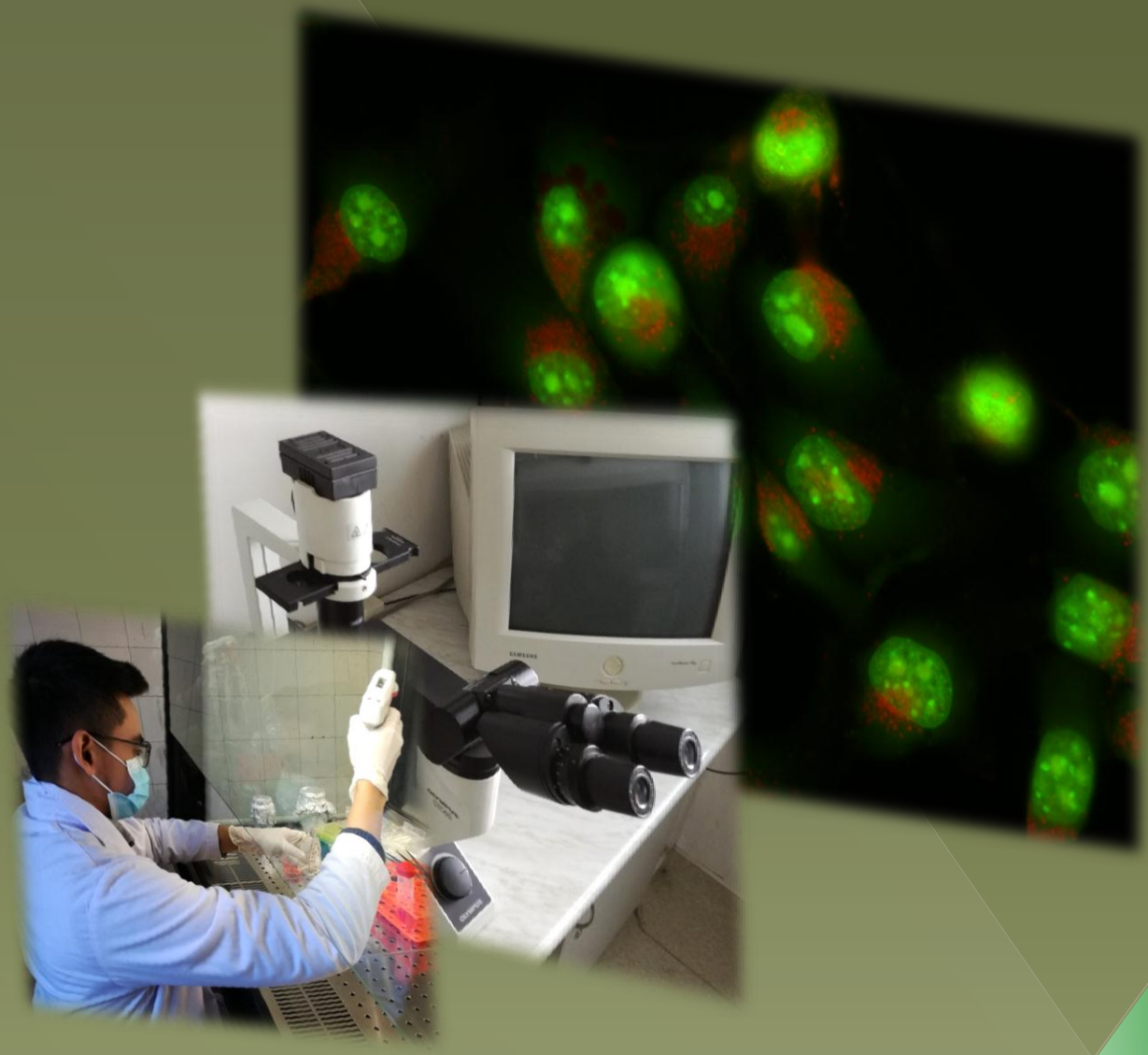
145 ГОДИНИ БЪЛГАРСКА АКАДЕМИЯ на НАУКИТЕ
1869-2014

Institute of Experimental Morphology, Pathology and Anthropology with Museum

PROCEEDINGS

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



JUNE 14 – 16, 2017
SOFIA, BULGARIA

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PROCEEDINGS

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

June 14 – 16, 2017

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

Edited by: Dimitar Kadiysky and Radostina Alexandrova

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THE EGHHTH WORKSHOP

"EXPERIMENTAL MODELS AND METHODS IN BIOMEDICAL RESEARCH"

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

UNDER THE AUSPICES OF

THE BULGARIAN ACADEMY OF SCIENCES

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THE PROGRAM OF THE WORKSHOP

Wednesday, 14 June 2017

9.30 – 9.45 OPENING CEREMONY

Session A

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

Assoc. Prof. Plamen Todorov, PhD
Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences

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

9.45 – 10.15
AO1. AUTOPHAGY IN NORMAL AND PATHOLOGICAL PROCESSES
Radostina Alexandrova[1], Abedulkadir Mahdi Abudalleh[1]
Milena Glavcheva[1], Desislav Dinev[1], Tanya Zhivkova[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
[3]Health Service, Gorna Malina Bulgaria

10.45 – 10.45
AO2. XENOTRANSPLANTATION AS AN INVESTIGATION MODEL OF THE FUNCTIONAL CHARACTERISTICS OF HUMAN OVATIAN TISSUE AFTER CRYOPRESERVATION
P. Todorov[1], E. Hristova[1], A. Chorbanov[2], N.Mihaylova[2], G. Rahimi[3], V. Isachenko[3]
[1]Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Institute of Microbiology, Bulgarian Academy of Sciences, Sofia,Bulgaria
[3]Cologne University, Cologne, Germany

10.45 – 11.00
AO3. EFFECT OF THE COMBINED EFFECT OF VITAMINS A, D3 AND ON THE MOTHER OF SMOOTH SPERMATOISOIDS IN THE CRYO CONFIRMATION PROCESS
Kiril Lazov, Desislava Gradinarska, Denica Daskalova
Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria

11.00 – 11.20 Coffee Break

11.20 – 11.50

АО4. ОСТРИ ИНТОКСИКАЦИИ С ФОСФОРОРГАНИЧЕН ПЕСТИЦИД – НУРЕЛЕ Д - ПРЕДСТАВЯНЕ НА ДВА КЛИНИЧНИ СЛУЧАЯ

Раденкова-Саева Ю., М.Петкова
Клиника по токсикология, УМБАЛСМ "Н.И.Пирогов",

11.50 – 12.05

AO5. NOVEL SUBSTRATES FOR THE DETERMINATION OF FIBROBLAST ACTIVATION PROTEIN α (FAPα) ACTIVITY

M. Dimitrova[1], I. Iliev[1], D. Tasheva[2], V. Lozanov[3], I. Ivanov[3]
[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Faculty of Chemistry and Pharmacy, Sofia University "St. Kl. Ohridsky", Sofia, Bulgaria*
[3]*Department of Medical Chemistry and Biochemistry, Medical University of Sofia, Sofia, Bulgaria*

12.05 – 12.20

AO6. SALINOMYCIN AMELIORATES HEMATOLOGICAL PARAMETERS AFTER CADMIUM-INDUCED INTOXICATION

Yordanka Gluhcheva[1], Emilia Petrova[1], Ivelin Vladov[1], Ekaterina Pavlova[1], Kalina Kamenova[2], Juliana Ivanova[3]
[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria;*
[2]*Faculty of Chemistry and Pharmacy, Sofia University 'St. Kliment Ohridski', Sofia, Bulgaria;*
[3]*Faculty of Medicine, Sofia University 'St. Kliment Ohridski', Sofia, Bulgaria*

12.20 – 12.40
Discussion

Session B

Chairpersons:

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

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

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

13.30 – 14.00

BO1. APPLICATION OF DIAGNOSTIC TECHNIQUES, DATA INTERPRETATION AND ANALYSIS OF LAWSONIA INTRACELLULARIS INFECTIONS

Kiril K. Dimitrov[1*], Ivan Dinev[1], Ismet Kalkanov[1], Katerina Todorova[2], Yosif Velkov[2], Boicho Nikolov[2]

[1]Pathologic Anatomy Unit, Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria
[2]Institute of Experimental Morphology, Pathology and Antropology with Museum, Bulgarian Academy of Science, Sofia, Bulgaria

14.00 – 14.15

BO2. DETECTION OF LAWSONIA INTRACELLULARIS IN WILD BOAR AND INDIGENOUS EASTERN BALKAN SWINE FROM CENTRAL STARA PLANINA

Kiril K. Dimitrov,' Ivan Dinev, Ismet Kalkanov
Pathologic Anatomy Unit, Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

14.15-14.45

BO3. TECHNIQUES FOR OBTAINING PRIMARY CULTURES OF COLORECTACAL CARCINOMA

Boyka Andonova-Lilova, Radostina Alexandrova
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

14.45-15.00

BO4. CANCER STEM CELLS

Desislav Dinev, Radostina Alexandrova
Institute if Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

15.00 – 15.20 Coffee Break

15.20 – 15.50

BO5. DETECTION OF MITE VARROA DESTRUCTOR IN BEE SAMPLES FROM DIFFERENT REGIONS OF BULGARIA

Delka Salkova[1], Kalinka Gurgulova[2], Ilian Georgiev[3]

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

[2]National Diagnostic & Research Veterinary Medical Institute „Prof. Dr G. Pavlov“, Sofia

[3]University of Forestry, Veterinary Medical Faculty, Sofia

15.50-16.05

BO6. СТЕРИЛИТЕТ

Стефани Димитрова, Надежда Стоянова

Медицински Университет София, Медицински факултет

16.05 – 16.20

BO7. КАКВО НЕ ЗНАЕМ ЗА СИНДРОМА НА КУШИНГ

Стефани Димитрова, Надежда Стоянова

Медицински Университет София, Медицински факултет

16.20 – 16.40

Discussion

Thursday, 15 June 2017

Session C

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

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

Secretary: Lora Dyakova, MSc
Institute of Neurobiology, Bulgarian Academy of Sciences

13.30 – 14.00

CO1. NON-SMALL CELL LUNG CANCER- NEW STRATEGIES OF TREATMENT

Lora Dyakova[1], Tanya Zhivkova[2], Daniela-Cristina Culita[3], Gabriela Marinescu[3], Luminita Patron[3], Radostina Alexandrova[2]

[1]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Institute of Physical Chemistry "Ilie Murgulescu", Romanian Academy, Bucharest, Romania*

14.00 – 14.30

CO2. GROWTH SUPPRESSION EFFECT OF NOVEL SCHIFF BASE COMPLEXES OF COPPER (Cu) TOWARD CANCER AND NON-CANCER CELL LINES

Milena Glavcheva[1], Zdravka Petrova[1,2], Tanya Zhivkova[1], Lora Dyakova[3], Gabriela Marinescu[4], Daniela-Cristina Culita[4], Luminita Patron[4], Radostina Alexandrova[1]

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Faculty of Biology, Sofia University "St "Kl. Ohridski", Sofia, Bulgaria*
[3]*Institute of Neurobiology, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Block 23, 1113 Sofia, Bulgaria*
[4]*Institute of Physical Chemistry "Ilie Murgulescu", Romanian Academy, Bucharest, Romania*

14.30 – 15.00

CO3. FERULIC ACID – ANTIPROLIFERATIVE AND PROAPOPTOTIC ACTIVITY IN HELA TUMOR CELLS

A. Georgieva[1], G. Yakub[2], M. Ignatova[2], N. Manolova[2], I. Rashkov[2], R. Toshkova[1]

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

15.00 – 15.20 Coffee Break

15.20 – 15.35

CO4. DIFFERENTIAL EXPRESSION OF INTEGRINS AND GALECTINS IN CANCER

Jordan Stoyloff

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Siences, Sofia, Bulgaria

15.35 – 15.50

CO5. SUBACUTE SCLEROSING PANENCEPHALITIS - DELAYED AND TERRIBLE SENTENCE AFTER A MEASLES INFECTION

A. Pavlova, S. Krumova

National Centre of Infectious and Parasitic Diseases, Department of Virology, NRL "Measles, mumps, rubella", Sofia, Bulgaria

15.50 – 16.20

CO6. INTRODUCTION OF DIFFERENT HIV-1 SUBTYPES AMONG HETEROSEXUALS IN BULGARIA

Reneta Dimitrova[1], Asya Kostadinova[1], Anna Gancheva[1], Lora Nikolova[1], Ivaylo Elenkov[2], Nina Yancheva[2], Mariyana Stoycheva[3], Daniela Nikolova[4], Tsetsa Doychinova[5], Liliya Pekova[6], Ivailo Alexiev[1]

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

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

[3]*Department of Infectious Diseases, Medical University, Plovdiv, Bulgaria.*

[4]*University Hospital „St. Marina", Medical University, Clinic of Infectious Diseases, Varna, Bulgaria.*

[5]*Department of Infectious Diseases, Medical University, Pleven, Bulgaria.*

[6]*Clinic of Infectious diseases, University Hospital, Stara Zagora, Bulgaria.*

16.20 – 16.35

CO7. LOW DRUG SOLUBILITY – PROBLEMS AND SOLUTIONS

Katerina Stoyanova

Faculty of Biology, Sofia University "St.Kliment Ohridski"

16.35 – 16.50

CO8. HISTORICAL REVIEW OF THE APPLICATIONS OF SEVEN DRUGS

Vasil Boyanov, Liliya Lazova

Medical University of Sofia

16.50 – 17.05

CO9. DEVELOPMENT OF ELECTROCONVULSIVE THERAPY FROM THE BEGINNING OF THE TWENTIETH CENTURY TO THE PRESENT

Vasil Boyanov, Liliya Lazova

Medical University of Sofia

17.05 - 17.30 Discussion

Friday, 16 June 2017

9.30 – 10.00

CO10. CELL SPECIFIC CYTOTOXIC EFFECT OF NEWLY SYNTHESIZED METAL COMPLEXES

Zdravka Petrova[1,2], Milena Glavcheva[1], Desislav Dinev[1], Tanya Zhivkova[1], Lora Dyakova[3], Abedulkadir Mahdi Abudalleh[1], Gabriela Marinescu[4], Daniela Cristina Culita[4], Luminita Patron[4], Radostina Alexandrova[1]

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

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

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

[4]*Institute of Physical Chemistry "Ilie Murgulescu", Romanian Academy, Bucharest, Romania*

10.00 – 10.30

CO11. Multidrug resistance if cancer cells – between pessimism and optimism

Radostina Alexandrova

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

Session D

Chairpersons:

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

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

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

10.30 – 11.00

DO1. BIOCHEMICAL AND HISTOLOGICAL STUDIES IN RAT MODELS WITH EXPERIMENTAL IMPLANTS BASED ON MODIFIED BETA – TCP

Veselin Nanev[1], Ivelin Vladov[1], Petar Dimitrov[1], Katerina Todorova[1], Neli Tsocheva-Gaytandzhieva[1], Radost Ilieva[2], Rumyana Gergulova[2], Margarita Gabrashanska[1]

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

11.00 – 11.20 Coffee Break

11.20 – 11.35

DO2. IN VITRO INVESTIGATION ON NEW MATERIALS FOR BONE IMPLANTS

Boyka Andonova-Lilova, Tanya Zhivkova[1]
Lora Dyakova[2], Abedulkadir Abudalleh[1], Diana Rabadzieva[3], Stefka Tepavitcharova[3], Radostina Alexandrova[1]

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences*
[3]*Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences*

11.35 – 11.50

DO3. INITIAL STUDIES OF CITRATE-BASED AMORPHOUS CALCIUM PHOSPHATES AS MATERIALS FOR BONE IMPLANTS

Tanya Zhivkova[1], Boyka Andonova-Lilova[1], Lora Dyakova[2], Abedulkadir Abudalleh[1], Diana Rabadzieva[3], Stefka Tepavitcharova[3], Radostina Alexandrova[1.]

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

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

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

11.50 – 12.20

DO4. CALCIUM PHOSPHATE LOADED BIOADHESIVE BIOPOLYMER BASED HYDROGEL SCAFFOLD: A NOVEL BIOMATERIAL FOR BONE TISSUE ENGINEERING

Probal Basu, Nabanita Saha, and Petr Saha

Centre of Polymer Systems, University Institute, Tomas Bata University in Zlin, Trida Tomase Bati, Zlín, The Czech Republic

12.20 – 12.40 Discussion

12.40 – 13.00 Closing Remarks

Session A

Chairpersons:

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

Assoc. Prof. Plamen Todorov, PhD
Institute of Biology and Immunology of Reproduction Acad. Kiril Bratanov" , Bulgarian Academy of Sciences

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

AO1. AUTOPHAGY IN NORMAL AND PATHOLOGICAL PROCESSES

Radostina Alexandrova[1], Abedulkadir Mahdi Abudalleh[1]
Milena Glavcheva[1], Desislav Dinev[1], Tanya Zhivkova[1], Lora Dyakova[2], Orlin Alexandrov[3]
[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Block 25, Sofia 1113, Bulgaria*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Block 23, Sofia 1113, Bulgaria*
[3]*Health Service, Gorna Malina Bulgaria*

AO2. XENOTRANSPLANTATION AS AN INVESTIGATION MODEL OF THE FUNCTIONAL CHARACTERISTICS OF HUMAN OVATIAN TISSUE AFTER CRYOPRESERVATION

P. Todorov[1], E. Hristova[1], A. Chorbanov[2], N.Mihaylova[2], G. Rahimi[3], V. Isachenko[3]
[1]*Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, 73 "Tzarigradsko shosse" blvd 1113 Sofia, Bulgaria, e-mail: plamen.ivf@gmail.com*
[2]*Institute of Microbiology, Bulgarian Academy of Sciences, "Acad. G. Bonchev" str, bl. 26, 1113 Sofia, Bulgaria*
[3]*Cologne University, Kerpener Str. 7, 50931 Cologne, Germany*

Chemo- and radiation therapy damage gonadal function (spermato- and oogenesis). Gamete and embryo freezing and storage for fertility preservation is offered to patients with oncological diseases before the onset of their treatment. The technology is well established and is used in assisted reproduction clinics. However, in certain cases like young (prepubertal) girls, in women to whom hormonal stimulation is contraindicated, and others, the cryopreservation of embryos and oocytes is not suitable and the only available approach for fertility preservation is ovarian tissue freezing. It is a relatively new method, and although it is still considered experimental, is already in clinical application in developed countries. Over 100 children have been born until now worldwide after cryopreservation and consecutive transplantation of ovarian tissue. Unfortunately, the success rate of the procedure is still low. A number of issues regarding the preservation of the ovarian cells function during the freezing process remain unanswered. Different ways to optimize the technology are also being

investigated. Interesting approach is the possibility to transplant the tissue to immunodeficient (SCID) mice with the purpose to trace the progress of angiogenesis, folliculogenesis and potential oocyte development.
In this report, the authors present a review of the research on cryopreservation and transplantation of human ovarian tissue and also their original results in the field. After programme freezing or vitrification, fragments of human ovarian tissue was grafted to SCID mice and their development was followed in vivo regularly. The degree of maturation and the morphology of the different types of follicles, revascularization (PECAM-immunohistochemistry) and the level of apoptosis in the explants (anti-caspase-3 immunofluorescence and flowcytometry with FITC-Annexin –V detection kit) were used as indicators for the functional properties of the tissue. The comparative analysis showed that the programme freezing is an effective method for cryopreservation of human ovarian tissue. Xenotransplantation of the ovarian fragments after thawing gives the opportunity for their contemplated assessment and in vivo maturation of the innate primordial follicles.

The research has been partially funded by the National Science Fund project B-01/10 "Isolation, characterization and in vitro differentiation of ovarian stem cells".

AO3. EFFECT OF THE COMBINED EFFECT OF VITAMINS A, D3 AND ON THE MOTHER OF SMOOTH SPERMATOISOIDS IN THE CRYO CONFIRMATION PROCESS

Kiril Lazov, Desislava Gradinarska, Denica Daskalova

Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov",
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73 Tsarigradsko shose Blvd., 1113 Sofia, Bulgaria
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Abstract
The purpose of this study was to trace the effect of the combined application of vitamins A, D3 and E on motility of sperm from a dog in the cryopreservation process. Ejaculates (n = 5) were manually obtained from clinically healthy dogs of different breeds aged between two and six years. Each ejaculate was divided into two parts - test and control. A standard Norwegian sperm diluent was used to dilute the samples, vitamin E (1 mg / ml), cholecalciferon (2000 UI / ml) and α-tocopherol acetate (1500 IU / ml) were added to the dilution media. Dilution of the semen was performed at a ratio of 1: 2-1: 3 to provide a final concentration of spermatozoa 100-200x10 ^{6} sperm / ml. Sperm motility (overall mobility, progression, rate) was assessed before and after cryopreservation using a computer-assisted asthma screening system (CASA). The cryopreservation of the samples was accomplished by an accelerated freezing procedure at a temperature of -150 ° C in cryo-fluids in a volume of 0.5 ml. The thawing was performed in a water bath at 65 ° C for 60-80 seconds, followed by 37 ° C for 3-5 minutes.
In the course of the experiment, differences in sperm motility were detected after thawing in favor of the test samples. The addition of the combination of biologically active substances (vitamins A, D3 and E) reliably preserves the total motility of the spermatozoa after cryopreservation, mainly at the expense of non-progressive motility gametes. Also, there was

a tendency for a higher percentage of progressively mobile sperm as well as fast and medium velocity sperms in the test samples compared to the controls.

Introduction

Cryopreservation of spermatozoa takes place in the temperature range below -150 ° C (quantum reaction zone), where the crystallisation and recrystallization processes are absent. Cryo-conservancy also hides its risks directed at deep-freezing sites. The low temperatures used have a detrimental effect on living objects. The freezing temperatures used in practice result in damage to the sperm, in case different protective methods and technologies are not used. The major damaging factors are related to the formation of intracellular ice crystals and sperm dehydration. As a result of the effects of the low temperatures, there are changes in the structure of the structure mainly of the plasma membrane (PM), mitochondria (M. O'Connell) and acrosome (Drobnis EZ et all), which has a negative effect on the sperm fertilization potential after thawing. Epigenetic influences of ultraviolet temperatures on the DNA molecule have also been identified. There is a correlation between the DNA-methylation status of fresh and cryopreserved sperm (Marta F. Riesco and Vanesa Robles). The process of cryopreservation also affects the concentration of reactive oxidation radicals (ROS). Depending on their concentration, nature and duration of action, ROS may have a beneficial or negative effect on sperm. Moderate levels of ROS mediate important physiological functions of spermatozoa such as: hyperactivity, capsitization, acrosome reaction, proper process of attachment of the sperm to the egg (Amrit Kaur Bansal and G. S. Bilaspuri). Endogenous sperm antioxidant systems prevent ROS-mediated lesions by metabolizing them to safe products. In this way a fine regulation is built between the processes of generating and neutralizing ROS. It was found that during the cryopreservation of the semen, the rate of ROS generation exceeds that of detoxification. As a consequence, sperm have oxidative stress and have a severe negative effect on sperm (Amrit Kaur Bansal and G. S. Bilaspuri). The main negative effects of oxidative stress are: sperm AF damage, DNA integrity impairment, oxidative metabolism blocking, oocyte interference processes, Mobility and sperm vitality, etc. (H. Gurler et all).

The working hypothesis of the experiment is based on the theoretical and practical knowledge related to the common mechanisms of all sperm damage in the cryopreservation process. Adding to the diluent an antioxidant (vitamin E) (Mohammad Baqer Minaei et all) is aimed at reducing damage due to oxidative stress. The mechanisms of impact of the other two components - vitamin D and vitamin A - have not yet been fully elucidated in the ultraviolet gametes storage process. Human spermatozoa has been found to have a receptor for vitamin D (VDR) as well as an enzyme that metabolizes it (CYP24A1). Expression levels of VDR and CYP24A1 in human spermatozoa serve as positive prognostic markers for sperm quality. VDR mediated increase in the concentration of intracellular $Ca^{2+,}$ resulting in increased sperm motility (Blomberg Jensen M et all). There are literature data from functionally conducted experiments that vitamin D increases the rate of sperm movement in some animal species (Corbett ST, Hill O, Nangia AK). The role of vitamin A in the protection of sperm in the process of cryopreservation and thawing is still unclear. Probably, because of its fat-soluble nature, vitamin A plays a role in stabilizing sperm AF and preserving membrane integrity under ultra-low temperature.

Materials and methods

Seed preparation and processing

Ejaculates (n = 5) were obtained from clinically healthy dogs of different breeds aged between two and six years. A method of manually obtaining semen was used.

Each sample was divided into two parts - test and control. Control samples were diluted to a standardized Norwegian cryoprotective medium (composition) (citation). The test samples were diluted with the same diluent with the addition of:

- Vitamin E - 1 mg / ml medium
- Cholecalciferon - 2000 IU / ml medium
- α-tocopherol acetate - 1500 UI / ml medium

Dilution of the semen was performed at a ratio of 1: 2-1: 3, so as to provide a final concentration of spermatozoa 100-200x10 ^{6} sperm / ml (ie 50-100x10 ^{6} cells in a volume of 0.5ml)

Evaluation of the resulting seed liquid

Motility of sperm from each ejaculate was evaluated by using a system for computer-assisted spermoanaliz (CASA) System Sperm Class Analyzer ® (Microptic, Spain), analytical module "Motility and concentration". Roofing glasses measuring 20x20 mm and 10 μl drip volume were used. The sperm from each sample were evaluated before and after cryopreservation. Sperm motility was assessed by monitoring progress (static, progressive, non-progressive,%) and rapid (medium, slow)% of at least 1000 sperm in at least 5 visual fields of each sample.

Cryopreservation and thawing

The test and control samples were dispensed in cryo-fluids in a volume of 0.5 ml. The equilibration was performed over a period of 3h at 4 ° C. The cryopreservation of the samples was performed using an accelerated freezing mode at a temperature of -150 ° C in a biofreeze. A minimum of 72h of the samples was provided before de-icing.

The thawing was performed in a water bath at 65 ° C with an exposure time of 60-80 sec (until the solid phase disappeared). The phials were then placed in a water bath at 37 ° C for 3-5 minutes and the motility of the frozen-thawed sperm was evaluated by CASA.

Results and discussion

Figure 1 shows CASA data on motility and progression of sperm from test and control samples before and after cryopreservation. A statistically significant difference ($p < 0.001$) was found in the percentage of static sperm between test and control samples after thawing. Samples with added combination of vitamins A, D3 and E showed significantly better overall mobility, demonstrated by lower percentage of static sperm after thawing. In control samples this percentage increased significantly after cryopreservation. Better survival of total sperm motility in the test samples after thawing is mainly provided at the expense of cells with non-progressive motility, which are significantly more than control samples ($p < 0.001$). There was a tendency for a higher percentage of progressive moving sperm in the test samples after thawing.

Fig. 1 Progression of spermatozoa before and after cryopreservation.

The CASA data on sperm velocity from the test and control samples before and after cryopreservation is presented in Figure 2. A tendency was observed for a higher percentage of fast and medium velocity sperm in the test samples compared to controls.

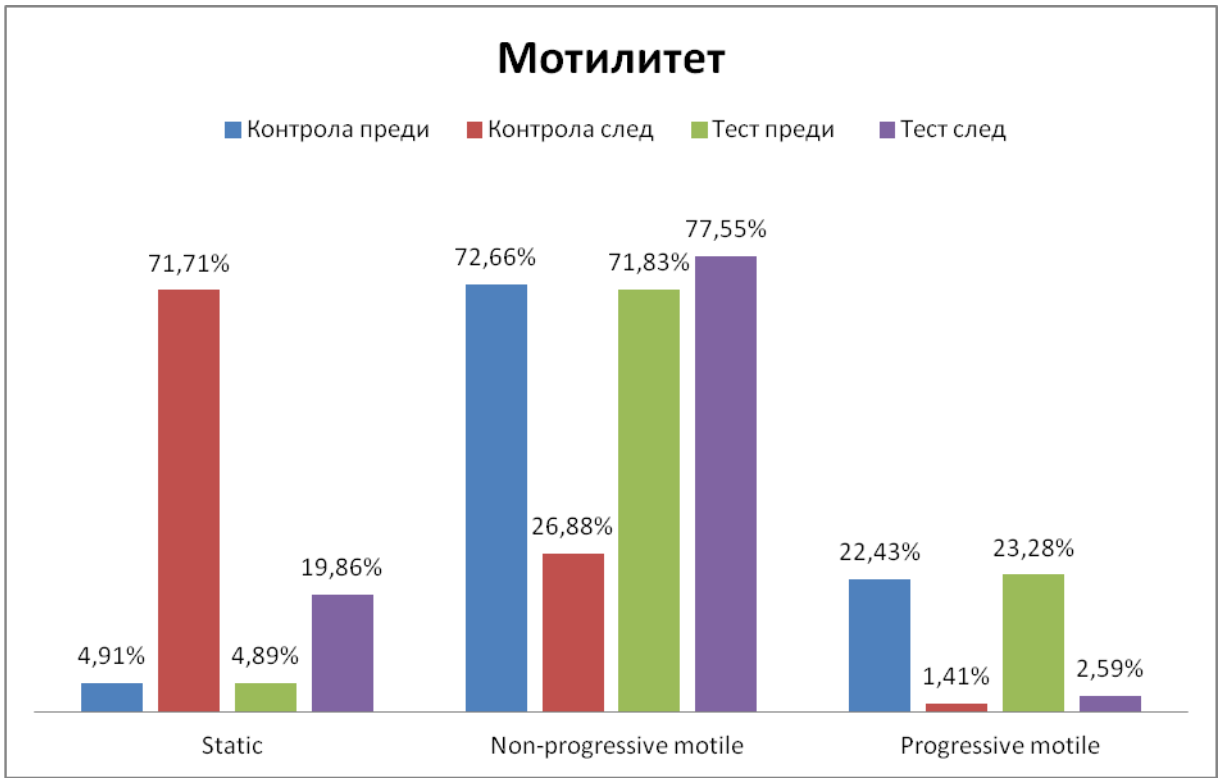
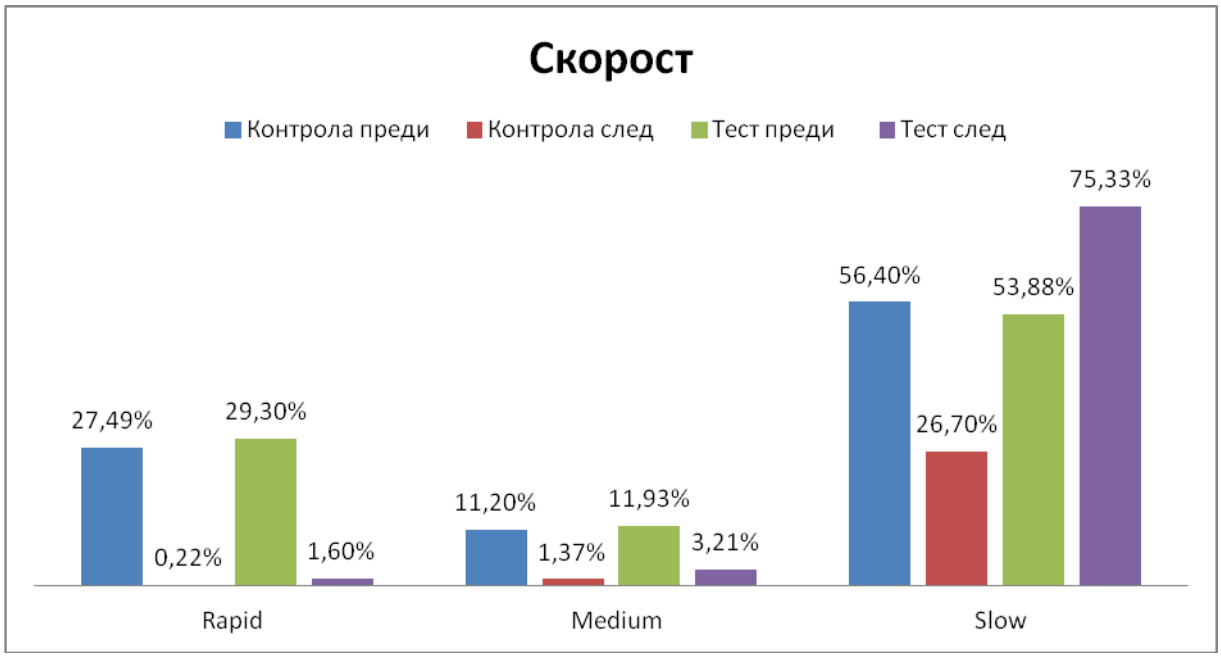


Fig.2. Sperm velocity before and after cryopreservation
(Conclusion)



In conclusion, the difference in sperm potency after thawing was found in favor of the test samples. The addition of the combination of biologically active substances (vitamins A, D3 and E) reliably preserves the total molecular capacity of the spermatozoa after cryopreservation.

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АО4. ОСТРИ ИНТОКСИКАЦИИ С ФОСФОРОРГАНИЧЕН ПЕСТИЦИД – НУРЕЛЕ Д - ПРЕДСТАВЯНЕ НА ДВА КЛИНИЧНИ СЛУЧАЯ

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Фосфорорганичните пестициди са широко използвани в селското стопанство и представляват значителен потенциален риск за човешкото здраве.

Авторите представят два клинични случая на тежки остри екзогенни интоксикации с фосфорорганичния пестицид - Нуреле Д при пациенти, хоспитализирани в Клиниката по токсикология, Отделение – възрастни, УМБАЛСМ "Н.И.Пирогов". Пациентите са мъже на възраст 63 и 72 години. Случаите на остри отравяния с фосфорорганичният препарат са анализирани по отношение на клинично протичане, придружаващи заболявания, лечение и изход.

Ключови думи: остро отравяне, фосфорорганични пестициди, Нуреле Д

AO5. NOVEL SUBSTRATES FOR THE DETERMINATION OF FIBROBLAST ACTIVATION PROTEIN α (FAPα) ACTIVITY

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Fibroblast activation protein α (FAPα; EC 3.4.21.B28) is a serine type proteinase, belonging to the S9b sub-family of post-proline cleaving enzymes. The enzyme is a type II transmembrane glycoprotein existing as a homodimer in its native form [6]. It hydrolyzes

polypeptide substrates possessing proline at P_1 position. FAPα has both exo- and endopeptidase activities and is more effective as an endopeptidase. Amongst its natural substrates are collagen type I [5] neuropeptide Y, B-type natriuretic peptide, substance P and peptide YY [3]. In normal human and mammalian tissues, the enzyme activity is restricted to single reactive fibroblasts, glucagon producing A-cells in the pancreatic islets and separated endometrial cells [reviewed in 8]. Comparatively high enzyme levels are found in mesenchymal cells during embryogenesis. Alternatively, FAPα expression is highly induced during different pathological processes like rheumatoid arthritis and osteoarthritis, idiopathic pulmonary fibrosis, liver fibrosis, cancer, etc. [8]. The most characteristic feature of FAPα is that it is up-regulated in stromal fibroblasts of over 90% of human epithelial tumors and in lots of sarcomas, but not in benign tumors [reviewed in 2 and 4]. Thus, the enzyme is considered as a valuable marker for different carcinomas. FAPα is structurally related to dipeptidyl peptidase IV (DPPIV, EC 3.4.14.5) and DPPIV-like enzymes (DPP8 and 9). They share 50% sequence identity in the entire sequence and 70% identity in the catalytic domain [1, 7] – a fact which impedes the design of specific substrates and/or inhibitors for the enzymes.

In the present work we describe the design (using molecular modelling), syntheses and testing of four novel substrates for the biochemical evaluation of FAPα activity – isonicotinoyl-D-Ala-Pro-4-nitroanilide (IAP), β-Ala-D-Ala-Pro-4-nitroanilide (AAP), β-Ala-Nle-Pro-4-nitroanilide (ANP) and D-Asp(D-Ala-Pro-4-nitroanilide) (DAP). The activities of all the substrates were measured using recombinant human FAPα and Michaelis-Menten constants were obtained. The substrates were compared to the most commonly used Z-Gly-Pro-4-methyl-7-coumarinylamide (GP-MCA), which was found to be a non-specific substrate cleaved off by a number of post-proline cleaving enzymes. The substrates were also tested in cell lysates of three types of cultured human cells – MCF-10A (normal epithelial cells of mammary gland), MCF-7 (low invasive mammary gland carcinoma) and MDA-MB-231 (highly invasive mammary gland carcinoma). The results show that ANP is a non-spesific substrate, whereas DAP and IAP have a potential to be used as specific FAPα substrates.

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AO6. SALINOMYCIN AMELIORATES HEMATOLOGICAL PARAMETERS AFTER CADMIUM-INDUCED INTOXICATION

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Cadmium (Cd) intoxications cause anemia and multi organ dysfunction. Chelation therapy is the most commonly used therapeutic strategy for the treatment of toxic metal poisonings [1].

Salinomycin is an antibacterial and coccidiostat ionophore therapeutic drug shown to form complex with Cd(II) *in vitro* [2]. To the best of our knowledge there is a lack of information regarding the potential application of this antibiotic for treatment of Cd-intoxication. The aim of the present study is to evaluate the effect of salinomycin on the erythrocytic parameters of mice after subacute Cd intoxication. Exposure of mice to Cd(II) acetate treatment for 14 days resulted in a significant decrease of RBC count and Hb content compared to the untreated controls. Subsequent administration of tetraethylammonium salt of salinomycinic acid to Cd-intoxicated mice for 2 weeks improved these parameters although they remained lower than the normal control values. Treatment of control mice with tetraethylammonium salt of salinomycinic acid led to a slight increase in RBC and Hb suggesting that the ionophorous antibiotic has a beneficial effect on erythropoiesis and could be used for treatment of Cd intoxication.

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Session B

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BO1. APPLICATION OF DIAGNOSTIC TECHNIQUES, DATA INTERPRETATION AND ANALYSIS OF LAWSONIA INTRACELLULARIS INFECTIONS

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Proliferative enteropathy (PE) in swine is a common group of acute and chronic conditions, prevailing in around 96% of farm sites worldwide. Usually the disease starts at 3-4 weeks the post weaning or in older 10-12 weeks old finisher/replacement pigs, wherein around 30 % of the affected animals reveal detectable lesions, causing sufficient economic losses (McOrist et al. 2003; Stege et al. 2000, 2004). The particular causative agent, as part of the epidemiological triad of Sniezko (1974), is pathogenic bacteria *Lawsonia intracellularis* (*L. intracellularis*) slow-growing and fastidious obligate intracellular organism (Gebharth et al., 1993, McOrist et al 1993). The current study provides a detailed overview of the potential for appilication of diagnostic techniques for detection of the etiological agent localization and morphological features in the development of lesions associated with proliferative enteropathy. Histochemical (Warthin-Starry silver stain), immunohistochemical (monoclonal antibody Law1DK), light and electron microscopic methods were used for specific detection. Fresh materials (grossly identified as PE positive) from distal ileum and mesenteric lymph nodes (n = 127) taken from slaughterhouses were processed according to the routine histological techniques. Microphotographs were taken with Leica DM 500B, Wetzlar, Germany and transmission electron microscope JEOL 1200 EX. Histopathogical observation revealed distinct proliferation of immature enterocytes in the crypts, increased mitotic figures and apoptosis. Considerable marked emplacement of argyrophilic organisms in the crypts of Lieberkuhn epithelium and immunolocalisation with Law1DK antibody was monitored in high percentage (from 74 to 88%, averagely 81%) of the examined specimens. Ultrastructural findings consisted of the presence of intestinal crypts containing numerous proliferating

immature epithelial cells, at the site of apoptosis and abundance of apical intracytoplasmic vibroid, curved bacteria, morphologically and metrically comparable with *L. intracellularis*. The results of our studies show that the methods used are reliable tools in the diagnosis and differential diagnosis of *L. aintracellularis* in pigs.

Key words: *Lawsonia intracellularis*, proliferative enteropathy, immunohistochemistry, electron microscopy

BO2. DETECTION OF LAWSONIA INTRACELLULARIS IN WILD BOAR AND INDIGENOUS EASTERN BALKAN SWINE FROM CENTRAL STARA PLANINA

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Lawsonia intracellularis (*L. intracellularis*) is an obligate intracellular bacterium causing proliferative enteropathy in pigs, horses and hamsters with prompt clinical findings. Although, subclinical or chronic infections have usually been described in wild, domestic pigs and wide range of hosts for this organism. The aim of this study was to identify the causative agent of Porcine proliferative enteropathy (PPE) in wild boar (WB) and free range raised authochtonous Eastern Balkan swine (EBS) and to evaluate the development of PPE associated lesions. Tissue samples from distal ileum were collected during the hunting/slaughter season from December 2014 until January 2015 from Central Stara planina region. A total of 9 samples from wild boars and 12 samples from EBS were examined, originating from 4 hunting grounds and 7 farms. Each of the farm had a history of sporadic cases of diarrhea and retarded growth. Tissues were examined for gross pathologic changes and by routine histology, silver staining (WS) and immunohistochemistry for detection of *L. intracellularis*. Grossly only one (11.1%) of the WB and 3 (25.0%) of the EBS samples were suspicious for PPE with resemblance of moderate thickening and slight corrugation. Microscopically 2 (22.2%) of the WB and 3 (25.0%) of EBS samples showed lesions typical for proliferative enteritis, while others were normal or with minor insignificant lesions. WS staining revealed presence of argyrophilic structures in the apical cytoplasm of the enterocytes and some cells of the round cellular infiltrate in 3 (33.3%) of the WB and 11 (91.7%) of the EBS samples. Low positive signal for LI was observed immunohistochemically among 88.9 % (n=8) of the WB and 83.3% (n=10) of EBS samples. The results from our research showed significant presence of LI in WB and EBS ileal samples.

Key words: proliferative enteropathy, wild boar, autochtonous swine, *Lawsonia intracellularis*

BO3. TECHNIQUES FOR OBTAINING PRIMARY CULTURES OF COLORECTACAL CARCINOMA

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BO4. CANCER STEM CELLS

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BO5. DETECTION OF MITE VARROA DESTRUCTOR IN BEE SAMPLES FROM DIFFERENT REGIONS OF BULGARIA

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BO6. СТЕРИЛИТЕТ

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Стерилитетът е състояние, при което не настъпва бременност в период до 1 година, въпреки редовния полов живот на двойка хетеросексуални партньори. Броят на двойките с репродуктивни проблеми се увеличава непрекъснато.Решаването на проблема се затруднява от нарастващата честота на сексуално предаваните инфекции, отлагане на раждането за по-напреднала възраст и др. Причините за безплодие могат да бъдат у жената, у мъжа, у двамата партньори или да са с неизяснен произход.
Стерилитетът у жената бива първичен и вторичен. Може да се дължи на нарушение във функциите на яйчниците, маточните тръби, маточното тяло или шийка, заболявания на влагалището или психични фактори.
Причините за стерилитет у мъжа най-общо се делят на претестикулярни, тестикулярни и посттестикулярни. Претестикулярните включват заболявания на хопоталамуса, хопофизата, приемане на екзогенни хормони или други лекарствени средства. Тестикулярните включват структурни и бройни хромозомни аномалии, билатерална анорхия, Сертоли-клетъчен синдром, орхит, травма, радиация, сисъемни заболявания, крипторхизъм, варикоцеле. Към посттестикулярните спадат нарушен транспорт на спермата, нарушена подвижност и функция на сперматозоидите и сексуална дисфункция.
Алгоритмът на действие за уточняване причината за стерилитет у двойката включва изследване на спермограма, Изследване на базалната температура, хормонални изследвания- FSH,LH, пролактин, естрадиол, прогестерон.; хистеросалпингография, фоликулометрия, изследване на цервикална слуз и тестове за съвместимост на

партньорите, лапароскопия, хистероскопия, пробно абразио,имунологично изследване на спермоантитела.
Наложени са определени изисквания относно спермодаването за спермограма, а критериите за нормоспермия са дадени от СЗО през 1999г. При спермограмата се изследват следните параметри: обем, цвят, мирис, време на втечняване, общ брой сперматозоиди в 1 мл, процент подвиждни сперматозоиди, морфология, фруктолиза, скорост на придвижване, наличие на аглутинати и др.

ВО7. КАКВО НЕ ЗНАЕМ ЗА СИНДРОМА НА КУШИНГ

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Харви Кушинг е роден в Охайо през 1869г. и е известен още като бащата на неврохирургията. По време на практиката си попада на Кушинг рефлекс, който описва връзката между кръвното налягане и интракраниалното. На 32-годишна възраст става професор по неврохирургия. Харви Кушинг въвежда локалната анестезия, с което оставя значим отпечатък в развитието на хирургията.

Съществуват два вида синдром на Кушинг – ендо- и екзогенен. Ендогенният от своя страна се разделя на АКТХ – зависим и АКТХ-независим. Екзогенният се разделя на медикаментозен и индуциран от системна употреба на алкохол.

Болестта на Кушинг (нарича се още централен синдром на Кушинг) се означава това болестно състояние, при което повишената глюкокортикоидната секреция настъпва вторично, вследствие на двустанна надбъбречна хиперплазия, зависима от АКТХ.
При синдрома на Кушинг увеличената кортикоидна секреция се дължи на първични патологични процеси в надбъбреците. Най-често това са аденоми или карциноми на надбъбречните жлези.
Най-честите болестни промени засягат белтъчната, мастната и въглехидратната обмяна. Настъпват изменения и в хемодинамиката, хемопоезата, кожни промени и хормонален дисбаланс. Наблюдава се затлъстяване от централен тип, мастна гърбица, луновидно лице, кожни стрии, остеопороза, артериална хипертония, психични изменения и други.
Важен тест в диагностиката е дексаметозоновият супресионен тест с ниски дози. Той се състои в следното: след приема на 2мг дексаметазон в часовете около полунощ се стига до недостатъчно потискане на секрецията на серумния кортизон, който се отделя около 8ч сутринта на следващия ден. Този тест решава въпроса дали има или не хиперкотицизъм, без да дава яснота за етиологията. Етиологичното определяна на синдрома става с помощта на други тестове: провеждане на дексаметазонов тест с високи дози – определя дали хиперкортицизмът е от централен тип, тест за стимулация с кортикотропин-рилизинг хормон. Чрез него при централния синдром на Кушинг се стига до покачване на АКТХ. Друг вид тестове са образните изследвания в зоната на хипофизата.
Лечението се провежда според причината за хиперкотицизма, неговата давност и настъпилите органни увреждания. При централен синдром на Кушинг – първият избор е трансназална/трансфеноидалната аденомектомия. При неуспех може да се направи облъчване на хипофизата с протони.

Session C

Chairpersons:
Prof. Reni Kalfin, PhD
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CO1. NON-SMALL CELL LUNG CANCER- NEW STRATEGIES OF TREATMENT

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CO2. GROWTH SUPPRESSION EFFECT OF NOVEL SCHIFF BASE COMPLEXES OF COPPER (Cu) TOWARD CANCER AND NON-CANCER CELL LINES

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CO3. FERULIC ACID – ANTIPROLIFERATIVE AND PROAPOPTOTIC ACTIVITY IN HELA TUMOR CELLS

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Ferulic acid (FA, 4-hydroxy-3-methoxycinnamic acid) is a natural phenolic compound known for its antioxidant, anti-inflammatory, immunoprotective, antiviral and anticancer properties. The present study aims to assess the antiproliferative and proapoptotic potential of FA against human HeLa tumor cells. The effects of different concentrations of FA on the cell viability were evaluated by enzyme-based colorimetric method (MTT assay) and the induced alterations in tumor cells morphology were examined by light microscopy. The apoptosis inducing ability of FA was studied by fluorescence microscopy after staining with the florescent dyes acridine orange/ethidium bromide and 4',6-diamidino-2-phenylindole (DAPI). The obtained results showed that FA induced cytopathic effects in HeLa cells and inhibited the cell proliferation in a dose- and time-dependent manner. Fluorescent microscopy studies revealed marked alterations in the morphology of FA-treated cells with signs of cell cycle arrest and apoptosis. These findings are in accordance with previous data indicating significant antitumor and proapoptotic activity of FA in human tumor cell lines with various tissue origin. However, the chemical instability and low water solubility of FA limit its potential clinical applications. The incorporation of FA in nanofibrous materials by modern electrospinning method has been proposed as a novel strategy to enhance the FA bioavailability through improving its solubility in water, stabilizing the compound from external stress and increasing the cellular uptake. The presented results are part of an ongoing study on the *in vitro* antitumor activity of different recently prepared formulations of FA-containing nanofibrous mats.

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CO4. DIFFERENTIAL EXPRESSION OF INTEGRINS AND GALECTINS IN CANCER

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Abstract

Pancreatic cancer cell lines show overexpression of integrin β6 and galectins -1 and -3, whereas galectin-8 is downregulated. There is no evidence on differential expression of integrins in gastric tumors, but galectin-3 is found to be upregulated. The α-V-β-6 integrin is strongly expressed in human cholangiocarcinoma (CC), but not in hepatocellular carcinoma (HCC). Integrins αvβ6, α6β4 and α3β1 are down-regulated in cholangiolocellular carcinoma (CoCC), in contrast to its high expression in CC. Galectins are also differentially expressed in cholangiocarcinoma and hepatocellular carcinoma. Galectin-8 expression in colorectal cancer is downregulated. Galectin-l and -3 are upregulated in colorectal cancer and correlate with the degree of dysplasia. High cytoplasmic expression of galectin-3 is mainly associated with thyroid tumors involving lymph node metastases. Higher expression of galectin-3 is found in follicular carcinomas, compared to follicular adenomas. Metastatic tumors have higher expression of galectin-3, than non-metastatic. There is no data on differential expression of integrins in bladder tumors. In renal cell carcinoma α-2, α-5, and α-6 integrins are differentially expressed and their expression depend on the histological type and tumor grade. Expression levels of integrins are higher in malignant and tumorigenic cell lines α5 MCF, relative to their expression in the nontumorigenic MCF-10F, Estrogen and α3 cell lines. A low serum level of galectin-3 is found in healthy individuals, whereas significant increase of galectin-3 levels is detected in breast cancer patients. Patients with metastases had higher galectin-3 concentrations, than patients with localized tumors. Known distinct topographical pattern of integrins is altered in transformed mucosa of laryngeal and oropharyngeal carcinomas, but the extent of changes is more marked in oropharyngeal tumors, which are known to be more infiltrating and diffusive and to have a bad prognosis.

Introduction

Cancer cells arise from normal ones in a multi-step process of progressive accumulation of genetic and epigenetic DNA alterations. Cancer cells may accumulate mutations, deletions or insertions of genetic material, which finally led to changes of expression of corresponding proteins. Differential expression of proteins is not unique for cancer. It is common in many diseases, as well as during embryogenesis. Knowledge of differential expression of proteins in cancer may be useful for developing tumor markers, as well as in therapy as target molecules.

In this paper we intend to review available information for differential expression of integrins and galectins in cancer. Adhesion of tumor cells on extracellular matrix proteins, on endothelial cells and among themselves (homotypic aggregation) is important for tumor cell spreading and invasion in distant organs – metastasis. Metastasis is a multistep process and almost every step include participation of integrins and galectins. Expression pattern of these proteins is different for every type of cancer. In some cases integrins and/or galectins are upregulated, while in others they are downregulated. There are also differences in expression patterns of individual members of galectin and integrin family.

Differential expression of integrins and galectins in digestive system tumors

Integrin β6 is overexpressed in pancreatic cancer cell lines. Immunohistochemistry demonstrate 20% to 70% overexpression of this protein in primary pancreatic tumors[28]. Ductal cells in cancerous pancreatic tissue had increased galectin-3 expression [42]. Hamster pancreatic cancer cells express high levels of galectin[44], but galectin-8 is found to be downregulated in pancreatic tumors [10]. Above data show that galectins -1 and -3 are upregulated in pancreatic tumors, whereas galectin-8 is downregulated.

As to our knowledge there is no evidence on differential expression of integrins in gastric tumors. Galectin-3 is upregulated in papillary and poorly differentiated adenocarcinoma. Moreover galectin-3 expression is significantly stronger in metastatic lymph nodes, than in the primary gastric cancers [32]. Preferential up-regulation of galectin-4 is observed in scirrhous gastric cancer cell lines investigated by Northern blotting [19]. Galectin-3 is found to be upregulated in gastric tumors, compared with normal tissue. Stronger galectin-3 signals are present in metastatic than in primary tumors. Galectin-4 is also upregulated in scirrhous gastric cancer.

Cholangiocarcinoma (CC) and hepatocellular carcinoma (HCC) are common primary hepatic malignancies. The α-V-β-6 integrin is strongly expressed in human CC, but not in HCC and therefore can be considered as a specific immunohistochemical marker in the differential diagnosis of primary liver tumors [38]. Little or no positivity for β6, β4 and α3 integrins is found in 91%, 91% and 52% of cholangiolocellular carcinoma (CoCC) and 100%, 98% and 81% of hepatocellular carcinoma (HCC), whereas intense positive staining for these integrins is demonstrated in 64%, 96% and 75% of cholangiocarcinoma (CCC). These results indicate down-regulation of αvβ6, α6β4 and α3β1integrins in CoCC, in contrast to its high expression in CCC [47]. By immunohistochemistry, 88% of cholangiocarcinoma (CC), 50% of sclerosing cholangitis (PSC), 13% of colorectal carcinoma metastases, and 80% of pancreatic carcinoma metastases presented α-V-β-6 integrin, whereas all hepatocellular carcinoma (HCC), combined CC/HCC and fibrolamellar HCC stain negative [39]. Alpha6 integrin and β1 integrin show increased expression in hepatocellular carcinoma, compared with non-cancerous liver tissue, although the α1 integrin do not show a significant change. Furthermore β1B integrin, a splicing variant of β1integrin, is overexpressed in hepatocellular carcinoma, while the β1A integrin isoform have not significant changes between hepatocellular carcinoma and surrounding non-cancerous liver tissue [37]. Alpha2, α3 and β1 integrins are downregulated in poorly differentiated hepatocellular carcinomas [22]. Poorly differentiated hepatocellular carcinomas are characterized by downregulation of α5 and β4integrins [22]. All cases of liver cell adenoma and well-differentiated HCC express the same set of integrins as in normal liver, i.e., VLA-α1 and VLA-β1. Poorly differentiated HCC expressed VLA-α1 and VLA-β1, but in

addition express VLA-α2, VLA-α3 and VLA-α6. Cholangiocarcinoma (ChC) express an identical integrin immunoprofile as observed in normal bile duct epithelium, i.e., VLA-α2, VLA-α3, VLA-α6 and VLA-β4, whereas poorly differentiated ChC show a markedly decreased expression of these integrins. VLA-α1 is absent from ChC, whereas VLA-β4 is never expressed by HCC. Differences in integrin receptor expression vary according to the cellular origin of the tumors and are associated with a poor differentiation [58]. Galectins are also differentially expressed in cholangiocarcinoma and hepatocellular carcinoma [49].

Expressions of integrins are deregulated in colorectal cancer cells [23]. Downregulation of ST6Gal-I glycosyltransferase leading to diminished α2-6 sialylation of integrins, inhibits cell adhesion to collagen I [46]. Decrease of the α3 integrin chain is found in colorectal adenomas, together with that of α6 and β4 chains in colorectal carcinomas [48]. Galectin-8 expression in colorectal cancer is downregulated [4]. All normal mucosae have strong nuclear expression of galectin 3 but this protein is downregulated in the neoplastic progression in following order: adenomas (60%), carcinomas (48%) and metastases (44%). Cytoplasmic expression of galectin-3 is down-regulated in colorectal adenomas (16%), but increased in colorectal carcinomas (64%) [41]. Galectin-l and -3 are expressed in variable amounts in the epithelial cells and the connective tissue of normal colon. However their expression is significantly increased in colorectal cancer and correlate with the degree of dysplasia [23].

Differential expression of integrins and galectins in thyroid, prostate and bladder tumors

Follicular thyroid cancer cell lines - FTC133, 236, 238, HTC, HTC TSHr, XTC, PTC4.0/4.2, TPC1 and anaplastic thyroid cancer cell lines ATC, C643, Hth74 express high levels of integrins α2, α3, α5, β1, β3 and low levels of α1, whereas papillary lines express a heterogeneous pattern of integrins, dominated by α5 and β1. ATC mainly express integrins α2, α3, α5, α6, β1 and low levels of α1, α4 and αV. Thyroid carcinoma cell lines of different histogenetic background display profoundly different patterns of integrins that appear to correlate with tumor aggressiveness [19]. In thyroid carcinomas, loss of polar topography of α3 β1 and new expression of α6 β4 in histopathologically aggressive cancers were observed [7]. High cytoplasmic expression of galectin-3 is mainly associated with thyroid tumors involving lymph node metastases [9]. Normal thyroid tissue doesn't express galectin-3, but higher expression is found in follicular carcinomas compared to follicular adenomas. Metastatic tumors have higher expression of galectin-3, than non-metastatic [46]. Normal thymocytes do not express galectin-3. Galectin-3 is also never expressed in benign lesions, but it is invariably detected in thyroid cancers [16]. Follicular adenomas and papillary carcinomas are intense positive for galectin-3,whereas anaplastic carcinomas, poorly differentiated carcinomas, medullary carcinomas and follicular carcinomas have lower expression of galectin-3. Hurthle cell carcinoma show scattered strong positivity whereas follicular adenomas, hyperplastic nodules, nodular goiters, and normal thyroid tissue are negative [13]. Higher levels of galectin-1 are found in all thyroid malignancies in contrast to benign tumors and normal tissue. Generally galectin-3 is upregulated in thyroid carcinomas but absent or weakly expressed in adenomas and normal tissue. Metastatic tumors show higher expression of galectin-3 than primary tumors [2]. DU-145/AR prostate cancer cells, stably transfected with androgen receptor cDNA, exhibit lower expression of α6 and β4 integrin subunits and higher expression of α2 and α5 [33]. Human prostate tumors display on their cell surface α6β1 and/or α3β1 integrins. Three prostate tumor phenotypes can be distinguished based on differential integrin expression. Type I coexpress both α6 and α3 subunits, type II exclusively express α6 integrin, and type III express α3 integrin only [43].

In prostate carcinoma there is a complete loss of β4 expression and the α6- and β1-integrin subunits [26]. The very late antigen integrins (VLAs) are alpha beta-heterodimeric transmembrane proteins. In normal and hyperplastic glands there are two staining patterns that differ according to the density of α6- and α2-integrins. VLA-6 immunoreactivity show band-like pattern in approximately 70% of normal and hyperplastic glands compared with VLA-2, which show the same pattern in only 5% of cases. In prostatic adenocarcinoma the band-like pattern significantly decrease with dedifferentiation and is consistently absent in grade III lesions. Compared with staining intensities in normal and hyperplastic conditions, grade I and II tumors maintain or overexpress the VLA-6 in 85% of cases, whereas the VLA-2 is downregulated in approximately 70% of cases. Grade III tumors are characterized by a heterogeneous expression of VLA-6 and VLA-2 proteins [21]. Normal prostatic glands have moderate immunostaining for galectin-3, localized in both nucleus and cytoplasm, whereas prostatic cancer cells have no staining for galectin-3 or its staining is present only in the cytoplasm. Prostatic intraepithelial neoplasia (PIN) cells also show only cytoplasmic expression of galectin-3 [56]. Galectin-1 is expressed in normal human prostates, prostatic intraepithelial neoplasia (PIN), primary adenocarcinomas and prostate cancer metastases [50].

As to our knowledge there is no data on differential expression of integrins in bladder tumors. Highly differentiated bladder tumors express galectin-7, while less-differentiated tumors
do not express galectin-7 [36]. Bladder squamous carcinoma and sarcoma tumor both show immunoreactivity for galectin-3 [3].

Differential expression of integrins and galectins in kidney, breast, ovary, lung and head and neck tumors

In renal cell carcinoma α-2, α-5, and α-6 integrins are differentially expressed and their expression depend on the histological type and tumor grade [55]. Galectin-3 is expressed in renal cell carcinomas (RCC), chromophobe RCC and oncocytomas [60].

Expression levels of integrins are higher in malignant and tumorigenic cell lines α5 MCF, relative to their expression in the nontumorigenic MCF-10F, Estrogen and α3 cell lines [5]. Decreased expression of integrins in human invasive breast cancer cell lines is accompanied with reduced cell adhesion and focal adhesion formation on fibronectin-coated surfaces [18]. MDA-MB-468LN (468LN), a variant of the MDA-MB-468GFP (468GFP) human breast cancer cell line, produces extensive lymphatic metastasis in nude mice and 468LN cells differentially express α9β1 integrin [31]. High β5 and αvβ5 integrin expression is observed in MDA-MB-231, non-metastatic MCF7 and non-breast cancer Hek-293 cells, while high β3 and αvβ3 integrin expression is restricted to only MDA-MB-435 cells [54]. α (v)β(3) integrins are consistently up-regulated in human breast cancer cell line MDA-MB-231 and its highly osteotropic B02 subclone [25]. Invasion is a complex process controlled by alteration of integrin levels. In this case increased expression levels of matrix metalloproteinases-2 and -9 are observed, which correlate with decreased expression of integrins α1 and β1 [57]. MCF-7 breast cancer cells stably transfected with protein kinase C-alpha (MCF-7-PKC-alpha cells) show anchorage-independent growth and exhibit increased tumorigenicity in nude mice. MCF-7-PKC-α cells express increased β3 and decreased β5 on their surface. Surface expression of αV on MCF-7-PKC-α cells is unchanged [6]. Integrins are transmembrane receptors that modulate cell adhesion. Each is a heterodimer of varying α and β subunits. In malignancy, loss of integrin expression may result in less adhesive cells more likely to metastasize. All integrin subunits are significantly reduced on breast cancer compared with benign cells. Loss of α1 β1, α2, α3, α6, αV and β5 are related to the presence of axillary metastasis. Integrin expression is reduced in breast cancer and may explain tumor progression [17]. Poorer differentiation of hepatocellular carcinoma is characterized by up-regulation of

integrins [22]. A low serum level of galectin-3 is found in healthy individuals, whereas significant increase of galectin-3 levels is detected in breast cancer patients. Patients with metastases had higher galectin-3 concentrations, than patients with localized tumors [25]. Normal mammary epithelia and benign tumors express low levels of galectin-3 in the cytoplasm. However, galectin-3 expression in the cytoplasm is greatly increased in cell lines propagated from malignant ascites and pleural effusions of late stage breast cancer [29]. In some of the studies it is found that galectin-3 is upregulated, other data show downregulation correlating with the histological grade of tumors. Differences in expression can be found among types of tumors, for example higher expression in fibroadenomas and fibrocystic disease lesions, but lower expression in infiltrating ductal carcinoma and in situ carcinomas.

Overexpression of integrin αVβ3 is connected with enhanced adhesion, proliferation, and motility of human ovarian cancer cells [30]. Integrin α3 is associated with the acquisition of malignant potential by clear ovarian cell adenocarcinoma [53]. Ovarian cancer cell lines (Hey, Ovcar3 and Peo.36) demonstrate significantly high expression of α3, α6, αV and β1 integrin subunits [1]. Expression of galectin-3 is significantly higher in leiomyosarcomas, than in leiomyomas [45]. Galectin-1, galectin-3 and galectin-8 are detected in most of the ovary tumors, whereas galectin-9 is found only in ovarian carcinoma [27].

Decreased expression of integrins is accompanied with reduced human invasive breast cancer cells adhesion and focal adhesion formation on fibronectin-coated surface [18]. Integrin subunits β1, α2, α3, and α6 are overexpressed in Calu-1 cells implicating a role of these integrins in the observed motile behaviors [61]. Freshly isolated human lung cancers show up-regulated expression of integrin β4 and integrin α9 [35]. Non-small-cell lung cancer cells (A549 adenocarcinoma) coexpress integrin heterodimers composed of β1, β3, β4, and β5 subunits, whereas small cell lung cancer cells (AE2 and H69) express only β1integrin heterodimers [9]. Galectin-3 is present in the cytoplasm of small cell lung carcinoma [14]. Galectin-3 over-expression in non-small cell lung carcinoma cell line, DLKP result in enhanced adhesion to extracellular matrix components, cell motility and in vitro invasiveness [34].Galectin-1 is downregulated in malignant (A549) lung epithelial cells [8]. Galectin-3 has cytoplasmic localization in small cell lung carcinoma. Its over-expression in this cell line lead to adhesion to extracellular matrix components and in vitro invasiveness. Po66-CBP is found in squamous cell metaplasia of the bronchi [51].

The squamous UM-SCC-1 carcinoma cells are more invasive than squamous cell carcinoma JHU-022-SCC cells, which is related to differential expression of the integrins α6β4, α3β1 and α2β1 [11]. Known distinct topographical pattern of integrins is altered in transformed mucosa of laryngeal and oropharyngeal carcinomas, but the extent of changes is more marked in oropharyngeal tumors, which are known to be more infiltrating and diffusive and to have a bad prognosis [40].

Metastatic melanoma (MM) cell line express markedly increased levels of β1, α2 and α3, but not α6 integrin subunit, compared to the primary melanoma (PM) cell line. The MM cell migration rate is significantly higher than that of the PM cell line on LN- or CN IV-coated substrates [59]. Reduced expression of galectin-3 is found in melanoma. On the contrary galaptin is overexpressed [52].

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CO5. SUBACUTE SCLEROSING PANENCEPHALITIS - DELAYED AND TERRIBLE SENTENCE AFTER A MEASLES INFECTION

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Subacute sclerosing panencephalitis (SSPE) is a rare chronic progressive degenerative form of brain inflammation with a different infectious causative agent that develops after an initial, uncomplicated measles infection during childhood and/or early adolescence. SSPE is so-called "slow" infection that develops for 6-7 years and it is due to a persistent defective measles virus that infects neurons and survives in latent form for years. The neurological finding is associated with neuronal degeneration, demyelination and microglia proliferation. Brain biopsies show data on astrogliosis, neuronal loss, dendrites degeneration, demyelination, neurofibrillary nodes, and infiltration of inflammatory cells. Patients typically have behavioral changes, myoclonus, dementia, visual disturbances and pyramidal and extrapyramidal signs. The disease has a gradual progressive course – about 95% of patients with SSPE have a lethal outcome within 5 years, while only 5% undergo spontaneous remission. The diagnosis of SSPE is based on the Dyken's criteria: (1) specific clinical symptoms (cognitive damage, dementia, extrapyramidal hyperkinesia, epileptic seizures, hemiparesis and visual disturbances), (2) high values of specific pathogenic measles IgG antibodies in the cerebrospinal fluid and serum, (3) proving of measles nucleic acid in a brain

biopsy material. This condition affects mostly children and young people. Incidence rates among ill patients are 4:100 000, and among immunized – 0.14:100 000. Its prevalence varies between different geographical areas and ethnic groups where genetic polymorphism exists for this particular infection. This is happening in almost all European countries, with priority in the Scandinavian countries, and less in England and the United States. The disease is more likely to affect urban boys (3-6:1). Children who have had measles before the age of 2 are at a higher risk. SSPE treatment is still undetermined.

Key words: subacute sclerosing panencephalitis, measles, neurological disorder

CO6. INTRODUCTION OF DIFFERENT HIV-1 SUBTYPES AMONG HETEROSEXUALS IN BULGARIA

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Background: From 1986 to the end of 2016, 2450 HIV-1 cases were registered in Bulgaria. 1259 (51.4%) of them were heterosexuals (HET). This is the biggest transmission group. The aim of the study was to define HIV-1 diversity in this group and to assess evolutionary history of dominating subtypes in our country.

Materials & Methods: During virology monitoring in National Reference Conformatory Laboratory of HIV were analyzed 322 HIV-1 *pol* gene sequences from HET individuals. HIV-1 subtypes were classified using internet based tools COMET v1.0, REGA v3, manual phylogenetic analysis with ML in FastTree 2 program. The credibility of topology were performed with SH criteria. Phylogenetic trees were displayed with FigTree v.1.4.2 program. Recombinant analysis were performed using bootscan with SimPlot. Time of the most recent common ancestor for analysis molecular clock were performed with Bayesian analysis in BEAST v.1.8.0. program.

Results: 125 (38.8%) sequences were defined as subtype B, 63 (19.6%) as CRF01_AE, 22 (6.8%) subtype C, 21 (6.5%) 02_AG and a variety of more than 30 different HIV-1 subtypes, circulating and unique recombinant forms. Molecular clock analysis showed that subtype B was introduced in Bulgaria around 1980 year. Demographic history showed increase of HIV cases (1980-1990), and then another growth to approximately 2012. The global phylogenetic analysis for the sybtypes B, 01_AE and 02_AG show that they has many introductions in Bulgaria from different countries of the world and overflow from different transmission groups.

Conclusions: We found high genetic diversity among HET with domination of non-B subtypes and phylogenetic link between Bulgarian sequences and such from around the world and different transmission groups. Non-B subtypes were more common in HET individuals compared to non-HET transmission groups. Our study indicated that providing of molecular epidemiological surveillance of HIV-1 diversity is of importance to better control HIV-1 epidemic in Bulgaria.

CO7. LOW DRUG SOLUBILITY – PROBLEMS AND SOLUTIONS

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More than 40% of the new chemical entities that emerge from modern drug discovery programs are characterized by poor water solubility. The slow and incomplete dissolution of such drugs in the gastro-intestinal fluids limits their oral bioavailability and presents a significant problem in drug development.

The aim of this presentation is, on the one hand, to explain the problems which occur throughout drug development and which are related to low drug solubility and, on the other hand, to summarize the common strategies to address low drug solubility, currently used by the pharmaceutical industry all over the world.

For each approach, included in this presentation, it is shown the way it works in order to improve the water solubility of hydrophobic drugs, along with the advantages and disadvantages of its application in drug delivery vehicles.

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CO8. HISTORICAL REVIEW OF THE APPLICATIONS OF SEVEN DRUGS

Vasil Boyanov, Liliya Lazova
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In medicine there are many examples of drugs developed for a given application and subsequently accidentally detected unexpected additional effects. In our paper we will discuss scientific and historical facts on how to validate the additional uses of seven drugs. These are: the antiaggregant effect of Aspirin, the viagra aphrodisiac effect of Viagra, beta blockers in the treatment of haemongioma, the analgesic effects of tricyclic antidepressants, BCG vaccine and Thalidomide in the management of neoplastic processes and the introduction of Ivermectin from veterinary medicine into the human.

CO9. DEVELOPMENT OF ELECTROCONVULSIVE THERAPY FROM THE BEGINNING OF THE TWENTIETH CENTURY TO THE PRESENT

Vasil Boyanov, Liliya Lazova
Medical University of Sofia

Electroconvulsive (ECT) therapy is a non-invasive biological method for the treatment of mental illness through artificially induced epileptic seizures with dosed electrical stimulation of the brain of the patient. It is done through unique, specific therapeutic mechanisms that can not be started by any other antipsychotic agent. From the early 1930s to the present, ECT has gone through various stages of its development, making it an indispensable treatment for some diseases. Today, ECT is a very convenient, safe and easy-to-use method with undeniable efficiency and great practical value.

CO10. CELL SPECIFIC CYTOTOXIC EFFECT OF NEWLY SYNTHESIZED METAL COMPLEXES

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CO11. Multidrug resistance if cancer cells – between pessimism and optimism

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Session D

Chairpersons:

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

Secretary: Tanya Zhivkova, MSc
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DO1. BIOCHEMICAL AND HISTOLOGICAL STUDIES IN RAT MODELS WITH EXPERIMENTAL IMPLANTS BASED ON MODIFIED BETA – TCP

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Abstract

Calcium-phosphate (Ca-P) cements are widely used as bone substitutes in orthopedic, reconstructive and maxillofacial surgery because they have good biocompatibility and extensive bone conductivity. Many bivalent trace metallic ions have demonstrated their beneficial effects in bones tissues engineering applications. A bone-related enzyme alkaline phosphatase (ALP) together with bone – related minerals (Ca, P, Mg and Zn) act actively in bone formation.

The aim of this study was to asses some bone turnover parameters (alkaline phosphatase, Ca, P, Mg and Zn) and histological response in rat models with experimental subcutaneous beta - TCP implants modified with doped trace elements (Zn and Mg). The newly synthesized three types cements - β-tricalcium phosphate doped with Zn/Mg were studied in a rat experimental model during 12 week. Slight deviations were observed in the studied bone turnover markers. There was an absence of inflammation and necrosis, suggesting that there were no toxic effects in the surrounding tissues and no disorders

observed during degradation of materials. Results obtained showed that TCP with dual dopants of Mg and Zn has the potential to be used in orthopedics and dentistry.

Key words: rats, Ca, P, Mg, Zn, ALP, β – TCP, histological study

Calcium-phosphate (Ca-P) cements are widely used as bone substitutes in orthopedic, reconstructive and maxillofacial surgery because they have good biocompatibility and extensive bone conductivity [4]. However, some problems with these materials have been reported – devices implanted within the living tissue must interact with the physiological functions of the host with development of foreign body reactions around the implanted material. Various studies have demonstrated that the addition of trace elements to Ca-P-materials can lend controlled degradation, increase the mechanical strength of the materials and positively influence the biological response [5]. These biogenic elements have been found to play important vital roles in the formation, growth, and repair of bone. Many bivalent trace metallic ions have demonstrated their beneficial effects in bones tissues engineering applications [5].

Literature sources show that Zn ions, Mg ions etc. play a vital role either in osteogenesis or in angiogenesis or in both cases. Zn is important essential trace element in bone development [3]. There are several important metalloenzymes that utilize Zn for structure, catalytic or regulatory actions. Essentially, their role is to create an alkaline environment that favors the precipitation and subsequent mineralization of these inorganic phosphates onto the extracellular matrix that the osteoblasts produce [13].

Mg and Zn dopants play a significant role toward improving cell-materials interactions of tricalcium-phosphates (TCP) [13].

Bone-related enzyme alkaline phosphatase (ALP) together with bone – related minerals (Ca, P, Mg and Zn) act actively in bone formation [11, 13, 14, 15]. TCP with dual dopants combined the benefits of Mg and Zn additives. The presence of Mg enabled TCP-Mg-Zn to obtain high mechanical property, low solubility and good cell-material interaction, while Zn additive stimulated the osteoblast response. TCP with dual dopants of Mg and Zn has the potential to be used in orthopedics and dentistry.

The aim of this study was to asses some biochemical parameters (alkaline phosphatase, Ca, P, Mg and Zn) and histological response to subcutaneous beta - TCP implants modified with doped trace elements (Zn and Mg) in rat model.

Materials and methods

Cements preparation

Three types of β-tricalcium phosphate (TCP) - pure β-TCP and ion-modified with zinc (Zn–β-TCP) and with magnesium (Mg-β-TCP) were used in the solid phases for cement samples preparation. The molar ratio of the modified β-TCP substances were Zn (Mg)/(Ca+Zn) = 0.13 and (Ca+Me)/P = 1.3. The tricalcium phosphates were prepared by our method published earlier [10]. Then they were mechanically activated in a planetary agate mill in order to increase the reactivity of the surface layer of the particles and only the fraction with particle sizes below 28 μm was used in the experiments. Thus prepared tricalcium phosphates were mixed with 4 additives, all of them with particle sizes below 28 μm - chemical reagents CaO, ZnO and MgO and natural polysaccharide xanthan gum, to form the

sample solid phases. The all liquid phases were 2.5% water solution of K_2HPO4. The molds were formed by mixing of solid and liquid phases at different ratio and homogenized for 6-15 minutes to form a plastic mass.

Animal design

20 Wistar male albino rats were used in the experiment. They were divided into 4 groups: the 1st group – without implantation, 2nd group - subcutaneous implantation of β - TCP, the 3rd group – subcutaneous implantation of Zn- β –TCP modified with MgO 10 mol%, ZnO 3 mol%, the 4th group – subcutaneous implantation of Mg-β- TCP modified with ZnO 3 mol%, CaO 3 ml% subcutaneously. The solid phase was prepared with xanthan 0.5% and liquid phase was K_2HPO_4 2, 5% in all materials.

Implantation

An aseptically surgical technique was used during the surgical procedure in the implantation. Rats were anesthetized with ketamine and xylazine in standard doses. After the anesthesia had taken skin effect, dorso-lumbal region was shaved. The incision was made into the skin and into the paravertebral (dorsal-back) muscle in the lower dorso-lumbal area, about 1cm from the midline parallel to the vertebral column. Sterilized implants were inserted subcutaneously. All animals were euthanized using an over dose of pentobarbital.

There were 3 evaluation periods – before the operation, at the end of the week 1 and 12 week after the implantation. Blood was collected from the abdominal aorta to check the activity of alkaline phosphatase enzyme, levels of Ca, Mg and Zn. The activity of the enzyme was determined spectrophotometrically. The levels of elements were determined by an atomic absorption.

Histological studies

Materials for histological observations were taken at the end of 12 week after the implantation. After surgical excision tissue samples with introduced implants were routinely fixed in 10% buffered formalin, rinsed in water and placed in 8% formic acid for demineralization. After dehydration in graded ethanol and xylene clearing, materials were embedded in paraffin. Tissue sections were stained in H&E and examined by light microscope (Leica DM 5000B, Wetzlar, Germany).

The sections were examined for the presence of a fibrous capsule and its thickness, newly formed vessels and occurrence of various types of inflammatory cells. Evaluation system of three categories was used to measure the microscopic observations. The inflammatory reactions were scored according to the following criteria (ISO_7405) regarding pharmacological agents used in dentistry [1,2,8].

Results

Surgical procedures were performed without complications, and the samples were introduced into subcutaneously without being damaged. All rats recovered well and showed no signs of illness within the following 12 weeks. No significant differences of macroscopical appearance and inflammatory reaction could be found between the examined groups. There were no significant differences in the tissue-implant reaction. No deaths were reported.

The interaction between an implant and the surrounding soft tissue can be considered vital for the final clinical performance implanted materials.

Serum Zn level before the operation was similar in the all groups. It was unchanged in the control group at the end of 1nd week compared to that before operation. Zn level was increased in the group 3 compared to the rest groups. At the end of the experiment (12 weeks post implantation) Zn level was continued to be higher than the other groups. The deviations of Zn were in the physiological limits (table 1 and 2).

Serum Mg level was equal in the all groups before operation. After the 1st week it was increased only in group 4 compared with that in the control group and groups 2 and 3. After the 12-nd weeks Mg level in the all groups was similar to those at the 1st week (table 1 and 2).

Serum P level was similar in the all groups before the operation. It was similar in the groups 2 and 3, and P was increased in the group 3 and 4 at the end of the 1st week. P content was increased in the group 2 and 4 compared to the level in the rest groups at the end of the experiment (Table 1 and 2).

Ca level was similar before the operation in all rats. After the 1st week Ca level in the all groups with dopants was increased like this elevation was the biggest in the group 4. The Ca levels at the end of the experiment were in the same limits as these in the 1^{st} week (table 1 and 2).

Alkaline phosphatase activity was similar in all rats before operation. At the 1st week it was increased in groups 3 and 4 compared to that in the control group. The enzyme was not changed in the gr.1 and 2 compared to that before operation. At the end of the experiment the enzyme activity was significantly increased in groups 2 and 4, as it was similar in the gr. 3 to the control (Table 3).

At the selected appropriate endpoints of 84 days of this biomedical evaluation the used materials did not produce any adverse local or systemic effects.

All mateials had shown good biocompatibility (fig.1A, B) and were assessed in first and second criteria - no reaction or slight reaction or moderate reaction (ISO_7405). Implant reabsorption and remodeling to a certain extent was found in some of the specimens. As dominant early responders to biomaterial implantation were observed few lymphocytes and plasmocytes, and tissue macrophages composing slight foreign body reactions. No necrosis, calcification, or residual effect were noticed in selected areas of implantation. According to Maher et al. [6] and Marcotte et al. [7] the formation of fibrous connective tissue around the implants is indicated that they were well tolerated by the surrounding tissues.

Discussion

There were no significant differences in the biochemical parameters before and after operations, which indicated that degradation of the Mg-Zn implants did not raise serum Mg levels non-significantly and Zn level – significantly. There was an absence of inflammation and necrosis, suggesting that there were no toxic effects in the surrounding tissues and no disorders observed during degradation. This correlated with serum biochemical measurements, which is in a good agreement with the study of Stoyanovich [12]. ALP level increased at the end of experiment and its helps locally by depositing phosphate or it helps in the formation of collagen matrix such a way that calcium precipitate at that side. This means

an elevated serum ALP can be due to rapid growth of bone since it is produced by bone-forming cells called osteoblasts. Enhancement of fast ALP activity was essentially related to new bone formation and calcification [9, 14]. Zinc serum ions as an active part of ALP enzyme, was raised at the end of the experiments with the dopants (group 2 and 4).

The formation of fibrous connective tissue around the implants was indicative, that they were well tolerated by the surrounding tissues.

TCP with dual dopants combined the benefits of Mg and Zn additives. The presence of Mg enabled TCP-Mg-Zn to obtain high mechanical property, low solubility and good cell-material interaction, while Zn additive stimulated the osteoblast response [4, 9]. TCP with dual dopants of Mg and Zn has the potential to be used in orthopedics and dentistry.

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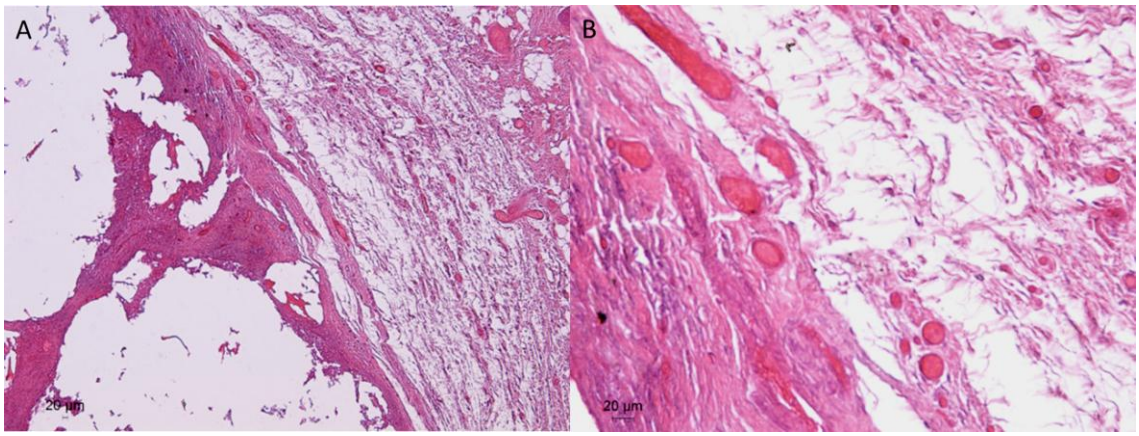
Figures and tables:

Fig. 1. Formation of a fibrous capsule with strands of fibrous tissue, adjacent to the implant surface; neovascularization in the implant contact zone /A and B/. Hematoxylin and eosin.



Serum levels of alkaline phosphatase (ALP), Ca, P, Zn and Mg with subcutaneous Ca-P-modified implants

Before operation Table 1

Groups	ALP SU/l	Ca mmol/l	P mmol/l	Mg mmol/l	Zn mmol/l
Group 1	10,00±0,02	6,90±0,87	2,79±0,11	1,42±0,07	0,77±0,47
Group 2	9,95±0,01	7,0±0,47	2,70±0,10	1,49±0,08	0,65±0,45
Group 3	10,12±0,67	6,97±0,56	2,90±0,20	1,42±0,11	0,67±0,11
Group 4	9,89±0,81	6,95±0,89	2,55±0,10	1,48±0,09	0,69±0,04

1 week post operation Table 2

Groups	ALP Su/l	Ca mmol/l	P mmol/l	Mg mmol/l	Zn mmol/l
Group 1	10,8±1,6	7,15±0,11	2,70±0,52	1,51±0,03	0,79±0,08
Group 2	10,2±0,99	7,94±1,25	2,99±0,58	1,82±0,74	0,85±0,15
Group 3	11,07±2,16	7,96±2,01	3,75±1,10	1,75±0,60	1,59±0,31
Group 4	11,5±1,48	8,56±1,33	3,69±0,87	2,40±0,33	0,75±0,14

12 weeks post operation Table 3

Groups	ALP SU/l	Ca mmol/l	P mmol/l	Mg mmol/l	Zn mmol/l
Group 1	11,5±2,41	7,95±2,93	2,70±0,5	1,41±0,03	0,90±0,08
Group 2	10,91±3,66	7,99±1,25	3,75±1,12	1,80±0,74	0,84±0,05
Group 3	13,99±2,16	8,42±2,14	2,94±0,81	1,65±0,50	1,67±0,31
Group 4	13,8±1,74	8,96±1,83	3,29±0,21	1,89±0,13	0,83±0,17

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DO2. IN VITRO INVESTIGATION ON NEW MATERIALS FOR BONE IMPLANTS

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DO3. INITIAL STUDIES OF CITRATE-BASED AMORPHOUS CALCIUM PHOSPHATES AS MATERIALS FOR BONE IMPLANTS

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DO4. CALCIUM PHOSPHATE LOADED BIOADHESIVE BIOPOLYMER BASED HYDROGEL SCAFFOLD: A NOVEL BIOMATERIAL FOR BONE TISSUE ENGINEERING

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Nearly 75 million people has found to be affected by bone related problem such as osteoporosis in Europe, USA and Japan. Research indicated that osteoporosis causes more than 8.9 million fractures annually [1]. Tissue engineering may become a possible approach to this problem. It includes the application of bioactive three dimensional scaffold which can serve as osteoconductive and osteoinductive agent and facilitates tissue regeneration process [2]. The present study involves the modification of previously developed bioadhesive biopolymer (bacterial cellulose (BC), carboxymethyl cellulose (CMC) and/or polyvinylpyrolidone (PVP)) based hydrogels with calcium phosphate (β-TCP and HA). The achieved modified hydrogels are round and off white and termed as "BC-CMC- β-TCP/HA" and "BC-PVP β-TCP/HA" respectively. These hydrogel scaffolds are analyzed on the basis of their structural and rheological properties. The Fourier Transform Infrared spectral (FTIR) analysis demonstrated the presence of all the ingredients in the all the four samples. SEM images of the modified samples reveal more denser structures than the control ones (i.e. BC-CMC / BC-PVP). Furthermore, all the hydrogel scaffolds exhibit significant elastic property. Hence, the obtained results suggesting the potential use of Calcium phosphate loaded bioadhesive polymer based hydrogel scaffold in the bone tissue engineering. Detail investigation about mechanical property, biocompatibility, biodegradability are in progress.

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