършва с цел, опазване на околната среда и безопасност на храните. Прилагат се регистрирани продукти за растителна защита - в съответните дози, подходящи за създалата се ситуация.

От гледна точка на здравеопазването, за хората е от значение системното регулиране на препаратите и съблюдаване на предписаните дози. По такъв начин може да се предотврати евентуалното повишаване съдържанието им.
Партньорството, взаимното разбиране, контрола, самоконтрола ще ограничат значително риска. Координацията и последователността следва да се гарантират, когато

на общностно равнище се прилагат разпоредбите на Базелската, Ротердамската и Стокхолмската конвенция и когато се участва в разработването на стратегическия подход при международното управление на химичните вещества и препарати (SAICM) в рамките на Обединените нации.

За в бъдеще, регламент REACH ще бъде подходящ инструмент, чрез който се прилагат необходимите мерки за контрол върху производството, пускането на пазара и употребата на изброените вещества, както и мерките за контрол върху съществуващите и новите химични вещества и пестициди, които проявяват
характеристики на устойчиви органични замърсители.

Нашето поведение за изграждане на един устойчив свят, който да е белязан от хармония между човешката дейност и чистата околна среда, ще даде отговор на въпроса как ще живеят нашите деца, бъдещите поколения и.... ще ги има ли?

GP1. ЛЕКАРСТВЕНИ ВЗАИМОДЕЙСТВИЯ НА ЧЕСТО СРЕЩАНИ ВЕЩЕСТВА

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