BULGARIAN ACADEMY
OF SCIENCES

PROCEEDINGS

OF THE THIRD WORKSHOP

ON EXPERIMENTAL MODELS AND METHODS IN BIOMEDICAL RESEARCH

23 – 25 APRIL, 2012

SOFIA, BULGARIA

THE THIRD 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

AND

SOCIETY OF IMMUNOLOGY

(UNION OF BULGARIAN SCIENTISTS)

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Supported by:

- *Bulgarian Ministry of Education, Youth and Science under the Grants DTK-02-70/2009; DDVU-02-24/2010, DMU 03/18*

Programme:

Monday, 23 April 2012

9.30 – 9.50 Registration

9.50 – 10.00 Opening Ceremony

Session A. Cell Biology

Chairpersons:

Assoc. Prof. Evelina Shikova-Lekova, MD, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

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

Secretary: Abdulkadir Abudalleh
Faculty of Biology, Sofia University "St .Kliment Ohridski"

10.00 - 10.20

AO1. ARE THE RPE-DERIVED CELL LINES RPE-1 AND RPE-J APPROPRIATE MODEL FOR INVESTIGATION OF CELLULAR LOCALIZATION OF BEST1 PROTEIN

V. Moskova-Doumanova, K. Mladenova, Z. Lalchev, J. Doumanov

10.20 - 10.40

АО2. СИСТЕМИ ЗА КЛЕТЪЧНО КУЛТИВИРАНЕ В УСЛОВИЯ НА СИМУЛИРАНА МИКРОГРАВИТАЦИЯ

Б. Арабаджиев, Р. Скробанска, А. Евангелатов, Н. Стефанова

10.40 - 11.00

AO3. COMPARATIVE ANALYSIS OF IN VITRO THREE-DIMENSIONAL FIBROBLAST CULTURING MODEL TO MURINE CONNECTIVE CUTANEOUS TISSUE

A. Evangelatov, R. Skrobanska, R. Pankov, S. Petrova

11.00 - 11.20 Break

11.20 - 11.30

АО4. ИЗОЛИРАНЕ НА ХЕПАТОЦИТИ

А. Георгиева

11.30 - 11.40

АО5. ЦЕНТРОФУГИРАНЕ В ПЛЪТНОСТЕН ГРАДИЕНТ, ОТДЕЛЯНЕ НА ЗРЕЛИ ОТ НЕЗРЕЛИ СПЕРМАТОЗОИДИ И ОЦЕНКА НА РИСКА ОТ ПРИЛАГАНЕ НА ТЕХНИКИ ЗА АСИСТИРАНА РЕПРОДУКЦИЯ ПРИ МЪЖЕ С НАРУШЕНА СПЕРМАТОГЕНЕЗА

Г. Ненкова

11.40 – 11.50

АО6. BRIEFLY ABOUT CELL DEATH

L. Dyakova, T. Zhivkova, A. Abudallech, B. Andonova-Lilova, J. Kojumdgian-Ivanova, R. Alexandrova

11.50 - 12.00

АО7. МЕТОДИ ЗА СИНХРОНИЗАЦИЯ НА КЛЕТКИТЕ

Й. Ралинска

12.00 - 12.30

АО8. КЛЕТЪЧНИ КУЛТУРИ – ЕКСПЕРИМЕНТАЛНИТЕ МОДЕЛИ, БЕЗ КОИТО НЕ МОЖЕМ

Р. Александрова

Session B. New Materials

Chairpersons:

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

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

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

13.30 - 13.50

BO1. BIOMEDICAL MATERIALS IN HUMAN BODY

V. Kolyovska, D. Deleva

13.50 - 14.00

BO2. ANIMAL MODEL FOR BONE IMPLANT RESEARCH: A RAT MODEL

M. Gabrashanska, M. Alexandrov, I. Yordanova, P. Dimitrov, I. Vladov, V. Nanev

14.00 – 14.20

BO3. ПРИЛОЖЕНИЕ НА ТЕХНОЛОГИИТЕ ЗА БЪРЗО ПРОТОТИПИРАНЕ В ТЪКАННОТО ИНЖЕНЕРСТВО: МОДЕЛ ЗА 3D ПРИНТИРАНЕ НА ОРГАНИ

Бойка Андонова-Лилова[1], Таня Живкова[1], Лора Дякова[2], Р. Александрова[1]

Session C. Parasitology

Chairpersons:

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

Assist. Prof. Delka Salkova, DVM, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

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

14.40 - 15.00

CO1. REVIEW OF THE METHODS FOR CONTROL OF CHICKEN COCCIDIOSIS

D. Salkova

15.00 - 15.10

CO2. COMPARATIVE ELECTROPHORETIC STUDIES OF PROTEIN EXTRACTS OF SIX TRICHINELLA ISOLATES BY SDS-PAAE AND PAAE

V. Dilcheva, S. Petkova, E. Gabev

15.10 – 15.30

CO3. EXPERIMENTAL MODELS OF FASCIOLOSIS AND CARCINOGENESIS

N. Tsocheva-Gaytandzhieva, R. Toshkova, I. Roeva, M. Topashka-Ancheva, A. Filchev, D. Salkova

15.30 - 15.40

CO4. THE DEADLY TROPICAL TRIANGLE

Dzh. Farandzha

15.40 - 15.50

CO5. МАЛАРИЯТА – ВЧЕРА, ДНЕС... А УТРЕ?

И. Иванова

15.50 - 16.00

CO6. КАКВО (НЕ) ЗНАЕМ ЗА ТЕНИИТЕ

В. Велчев

16.00 – 16.10

CO7. STRANGE PARASITES

A. Zreik

Tuesday, 24 April 2012

Session D. Molecular Biology, Biophysics and Biochemistry

Chairpersons:

Assoc. Prof. George Miloshev, PhD
Institute of Molecular Biology, Bulgarian Academy of Sciences

Assist. Prof. Roumiana Todorova, PhD
Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences

Secretary: Zina Ivanova
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

9.50 - 10.20

DO1. ФИЗИКА НА ЖИВАТА МАТЕРИЯ
А. Петров

10.20 – 10.40

DO2. DOES THE BUDDING YEAST LINKER HISTONE Hho1p INFLUENCE SURVIVAL AND MORPHOLOGY OF CHRONOLOGICALLY AGING CELLS?
K. Uzunova, M. Georgieva, G. Miloshev

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10.40 - 11.00

DO3. ANTIOXIDANT EFFECTS OF BULGARIAN PROPOLIS AND ITS COMPONENTS CAPE AND CHRYSIN ON Ty1 TRANSPOSITION IN YEAST *SACCHAROMYCES CEREVISIAE*
M. Georgieva, O. Krastanova, V. Bankova, M. Pesheva

11.00 – 11.20 Break

11.20 - 11.40

DO4. ОПРЕДЕЛЯНЕ НА АНТИОКСИДАНТНА АКТИВНОСТ НА ПЧЕЛНИ ПРОДУКТИ (МЕД И ПЧЕЛНО МЛЕЧИЦЕ) ОТ БЪЛГАРСКИ ПРОИЗХОД, ЧРЕЗ НОВ ТЕСТ
М. Димитров, М. Пешева

11.40 - 12.00

DO5. ESTIMATION OF THE INTRINSIC STRUCTURAL DISORDER OF NATIVE EWS AND ITS REPORTED FUSION ONCOGENIC PROTEINS WITH ANALYSIS OF THE FUNCTIONAL REGIONS

R. Todorova

12.00 - 12.20

DO6. LUNG MATURITY ASSESSMENT BY *IN VITRO* ANALYSES OF GASTRIC ASPIRATES FROM NEWBORN INFANTS

M. Bangyozova, A. Jordanova, A. Tsanova, J. Doumanov, E. Christova, Z. Lalchev

12.20 - 12.30

DO7. ВЪЗСТАНОВЯВАНЕ НА РАНА – ФИЗИОЛОГИЯ И МОЛЕКУЛЯРНИ МЕХАНИЗМИ НА РЕГУЛАЦИЯ

А. Тасев

Session E. Virology

Chairpersons:

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

Assist. Prof. Petia Genova, PhD
*Department of Virology, National Centre of Infectious and Parasitic Diseases***,**

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

13.30 – 13.50

EO1. HIV-1 POL GENE SEQUENCING AND PHYLOGENETIC ANALYSIS OF THE HIV-1 EPIDEMIC IN BULGARIA REVIEWED PRESENCE OF A BOUQUET OF VARIOUS SUBTYPES AND COMPLEX RECOMBINANT FORMS

I. Alexiev, D. Beshkov, V. Georgieva, L. Karamacheva, I. Elenkov

13.50 - 14.10

EO2. NANOTECHNOLOGICAL APPROACH FOR THERAPY OF HIV/AIDS BY DESTRUCTION OF THE VIRUS NAVIGATION SYTEM (HIGINS)

E. Gabev

14.10 – 14.20

EO3. OUR EXPERIENCE IN USING NANOTECHNOLOGIES FOR IMPROVING THE THERAPY OF HIV/AIDS

E. Gabev

14.20 - 14.40

EO4. DETERMINATION OF HEPATITIS C VIRAL LOAD BY TAQMAN PCR IN HIV-HCV COINFECTED PERSONS

E. Golkocheva-Markova, I. Alexiev, A. Kevorkyan, P. Teoharov

14.40 – 15.00 Break

15.00 – 15.20

EO5. ДИФЕРЕНЦИАЛНО-ДИАГНОСТИЧНИ ПОДХОДИ ПРИ ВИРУСНИТЕ ИНФЕКЦИИ

Ст. Иванова, А. Тошев, З. Михнева

15.20 – 15.40

EO6. ANIMAL PAPILLOMAVIRUSES AS MODELS FOR TESTING OF HPV VACCINES

E. Shikova, Z. Ivanova

15.40 – 16.00

EO7. HUMAN CYTOMEGALOVIRUS (HCMV) AND EPSTEIN-BARR VIRUS (EBV) INFECTIONS LEAD TO INCREASED RISK OF BREAST CANCER

P. Genova-Kalou, J. Ivanova

16.00 - 16.20

EO8. MuLV - BASED ANIMAL MODELS TO STUDY RETROVIRUS - INDUCED NEUROLOGIC DISEASE

E. Shikova

Wednesday, 25 April 2012

Session F. Medicine

Chairpersons:

Assoc. Prof. Reneta Toshkova, MD, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Assist. Prof. Albena Alexandrova, PhD
Institute of Neurobiology, Bulgarian Academy of Sciences

Assist. Prof. Milena Georgieva, PhD
Institute of Molecular Biology, Bulgarian Academy of Sciences

9.50 – 10.10

FO1. IN VITRO SYSTEMS FOR STUDYING DRUG METABOLISM: ORGAN SLICES
A. Alexandrova

10.10 – 10.30

FO2. AN EXPERIMENTAL MODEL OF SODIUM NITRITE-INDUCED HYPOXIA
E. Petrova, V. Ormandzhieva, Y. Gluhcheva, V. Atanasov, E. Pavlova, St. Dimitrova, B. Eremieva, M. Dimitrova, D. Kadiysky

10.30 - 10.40

FO3. REVIEW: ADRENERGIC RECEPTORS AND CARDIAC MYOCYTE APOPTOSIS
M. Vapireva

10.40 – 11.00 Break

11.00 – 11.20

FO4. ЕЛЕКТРОПОРАЦИЯ. ПРИЛОЖЕНИЕ В МЕДИЦИНАТА И МОЛЕКУЛЯРНАТА БИОЛОГИЯ.
Д. Мартинов, Е. Стоилкова

11.20 - 11.40

FO5. PLATELET AGGREGATION INHIBITORY EFFECT OF PHOSPHOLIPASE A_2 FROM *VIPERA AMMODYTES MERIDIONALIS* VENOM

Y. Goranova, S. Stoykova, V. Atanasov, D. Danchev, S. Petrova

11.40 – 11.50

FO6. IDENTIFICATION OF CRYSTALS IN RHEUMATOLOGY

A. Ivanov, M. Geneva, L. Simeonov

11.50 - 12.00

FO7. ANTI-INFLAMMATORY EFFECT OF ROSEMARY OIL IN MODEL SYSTEM

M. Draganova-Filipova, P. Zagorchev

12.00 - 12.10

FO8. DIETARY EXPERIMENTAL MODELS FOR THE STUDY OF THE DIFFERENT STAGES OF OBESITY AND METABOLIC SYNDROME IN RATS

P. Angelova

12.10 – 12.20

FO9. CERTAIN DETERMINANTS OF THE IRRELEVANCE BETWEEN CLINICAL AND EXPERIMENTAL SEPSIS

D. Popov

12.20 - 12.30

FO10. EXHALED NITRIC OXIDE - MEASUREMENT AND CLINICAL APPLICATION

B. Atanasova, D.Vasilev, M.Sirakova, S. Mandadzhieva

13.30 – 13.40

FO11. EXPERIMENTAL MODEL OF A COMPOUND LIVING SKIN EQUIVALENT OF NEONATAL CELLS

K. Tokmakova, P. Molchovski, A. Stanchev, W.Y.Ip, G. Tipoe

13.40 – 13.50

FO12. ПРИЛОЖНАТА КИНЕЗИОЛОГИЯ – ИНТЕГРАТИВЕН МЕТОД ЗА ОЦЕНКА НА ЗДРАВЕТО

Е. Тодорова

13.50 – 14.10

F13. EXPERIMENTALLY INDUCED DIABETES MELLITUS AND ITS EFFECTS ON ARGININE-VASOPRESSIN AND ANGIOTENSIN II - ELICITED MYOMETRIAL CONTRACTILITY

T. Georgiev, P. Hadzhibozheva, R. Kalfin, A. Tolekova

14.10 - 14.40

F14. GENDER DIFFERENCES IN SUSCEPTIBILITY TO TYPE 2 DIABETES IN RAT EXPERIMENTAL MODELS

M. Yakovlieva, S. Mihaylova, A. Anastasov, T. Tacheva, T. Vlaykova, K. Trifonova, A. Tolekova

Session G. Cancer Research

Chairpersons:

Prof. Dimitar Kadiysky, MD, PhD, DSc
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Assist. Prof. Yordanka Gluhcheva, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Assist. Prof. Emilia Petrova, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

14.50 – 15.10

GO1. A NEW APPROACH FOR LOCAL TREATMENT OF SOLID TUMORS, INVOLVING GOLD NANOPARTICLES

L. Yossifova, E. Gardeva, R.Toshkova, N. Nedyalkov, M. Alexandrov, P. Atanasov

15.10 - 15.30

GO2. ЦИТОТОКСИЧЕН И АНТИПРОЛИФЕРАТИВЕН ЕФЕКТ НА ПОЛИБУТИЛЦИАНОАКРИЛАТНИ НАНОЧАСТИЦИ IN VITRO.

М. Петкова, Р. Скробанска, Г. Йорданов

15.30 – 15.50

GO3. IN VITRO STUDIES ABOUT THE INHIBITION OF THE PROLIFERATION ACTIVITIES OF SOME GASTROPODAN HEMOCYANINS

P. Genova-Kalou, Y. Raynova, K. Idakieva

15.50 – 15.10

GO4. PLANT EXTRACTS WITH ANTITUMOR ACTIVITY

B. Petkova, V. Moskova-Doumanova, M.Dimitrova, T. Topouzova-Hristova, V. Kapchina

16.10 – 16.20

GO5. РАКОВИ ЗАБОЛЯВАНИЯ И СИНДРОМ НА ДАУН

Т. Владимиров

16.20 – 16.30

CLOSING CEREMONY

Poster Session

BP1. MESENCHIMAL STEM CELLS – A NEW HOPE FOR THE TREATMENT OF BONE DEFECTS

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

BP2. СТВОЛОВИ КЛЕТКИ

З. Димитрова

CP1. PHARMACO-TOXICOLOGICAL METHODS FOR TESTING ON SOME ANTIHELMINTICS

E. Arnaudova, M. Gabrashanska, D. Salkova, V. Nanev, N. Tzocheva-Gaitandhjieva, D. Hrusanov, I. Vladov, B. Arnaudova, B. Georgiev

DP1. СЪЩНОСТ НА РЕКОМБИНАНТНИТЕ ВАКСИНИ

Б. Трайкова

DP2. ГМО – ЗАЩО НЕ?

Г. Донкова

DP3. ХИПОТЕЗИ И ТЕОРИИ ЗА ПРОИЗХОДА НА ЖИВОТА

А. Фесток

EP1. COMBINED APPLICATION OF POLYPHENOL EXTRACT FROM *GERANIUM SANGUINEUM* L. AND PROTEASE INHIBITOR AS A NEW APPROACH FOR TREATMENT OF LETHAL EXPERIMENTAL INFLUENZA INFECTION IN MICE

Toshkova R., L. Yossifova, E. Gardeva, J. Serkedjieva, S. Apostolova

FP1. INSULINOMA CELL LINES

T. Zhivkova, L. Dyakova, B. Andonova-Lilova, J. Kojumdgian-Ivanova, R. Kalfin, A. Tolekova, R. Alexandrova

GP1. НАНОТЕХНОЛОГИИ В ЛЕЧЕНИЕТО НА РАКОВИТЕ ЗАБОЛЯВАНИЯ

Ж, Коюмджян-Иванова[1], Р. Александрова[1], Т. Живкова[1], Л. Дякова[2], Б. Андонова-Лилова[1], М. Симеонова[3], С. Пашевич[4], А. Денисов[4], В. Кульчицкий[4]

GP2. IN VITRO EFFECTS OF NANO-STRUCTURED HYBRID MATERIALS CONTAINING QUATERNIZED CHITOSAN AND GOSSYPOL ON HELA HUMAN CARCINOMA CELL LINE

Toshkova R.*, L. Yossifova*, E. Gardeva*, M. Ignatova, M. Alexandrov*, N. Manolova**, I. Rashkov****

GP3. ТРАНСФОРМИРАНИ С ПАПИЛОМАВИРУСИ ЧОВЕШКИ ТУМОРНИ КЛЕТКИ И ТЯХНОТО ПРИЛОЖЕНИЕ ПРИ ПРОУЧВАНИЯ ВЪРХУ АНТИТУМОРНАТА АКТИВНОСТ НА НОВОСИНТЕЗИРАНИ МЕТАЛНИ СЪЕДИНЕНИЯ

А. Абудаллех, Ж. Коюмджян-Иванова, Т. Живкова, Л. Дякова, Б. Андонова-Лилова, М. Александров, Г. Милошев, М. Георгиева, Р. Калфин, Г. Маринеску, Д. Кулита , Л. Патрон, Р. Александрова

23 April 2012 (Monday)

Registration 9.30 – 9.50

Opening Ceremony 9.50 – 10.00

Session A. Cell Biology

Chairpersons:

Assoc. Prof. Radostina Alexandrova, PhD

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

Assoc. Prof. Evelina Shikova-Lekova, MD, PhD

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

Secretary: Abdulkadir Abudalleh

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

AO1. ARE THE RPE-DERIVED CELL LINES RPE-1 AND RPE-J APPROPRIATE MODEL FOR INVESTIGATION OF CELLULAR LOCALIZATION OF BEST1 PROTEIN

Veselina Moskova-Doumanova[1], Kirilka Mladenova[1], Zdravko Lalchev[2], Jordan Doumanov[2]

[1] Department of Cytology, histology and embryology, Biological faculty, Sofia University "St. Kl. Ohridski", 8 Dragan Tzankov Blvd., 1164 Sofia, Bulgaria

[2] Department of Biochemistry, Biological faculty, Sofia University "St. Kl. Ohridski", 8 Dragan Tzankov Blvd., 1164 Sofia, Bulgaria

E-mail: moskova@biofac.uni-sofia.bg

The human eye is a complex system, which detects, amplify, extract and process the light to the brain. The retina is the first station of the visual system and possesses an orderly laminated structure. The nuclei and processes of the retinal cells are segregated into anatomically distinctive layers [1]. The photoreceptive cells or photoreceptors are present in the outer region of the retina [2], on the farthest position to the incoming light. These are the cells responsible for the capturing of the photons, amplifying the signal and sending it to the brain via synapses.

The life of the photoreceptors is supported by the retinal pigment epithelium (RPE) cells - a monolayer of pigmented cells, forming a part of the blood/retina barrier [3]. These are polar cells with apical membrane facing the photoreceptor outer segment and basolateral surface interacting with choriocapillaris [4], an apico-basal polarity, characteristic for a transporting epithelium [2]. The RPE cells transport ions, water and metabolic end products from the subretinal space to the blood and take up the nutrients from the blood and deliver them to the photoreceptors. Tight junctions ensure a polarized distribution of ionic channels and membrane receptors in the apical and basolateral plasma membrane domains [4].

Epithelial ion transport is essential for visual functions. Defective chloride transport is associated with Best's vitelliform macular degeneration (BVMD), one of the most frequently encountered autosomal-dominant retinal dystrophies [5], caused by degeneration of RPE [3]. Best disease is a monogenic disorder caused by mutations in the *BEST1* gene (previously known as *VMD2*) [6, 7]. This gene encodes a retina-specific protein, called bestrophin-1 or Best1 [8].

Best1 is a transmembrane protein, predominantly expressed on the basolateral surface of the RPE cells [9]. It acts as a Ca^{2+} activated Cl^- channel [10], a regulator of $Ca2^+$ transport [11] or both. There are also evidences that it plays a role in HCO_3^- transport as a channel [12], in a cell volume regulation, maintenance of cellular pH [13] and in eye development [14].

We were interested in investigation of influence of disease-causing mutations in the Best-1 protein (found in patients, clinically diagnosed with BVMD) on the cellular localization of the protein. Our model systems were two commonly used RPE- derived cell lines – human line RPE-1 and rat line RPE-J. We followed cellular expression of Best1 by confocal microscopy. Polarity of the cells was evaluated by the development of the tight junctions, separating apical and basolateral domains of the cells.

Although in the eye RPE is a monolayer, images of the RPE-1 cell line, obtained by us, revealed that these cells grow as pseudo-stratified epithelium, with parts of one cell over the neighboring. The cells were polarized, as indicated the presence of well developed tight junctions in their apical part (detected immunochemically by tight junction marker ZO-1). But in some cases the ZO-1 signal was over the nuclei of the cells, confirming the pseudostratification. Besides, cells were not thick enough for evaluation of the membrane localization of the protein by confocal microscopy – they were only around 2μm high.

Our second model system was rat cell line RPE-J. Cells were transiently transfected with normal human Best1 and some mutants. Confocal images revealed well established tight junctions, indicating the polarization of the cells. Despite this, even five days after the establishment of polarization, we observed non-polar expression of the Best1 proteins (normal and mutants) inside the cells, and on the basal and apical membranes. We observed perinuclear clustering of the signal, but colocalization with marker for trans-Golgi apparatus revealed that the Best1 protein is not retained in the Golgi, but the colocalization is probably due to the protein molecules in the biosynthetic pathway.

In conclusion, we consider that these two RPE-derived cell lines are not an appropriate model for investigation of cellular localization of the Best1 protein by the methods of confocal microscopy.

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АО2. СИСТЕМИ ЗА КЛЕТЪЧНО КУЛТИВИРАНЕ В УСЛОВИЯ НА СИМУЛИРАНА МИКРОГРАВИТАЦИЯ

Б. Арабаджиев, Р. Скробанска, А. Евангелатов, Н. Стефанова

Биологиески ф-т на СУ „Св. Климент Охридски“, катедра „Цитология, хистология и ембриология“, София, бул. „Драган Цанков“ №8, ПК:1164

E-mail n_stefanova@biofac.uni-sofia.bg

През последните години експериментите върху поведението на различни биологични обекти в условия на микрогравитация добиват все по-голяма популярност. Една от причините за това е развитието на космическите технологии, позволяващи по-дълги престои на астронавти в околоземна орбита. Установено е, че при бозайниците продължителното пребиваване в условия на микрогравитация причинява редица здравословни проблеми, най-съществените от които са загуба на костно вещество, атрофия на мускулите, забавено зарастване на раните, понижена синтеза на

извънклетъчен матрикс, повишена пропускливост на кръвоносните съдове и др. [1,2,3]. Ценна информация за причините и молекулните механизми на възникване на тези патологични изменения може да бъде получена от експерименти с клетъчни култури в условия на симулирана микрогравитация. Такива експерименти също така могат да хвърлят светлина върху някои фундаментални научни въпроси, касаещи влиянието на слабите механични въздействия върху поведението на биологичните системи.

Съществуват няколко типа системи за клетъчно култивиране в условия на симулирана микрогравитация:

Параболични полети. При тези системи експериментите се извършват на борда на самолет, който се движи по траектория, състояща се от последователни издигания и спускания, като за кратки периоди – от порядъка на 15-20 s – траекторията му описва параболична дъга, при която центробежната сила, действаща върху самолета, компенсира гравитационната сила (Кеплерова траектория) [4]. Основният недостатък на тези системи е, че периодът, през който резултатното ускорение, изпитвано от тестовия обект, е приблизително 0 m/s^2, е кратък и освен това е сравним с периода на издигане, при който резултатното ускорение е по-голямо от земното (около 1.8×g).

Free-fall машини. Това са апарати за симулирана микрогравитация, които позволяват по-продължителни експерименти. При тях изследваният обект бива издиган с голяма скорост на височина около 1 m и оставян да пада свободно (в продължение на около 900 ms), след което цикълът се повтаря. Тъй като времето, за което пробата се издига, е изключително кратко (от порядъка на 20 ms), се приема, че клетките не могат да регистрират увеличеното ускорение (около 20×g) и съответно то не предизвиква реакция. [5]

Магнитна левитация. При тези системи изследваният обект се поставя в силно градиентно магнитно поле. Тъй като обектите с диамагнитни свойства, каквито са биологичните обекти, се отблъскват от магнитното поле, възниква магнитна сила, насочена по посока на областите с по-малка сила на полето. В системите за магнитна левитация тази сила компенсира гравитационната сила, действаща върху изследвания обект и той се намира в състояние на стабила левитация [6].

Ротационни системи за клетъчно култивиране (едноосеви клиностати). Това са едни от първите разработени системи за симулирана микрогравитация, при които изследваният обект се върти около една ос, перпендикулярна на посоката на вектора на гравитацията. Поради високата скорост на въртене (от 30 до 150 rpm при клиностатите за култивиране на животински клетки) се приема, че клетките не могат да регистрират промяната в посоката на вектора на гравитация за периода на едно завъртане и сумарната големина на този вектор за периода им на реакция е близка до 0. От важно значение при тези системи е големината на работната камера, тъй като центробежната сила нараства с увеличаване на радиуса и това може да доведе до компроментиране на резултатите [7].

Random positioning machines (RPM). Тези системи се базират на принципа на едноосевите клиностати, но при тях ротацията на пробите се извършва във всички посоки. По този начин се постига още по-добро разбъркване на посоката на гравитационния вектор [8,9]. За сега се смята, че RPM машините са едни от най-удачните моделни системи за култивиране на животински клетки в условия на симулирана микрогравитация.

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AO3. COMPARATIVE ANALYSIS OF IN VITRO THREE-DIMENSIONAL FIBROBLAST CULTURING MODEL TO MURINE CONNECTIVE CUTANEOUS TISSUE

A. Evangelatov[1], R. Skrobanska[1], R. Pankov[1], S. Petrova[1]

[1] Department of Cytology, Histology and Embryology, Biological Faculty, Sofia University, 8 Dragan Tzankov Str., Sofia 1164, Bulgaria

Conventional monolayer culturing is the traditional method for culturing cells outside of the living organism. Studies from the past 10 years have shown that the transfer from the soft, elastic environment of tissues to the stiff, rigid surface of the culturing vessels causes changes in cellular signaling as an answer to the new environmental conditions. In recent years there has been an intensive development of three-dimensional (3D) culturing systems made either of synthetic fibers or natural components of the extracellular matrix with the aim to mimic the conditions of the living organism. The main difference and drawback of these 3D cultures is that they lack the natural organization of the extracellular matrix.

Our goal was to create a three-dimensional culturing model without using exogenous scaffold of matrix proteins. We developed a 3D culturing model, using the GD25β1 fibroblast cell line that can proliferate past 100% confluence. This culturing model provides a naturally synthesized and organized by the fibroblasts themselves extracellular matrix that contains a large amount of matrix proteins like fibronectin and collagen and provides closer to in vivo conditions. We have shown that fibroblasts grown in this culturing model acquire quiescent state-like properties like suppressed α-smooth muscle actin expression and lower number of dividing cells compared to monolayer culture. We believe that this culturing model is a better representation of normal tissue compared to the commercially available 3D culturing systems like Matrigel gels, collagen or fibrin gels, synthetic polymer fibers, etc.

Investigating the similarity of this in vitro 3D culturing model to healthy tissue we had to check for correlation between the biochemical data from the 3D model and healthy tissue. We choose to compare our 3D model to murine connective cutaneous tissue since we believe they are in close approximation. Healthy tissue (termed 3D tissue) was compared to the primary cell line, acquired from the same tissue (2D tissue). We investigated the level of expression of cyclin D1 in 3D tissue as well as after transition to 2D tissue and compared the data to GD25β1 fibroblasts grown as a monolayer as well as a three-dimensional culture. We also investigated the FAK/Src/ERK signaling cascade as a major pathway involved in regulation of cyclin D1.

АО4. ИЗОЛИРАНЕ НА ХЕПАТОЦИТИ

Алмира Георгиева

Институт по Невробиология – Българска академия на Науките, ул. Акад Г. Бончев, бл. 23, София, България

АО5. ЦЕНТРОФУГИРАНЕ В ПЛЪТНОСТЕН ГРАДИЕНТ, ОТДЕЛЯНЕ НА ЗРЕЛИ ОТ НЕЗРЕЛИ СПЕРМАТОЗОИДИ И ОЦЕНКА НА РИСКА ОТ ПРИЛАГАНЕ НА ТЕХНИКИ ЗА АСИСТИРАНА РЕПРОДУКЦИЯ ПРИ МЪЖЕ С НАРУШЕНА СПЕРМАТОГЕНЕЗА

Галина Трайкова Ненкова

Лаборатория "Свободно-радикални процеси", Институт по Невробиология – Българска академия на Науките, ул. Акад Г. Бончев, бл. 23, София, България

E-mail: *g_alina_n@abv.bg*

В спермата има два основни източника на активни форми на кислорода (АФК) – левкоцитите и сперматозоидите. Не всички сперматозоиди обаче продуцират високи нива на АФК. Възможни са нарушения в сперматогенезата, при които се получават сперматозоиди с морфологични изменения, част от които са пряко свързани с продуцирането на свободни радикали (СР). Установено е, че най-много СР се

генерират, когато в еякулата преобладават сперматозоиди с неправилна форма на главата, а също и такива с проксимални цитоплазмени остатъци.

Достъпен метод за разделянето на сперматозоидите с различни морфологични дефекти е центрофугирането в плътностен градиент. Използват се разтвори с плътност 90%, 70% и 47%. Разтворите се надслояват един върху друг, като накрая се добавя спермата (в случая се работи само с материали, в които не е установено наличие на левкоцити). Следва центрофугиране на стайна температура при 500g в продължение на 20 минути. Получават се четири фракции: първа – между семенната плазма и разтвора с плътност 47%, втора – между разтворите с плътност 47% и 70%, трета – между разтворите с плътност 70% и 90% и четвърта – утайката във фазата от 90%. За да се направи микроскопска оценка на концентрацията, подвижността и морфологията на сперматозоидите в различните фракции, е необходимо всяка фаза поотделно да се отпипетира в чиста епруветка, след което да се разреди със същия обем BWW среда. Пробите се центрофугират при 500g в продължение на 7 минути. Получените утайки се ресуспендират в 1 мл BWW среда. В аликвоти от тези суспензии се определят концентрацията, подвижността, морфологията на сперматозоидите, както и нивата на АФК.

Направената оценка на морфологията по стриктните критерии на Крюгер показва, че в първата фракция се съдържат незрели сперматозоиди - с неправилна форма на главата и с проксимални цитоплазмени остатъци, във втората фракция преобладават незрелите сперматозоиди с проксимални цитоплазмени остатъци, в третата се срещат както нормални, така и анормални по отношение на морфологията сперматозоиди, а в четвъртата фракция преобладават сперматозоидите с нормална морфология. Подвижността на сперматозоидите в първа и втора фракция е много по-ниска отколкото в трета и четвърта. Най-високи нива на АФК са измерени във втора, а най-ниски в четвърта фракция. Това се дължи на факта, че незрелите сперматозоиди с проксимални цитоплазмени остатъци се отличават с висока NADPH-оксидазна и G6PD-зна активност. Колкото повече сперматозоиди има във втора фракция, толкова по-малък е броят на подвижните сперматозоиди в четвърта фракция. Тази зависимост може да се обясни по следния начин: В сравнение с другите клетки сперматозоидите съдържат по-малко цитоплазмени антиоксиданти, тъй като голяма част от цитоплазмата им се отстранява при тяхното зреене. Освен това по време на сперматогенезата в семинифериите каналчета и в епидидимиса (надсеменника) мъжките полови клетки не са в контакт със семенната плазма, която също съдържа необходимите количества антиоксидантни ензимни системи. Това прави зреещите спрматозоиди много чувствителни към оксидативен стрес. В случай, че в сперматогенезата има нарушения, водещи до формирането на незрели сперматозоиди с проксимални цитоплазмени остатъци, нивата на АФК рязко се покачват. В семинифериите каналчета и в епидидимиса сперматозоидите са разположени в тесен контакт помежду си (липсва семенна плазма). Това прави възможно възникването на оксидативен стрес в зрели сперматозоиди (които нормално не генерират АФК), тъй като незрелите са в непосредствена близост. Важно е обаче да се отбележи, че увреждането на зрели сперматозоиди се наблюдава само в случай, че незрелите са над определен критичен праг. Ето защо в еякулата на мъже с аномалии в сперматогенезата се откриват зрели сперматозоиди с нарушена подвижност и с ДНК увреждания.

От тези факти става ясно, че инвитро и ICSI процедурите (при които се подбират възможно най-добрите сперматозоид по отношение на морфологията) крият голям

риск, тъй като дори клетки с нормална морфология могат да са с увреден генетичен материал.

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AO6. BRIEFLY ABOUT CELL DEATH

Lora Dyakova[1], Tania Zhivkova[2], Abdulkadir Abudallech[2,3], Boyka Andonova-Lilova[2], Janette Kojumdgian[2], 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
[3]Biological Faculty, Sofia University, 8 Dragan Tzankov Str., Sofia 1164, Bulgaria

AO8. КЛЕТЪЧНИ КУЛТУРИ – ЕКСПЕРИМЕНТАЛНИТЕ МОДЕЛИ, БЕЗ КОИТО НЕ МОЖЕМ

Радостина Александрова

Институт по експериментална морфология, патология и антропология с музей, БАН, София, България

Session B. New Materials

Chairpersons:

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

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

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

BO1. BIOMEDICAL MATERIALS IN HUMAN BODY

Vera Kolyovska, Denislava Deleva

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 25, 1113 Sofia, Bulgaria, E-mail: verakol@abv.bg

A biomaterial is any matter, surface, or construct that interacts with biological systems. The development of biomaterials, as a science, is about fifty years old. The study of biomaterials is called biomaterials science. It has experienced steady and strong growth over its history, with many companies investing large amounts of money into the development of new products. Biomaterials science encompasses elements of medicine, biology, chemistry, tissue engineering and materials science.

In terms of technology, it's helpful to distinguish body implants, first, from transplants and second, from synthetic organs. Transplants are also a form of "implantation" but they involve purely biological body parts, in fact, duplicates of the body part being replaced, except they come from another individual. Synthetic organs, such as an artificial heart, are obviously body implants; the main distinction is that they are entire organs, typically very complex entities, whereas most body implants are either much simpler pieces of the body, such as a hip replacement, or electronic devices not intended to replace any existing body part.

The many forms of implantation

The business of body implantation – inserting devices inside the human body – is already decades old. For example, research that eventually led to heart pacemakers and defibrillators goes back to 1889. The modern pacemaker dates to 1957 and 1958. In the last twenty years or so, the increasing sophistication of implant techniques and the reduction of body rejection have widened the application of implanted devices. This is particularly true for uses in the brain, such as monitoring and control of epileptic seizures and neurological pacemakers to combat severe cases of depression. The list of what's already being done with implants is impressive and perhaps a little surprising.

Keep in mind that most forms of implantation for the human body can also be applied to animals and eventually to plants. In fact, most of the technology now is use was developed and tested on animals.

Brain (or neural) implants: Pacemakers for the brain have been in use since 1997, mostly for alleviation of severe pain or diseases such as Parkinsons. Research in this area is rapidly developing new techniques for monitoring and controlling various aspects of brain function, including the insertion of computer chips (CPUs).

Sensory implants: Sometimes called cognotechnology, the replacement or augmentation of body senses by implanted devices has generally focused on sight (implanted replacement for eye parts) and hearing (cochlear implant).

Spinal implants: The use of replacement segments for the spine (bone and cartilage) are part of clinical procedures. Most spinal implants are intended to relieve pain. Electronic devices are used to regulate muscular and nerve signals that cause back-pain.

Organ stimulation implants: The most familiar implants of this kind are heart pacemakers and defibrillators, which help to restore or maintain normal heartbeats. Similar devices are being developed for other organs.

Subcutaneous implants: Placing devices just under the skin (more precisely) under the subcutaneous layer, is the preferred method for many kinds of monitoring and body support devices. New methods of drug delivery use this approach, as do personal identification devices (ID chips), and soon, a large number of convenience implants.

Repair implants: This is probably the oldest and most prevalent form of implant, which involves repairing or supporting bones and other structures in the body. Stents, braces, rods, pins, heart valves, hip prothesis, and knee replacements are examples of the implanted items.

Dental implants: Caps, crowns, plugs, posts and other dental implants are so common that they probably don't even to come to mind as being implants. Modern dental surgery also replaces teeth and jaw bones with artificial substitutes.

Cosmetic implants: This is an area of implantation that often merges with the techniques of plastic surgery. Breast enlargement with silicon (or other) implants gets the most attention, but almost all elements of the visible body (ears, nose, hands…) are becoming targets for body implants.

Many problems and concerns. While most people accept dental implants without scruple, other forms of implants are highly controversial – particularly those involving the brain and the placement of monitors inside the body. Issues of privacy, security, and mind-control are always lurking behind any type of invasive procedure and especially for implantation of electronic devices. Within the next decade or two, the rapid growth of cosmetic and convenience implants will undoubtedly provoke a public backlash in many countries, if for no other reason than the practice of implantation for non-medical reasons seems "unnatural". The effects of application of biomaterials are experimental stage and do not guarantee one hundred percent safety of the recipient. These materials are expensive and so far no clear long-term effects of taking them, and some of their possible disadvantages as immunologic incompatibility, integrity, corrosion, etc.

For some people, their body is a temple, a temple to be preserved and undefiled. Yet, for a variety of reasons, an ever increasing number of people are willing to park electronics and other non-biological articles inside their bodies.

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BO2. ANIMAL MODEL FOR BONE IMPLANT RESEARCH: A RAT MODEL

M. Gabrashanska, M. Alexandrov, I. Yordanova, P. Dimitrov, I. Vladov, V. Nanev

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. G. Bonchev Bl. 25, Sofia 1113, Bulgaria;

E-mail: m_gabrashanska@abv.bg

The development of orthopedic and dental implants has taken place for many years. For establishment whether a new biomaterial conforms to the requirements of biocompatibility and stability prior to clinical application, it must be test initially in vitro and in vivo. Animal models are essential for evaluating biocompatibility, tissue response and mechanical function of tested materials prior to clinical use after in vitro testing. They allow the evaluation of these materials in loaded /unloaded situations during long time durations and in different tissue qualities and ages. There are a lot of models for testing implant materials in vivo, ranging in purpose from the assessment of protein adsorption and soft tissue adherence to the integration of bone and the dissemination of implant wear particles. For testing the implants it is necessary to use a model which is reproducible and its dimensions are comparable to those used in humans. The number and size of the implant to be tested will influence the species of an animal chosen for a study. It is important that control implants are included in the study. These implants should be of a material already in clinical use. To draw accurate conclusion regarding the effects of implant modifications, one must determine the implant surface characteristics with regard to the chemical composition of the material and the surface topography. There are several factors for choosing the animal experimental model. They are: cost and care for animals, availability, acceptability to society, ease of housing, resistance to infection and disease, biological characteristics analogous to humans, tolerance to surgery, adequate facilities and staff and existing database of biological information for the species as well as suitable lifespan. In addition for understanding bone-implant interactions, such as bone microstructure and composition, bone modeling and remodeling properties are important.

Test samples and control material preparation are in compliance with ISO 10993- 12. Each material is manufactured, processed, cleaned of contaminants and sterilized by the method intended for the final product and this is confirmed in the study documentation. The test period is determined by the likely clinical exposure time or be continued until or beyond a steady state has been reached with respect to the biological response. Implantation in bone tissue may need longer observation periods before a steady state is reached.

Implantation surgery is performed under general anaesthesia (An and Friedman 1999).

Before the animal killing blood samples for biochemical studies are taken. The animals are euthanized and the implant together with sufficient unaffected surrounding tissue to enable evaluation of the local histopathological response is taken. For implants in bone the interface between the tissue and the material is of special interest. So that samples of soft and hard tissues are taken for biochemical study.

The following items are needed for bone implant evaluation (International Standard ISO 10993-6. 1994):

1. Description of test and control materials
2. Description of animals (species, sex, age, body weight)
3. Description of implantation – surgical procedure, anaesthesia, post-surgical analgesia, location and number of implants per animal
4. Description of retrieval technique
5. Description of implant evaluation – gross observation of implants, tissues and organs surrounding implants
6. Histological procedure –techniques for fixation and preparation of the histological sections, results of histological evaluation of implant site

7. Macroscopic and microscopic evaluation
8. Biochemical studies of the blood, hard and soft tissues- levels of Ca, P, Mg, Zn, enzymes and etc.
9. Comparative evaluation of the local effects after examination and statistical analysis in terms of the biological responses to test and control materials.

According the international standards the species suitable for testing biomaterials for bone implantation are dogs, sheep, goats, pigs, rabbits and rats. In general small laboratory animals such as mice, rats, hamsters or rabbits are preferred. But no single animal model will be appropriate for all purposes (Pearce et al 2007). Our studies are performed on a rat model modified by us.

For rats the test periods for long-term implantation are 12, 26 or 52 weeks. Male mature Wistar rats with an average body weight of 300 g are used. According our study the number of implants per a rat is 3. The defects are created in tibia or/and femur. Surgical cavities are 2 mm wide and 4 mm deep. The cavities are filled with test or control materials. At the end of the experimental period the animals are euthanized with an overdose of anaesthetic or by some other humane method. After that the above mentioned items are followed.

All animal studies perform in a facility approved by a national recognized organization and in accordance with all appropriate regulations dealing with laboratory animal welfare

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ВО3. ПРИЛОЖЕНИЕ НА ТЕХНОЛОГИИТЕ ЗА БЪРЗО ПРОТОТИПИРАНЕ В ТЪКАННОТО ИНЖЕНЕРСТВО: МОДЕЛ ЗА 3D ПРИНТИРАНЕ НА ОРГАНИ

Бойка Андонова-Лилова[1], Таня Живкова[1], Лора Дякова[2], Р. Александрова[1]

[1]Институт по експериментална морфология, патология и антропология с музей, БАН

[2]Институт по невробиология – БАН

ВР1. МЕЗЕНХИМНИ СТВОЛОВИ КЛЕТКИ – НОВА НАДЕЖДА ЗА ЛЕЧЕНИЕ НА КОСТНИ ДЕФЕКТИ

Радостина Александрова[1], Бойка Андонова-Лилова[1], Таня Живкова[1], Лора Дякова[2], Диана Рабаджиева[3], Стефка Тепавичарова[3]

[1]Институт по експериментална морфология, патология и антропология с музей- БАН;
[2]Институт по невробиология – БАН;
[3]Институт по обща и неорганична химия – БАН

Костно-ставните заболявания са сериозен здравен проблем, който силно влошава качеството на живот на страдащите. По данни на Световната здравна организация през 2020 г. процентът на хората с такива заболявания ще се удвои. Дегенеративните и възпалителните процеси в областта на ставите и костите, костните фрактури, болките в областта на гърба и кръста, остеопорозата, сколиозата и други мускулно-скелетни патологии в крайна сметка пораждат необходимостта от използване на различни (постоянни, временни, биоразградими) медицински устройства за възстановяване на нарушената функционална активност на опорно-двигателния апарат на пациентите. Въпреки че автогенните костни импланти са приети за златен стандарт при възстановяването на костни дефекти, а алогенните присадки са все още широко прилагани в медицинската практика, съществуващите ограничения и трудности при прилагането на тези два терапевтични подхода налагат необходимостта от разработването на биоматериали за костни заместители. През последните десетилетия бяха създадени редица материали (керамики, метали, биоактивни стъкла, полимери, композити и др.), които с различен успех намериха приложение в клиничната практика. Свързаните с използването им недостатъци обаче с все по-голяма острота поставят въпроса за създавеане на нови, усъвършенствани материали с добра биологична поносимост, остеокондуктивност и остеоиндуктивност, материали, чиито свойства се доближават в максимална степен до тези на функционално активната здрава кост и могат да бъдат успешно изпозвани за възстановяване на различни костни дефекти. Специален интерес през последните години предизвиква един нов подход – т.нар. тъканно инженерство, който съчетава прилагането на триизмерни матрици от биоматериали и култивирани в тях мезенхимни стволови клетки, при което се създава реална възможност за ефективно възстановяване на големи костни загуби. Изследванията в тази нова и обещаваща област са все още в съвсем ранен етап на лабораторни изпитания и in vivo тестове с експериментални животни. Съвместните интердисциплинарни проучвания на специалсти от различни области на човешкото познание, насочени към създаване на подходящи биоматериали и разработване на методи за манипулиране на мезенхимни стволови клетки, без съмнение ще допринесе за превръщането на тази многообещаваща възможност в клинична реалност.

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BP2. СТВОЛОВИ КЛЕТКИ

З. Димитрова

Session C. Parasitology

Chairpersons:

Assoc. Prof. Radostina Alexandrova, PhD

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

Assist. Prof. Delka Salkova, DVM, PhD

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

Secretary: Ivelin Vladov

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

CO1. REVIEW OF THE METHODS FOR CONTROL OF CHICKEN COCCIDIOSIS

D. Salkova

Institute of experimental morphology, pathology and anthropology with museum, Bulgarian Academy of Sciences Acad. G. Bontchev Str., Bl.25, 1113 Sofia, Bulgaria

E-mail: dsalkova@abv.bg

Chicken coccidiosis is an intestinal infection caused by the intracellular protozoan parasite of the genus Eimeria. Seven species have been recognized to infect chickens: *E. tenella, E. necatrix, E. acervulina, E. maxima, E. brunette, E. mitis* and *E. praecox.* It is the major parasite disease of poultry, with substantial economic burden estimated to cost the industry more than $ 800 million in annual losses worldwide (Williams, 1998).

The life cycle of *Eimeria* comprises intracellular, extracellular, asexual and sexual stages, so it is not surprising that host immunity is also complex and involves many facets of nonspecific and specific immunity (cellular and humoral immune mechanism).

The effective use of anticoccidial drugs over the past 50 years has played a major role in the growth of poultry industry and has allowed the increased availability of high quality, affordable poultry products to the consumer.

These anticoccidial can be classified as:

1.Chemicals which has specific modes of action against parasite metabolism, such as amprolium, clopidol decoquinate, halofuginone.

2.Polyether ionophore such as monensin, lasalocid, salinomycin, narasin, and maduramycin), which act throw general mechanisms of altering ion transport and disrupting osmotoic balance.

Vaccination

Avian coccidia are highly immunogenic, and primary infections can stimulate solid immunity to homologous challenges. Live vaccine for coccidiosis control have been used to a limited degree by the poultry industry for about 50 years. Three groups of live vaccines are used in control of disease:

1) Live, virulent strains

These vaccines comprise a varible number of wild type strains depending on their formulation and field of application. The advantage of live virulent vaccines is that they can provide equal or superior performance compared with prophylactic medication when given in gel form on day 1 of age because this method ensures the synchronous exposure of all birds to small uniform number of oocysts. The disadvantage of virulent vaccines is a risk of introducing unwanted *Eimeria* species in the environment.

2) Live, attenuated strains

This type of vaccines can be obtained by repeated selection for early maturation (precociousness) or serial passage through embryonated eggs. The advantage of live attenuated vaccines is that they have low productive potentials, thus avoiding crowding in the specific mucosal areas of infection and resulting in the development of optimal immunity with minimal tissue damage and there is no risk of introducing *Eimeria* species into the environment.

3) Live, tolerant to ionophores

There is a new development that made live *Eimeria* strains relatively tolerant to ionophores.

Recombinant vaccines

Due to emergence of drug-resistant *Eimeria* strains and high cost in manufacturing live vaccines much research has focused on recombinant vaccination strategies as potential alternative methods of disease control.

Alternative controls including nutritional and probiotics (immunomodulators) or natural-feed additives

Natural medicinal products as feed supplements have been widely used as growth and health promotors in farm animals in China. The immunoactive components of these plants and fungi include polysaccharides, glycosides, alkaloids, volatile oils, and organic acids, of which polysaccharides are considered to be the most important (Li, 2000).

Mushroom and herb polysaccharides

The polysaccharide extracts from 2 mushrooms, *Lentinus edodes* (LenE) and *Tremella fuciformis* (TreE), and a herb, *Astragalus membranaceus* (AstE) when given as supplement feed to chickens resulted in a significant impact on the inductive responses against *E. Tenella* infection in chickens by enhancing both cellular and humoral immunity.

Sources of fats

Sources of fats containing high concentrations of n-3 fatty acids (n-3 FA)(docosahexaenoic acid, eicosapentaenoic acid, and linolenic acid), such as fish oils, flaxseed oil, and whole flaxseed, when added to starter rations and fed to chicks from 1 day of age, effectively reduced lesions resulting from challenge infections with *E. Tenella*, but not *E. maxima* (Allen et al. 1997).

Herbs

Artemisinin is a Chinese herb isolated from *Artemisia annua*; it is a naturally occurring endoperoxide with antimalarial properties. It has been found effective in reducing oocyst output from both *E. acervulina* and *E. tenella* infections when fed at levels of 8.5 and 17 ppm in starter diets (Allen et al. 1997). The mode of action is also thought to involve oxidative stress.

Vitamin A

It is known that nutrition plays a significant role in the development of the chicken immune System. Essential nutrients such as vitamins may affect both humoral and cell-mediated immune responses

Betaine

Betaine is another dietary supplement, a naturally occurring amino acid derivative, that has been investigated as potential enhancing agent against coccidiosis.

Probiotics enhance gut defensive mechanisms

It is known that the gut microflora constitutes an important component of these first lines of defence in both humans and animals. Probiotic supplementation of the intestinal microflora has been shown to enhance gut defensive mechanisms in poultry (La Ragione et al. 2004).

CpG oligodeoxynucleotides (ODNs)

The short ODNs containig unmethylated CpG motifs have been shown to be effective immunoprotective agents in mammalian models by inducing both innate and adaptive immune responses (Krieg, 2002).

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CO2. COMPARATIVE ELECTROPHORETIC STUDIES OF PROTEIN EXTRACTS OF SIX TRICHINELLA ISOLATES BY SDS-PAAE AND PAAE

Valeria Dilcheva, Svetlozara Petkova, Evgeni Gabev

Institute of experimental morphology, pathology and anthropology with museum, Bulgarian Academy of Sciences Acad. G. Bontchev Str., Bl.25, 1113 Sofia, Bulgaria

Comparative electrophoretical studies on protein exstracts from six Trichinella isolates have been carried out with the purpose of obtaining novel data about their speciescharacterization. The methods of PAAE and SDS-PAAE have been used.The electrophoretical analysis of the water-soluble proteins of the six isolates: ISS03, ISS13, ISS02, ISS10, ISS029, ISS035 hasyielded as a result a heterogeneity both of the number and the electrophoretical mobility of the individual protein fractions. On the basis of the obtained results as well as on the data from studies of some authors [1,2,3]. The six isolates under study

belong to the species T. spiralis, T. pseudospiralis, T. nativa, T. britovi, T. nelsoni, T. murrelli.

The electrophoretical investigations in gels and the widely used synthetic polymers among which the PAA gel is the one with greatest applications have proven especially prospective. According to some authors the method of electrophoresis in PAA gel is a reliable biochemical method by which differences between the helminthes species can be registered and it is recommended as a diagnostics test especially in the species of a disputable individuality and those parasitizing in varying hosts.

The aim of the present study is to conduct comparative electrophoretical studies on protein extracts from six Trichinella isolates ISS03, ISS13, ISS02, ISS10, ISS029, ISS035 and to obtain a maximum number of protein fractions as well as the typical ones for each isolate.

The thin-layer PAAE was carried out on an apparatus for vertical electrophoresis with three electrophoretical investigations being performed for each of the six Trichinella isolates. The phoregrams of the six Trichinella isolates showed a total reproducibility of the results from the separation of the water-soluble proteins.

The electrophoretical analysis of the water-soluble proteins of the six Trichinella isolates has established aheterogeneity both of the number and the electrophoretical mobility of the separate protein fractions in the presumed zones: cathode, central and anode ones.

Based on the differences established in the number of the protein components of each of the six isolates under study (a different number of the polypeptide fraction, and their different electrophoretical mobility) as well as the studies of Pozio at al.[4] we can assume that the water-soluble proteins of the isolates ISS03, ISS13, ISS02, ISS10, ISS029, ISS035 belong to the species T.spiralis, T. pseudospiralis, T. nativa, T. britovi, T. nelsoni and T. murrelli and contain both shared protein components and also unique ones for the separate isolates which can be successfully utilized for the determining of different strains and isolates upon observing the same conditions: 10,5 % PAA gel and length of the track of the electrophoresis sample of 10 cm.

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CO3. EXPERIMENTAL MODELS OF FASCIOLOSIS AND CARCINOGENESIS

N. Tsocheva-Gaytandzhieva[1], R. Toshkova[1], I. Roeva[2], M. Topashka-Ancheva[3], A. Filchev[4], D. Salkova[1]

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 25, 1113 Sofia, Bulgaria;*
[2]*Institute of Microbiology, BAS, Sofia;*
[3]*Institute of Biodiversity and Ecosystem Research, BAS, Sofia;*
[4]*University of Forestry, Faculty of Veterinary Medicine, Sofia;*
E-mail: tsocheva_n@abv.bg

A new property of the mature trematode *Fasciola hepatica* was established in previous investigations of ours – its cell growth inhibiting effect on the development of experimental liver carcinogenesis with diethylnitrosamine (DENA) (Tsocheva, 1986). This formed the basis for a hypothesis on the possible roles of some biologically active substances, inhibitors of cell proliferation, of parasite or host origin, in the pathogenesis of the interaction fascioliasis - carcinogenesis.

Later, biologically active substances (BASes) were isolated from the tissues of *Fasciola hepatica* and from *F. hepatica* infected rat liver and their inhibiting effect on the proliferation of primary hepatocyte and lymphocyte cell cultures was established (Tsocheva et al., 1991; 1992).

The aim of the present study is to investigate the antitumor effect of mature *F. hepatica* and the newly isolated BASes on DNA synthesis *in vivo* on tumor bearing mice and *in vitro* on tumor cell cultures.

The effect of *F. hepatica* on cell proliferation is investigated under simultaneously or continuous application of experimental fascioliasis and solid hepatoma-22 in C_3H mice.

Wistar rats are orally infected with 15 metacercariae of *F. hepatica* for obtaining mature parasites 4 months later. The mature liver flukes and livers from the infected animals and from the bred under the same conditions healthy Wistar rats are processed to obtain the BASes. Thermostabile BASes are isolated from the liver of healthy and *F. hepatica* infected rats by modified method of Bardos et al. (1968). Thermolabile BASes are isolated by modified method of Verly et al. (1971) from the liver of healthy and *F. hepatica* infected rats and mature *F. hepatica* tissues using the fraction with a final concentration of ethanol between 70% and 87% (v/v).

The effects of thermostabile BASes are investigated *in vivo* on ascite leyomiosarcoma bearing BALB/c mice by calculating thymidine and mitotic tumor indexes. The effects of thermolabile BASes are investigated *in vitro* on hepatoma MC29 and myeloma cell cultures cell proliferation by [^{3}H]-Thymidine test. The results are statistically processed after variation analysis and the Student t-test.

Hepatoma-22 growth is inhibited *in vivo* at the background of chronic fascioliasis and the inhibiting effect is confirmed by the differences in the size or the lack of the tumors in *F. hepatica* infected C_3H mice under different experimental conditions.

Tumor growth inhibiting effects of all the newly isolated BASes (thermostabile and thermolabile) are established *in vivo* and *in vitro* in different degrees:

In vivo antitumor effect of the thermostabile BASes isolated from *F. hepatica* infected rat liver is established on ascite leiomiosarcoma JSM in BALB/c mice. The cell growth inhibiting effect of the BAS isolated from *F. hepatica* infected host liver is stronger than the effect of BAS isolated from healthy rat liver. The three-fold treatment with BAS isolated from

F. hepatica infected rat liver causes strong decreasing of thymidine index and mitotic index of the sarcoma cells and significantly decreasing of the tumor volume compared to the control.

The newly isolated thermolabile BASes have a strong inhibiting effect on DNA synthesis in hepatoma MC29 cell culture. The cell growth inhibiting effect of the BAS isolated from *F. hepatica* infected host liver is stronger than the effect of BAS from healthy rat liver. The strongest cell growth inhibiting effect is manifested by BAS isolated from *F. hepatica* tissues. Treatment of myeloma cell culture with the BASes does not cause a marked reduction of ^{3}H-Thymidine incorporation into the cells compared to the controls.

Tissue specific and species non-specific activities of the BASes are found out: Tissue specific activity is established for the thermolabile BASes isolated from normal and *F. hepatica* infected rat liver. They both inhibit the DNA-synthesis in hepatoma MC29 cells but do not affect it in myeloma cells. Species non-specificity of the newly isolated thermolabile BASes is established: Cell cultures from chicken and hamster tissues are influenced from the BASes isolated from rat liver tissue and from the tissues of lower animal – parasitic warm. The BAS isolated from *F. hepatica* tissues shows tissue non-specific activity. It has an inhibiting effect on the proliferation of various mammalian cells but it shows selective inhibiting effect on the tumor cells investigated: slight effect on myeloma cells and strong inhibiting effect on proliferation of hepatoma MC29 cells.

According to the results obtained from the present and previous our investigations, we suggest that the mature helminth *F. hepatica* consists of/or contains endogenous inhibitors of cell proliferation, similar to mammalians' with which it acts upon the host and uses them as a mean of autodefence during its parasitic way of life. Also, the liver cells metabolism in *F. hepatica*-infected host is changed as a result of the parasite migration through the liver tissue. Thus, either an activation of endogenous production of inhibitors of cell proliferation is induced or the permeability of some substances through the liver cell membranes is increased.

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CO4. THE DEADLY TROPICAL TRIANGLE

Dzhem Farandzha
Medical Faculty, Sofia University "St. Kliment Ohridski"

Burkitt's lymphoma is the most common childhood cancer in central Africa where it accounts for 50%-75% of all malignancies in children. Most patients have jaw deformations because of a large tumor with corresponding localization. The disease was first described in the 1950s by a surgeon named Denis Parsons Burkitt who served in the region where the disease is still most prevalent, and is also endemic for malaria. The involvement of some infectious agent was initially suggested, but research now indicates a more complex etiology with multiple factors present.

Burkitt's lymphoma is the most rapidly growing human tumor, occurring primarily in children. It presents with a monoclonal proliferation of B cells. Three types are present: endemic (African), sporadic (non-African), and immunodeficiency-related form. Although the non-African form is not as common as the endemic type, Burkitt's lymphoma constitutes 30% of all childhood lymphomas in the United States. Enlargement of the jaw or facial bones is a characteristic trait of the African form. This form is associated with Epstein-Barr virus in 95% of the cases. It is found in two main regions: 1. in central equatorial Africa, the region between 10^{o}N and 15^{o}S, where the tumor is common in areas with low elevation and high humidity, with average temperatures above 26^{o}C; 2. in New Guinea. It is three times more common in males, with maximum frequency in 4-7 year olds, which decreases as the land elevation increases. Since malaria is also widespread in these regions, it has been hypothesized that malaria activates the oncogenic properties of the Epstein-Barr virus: Plasmodium species stimulate the mononuclear phagocyte system, thus enhancing the immune response of the host and the population of B lymphocytes. This makes the cells more prone to abnormal change by EBV.

The human herpesvirus type 4 (HHV-4), more commonly known as the Epstein-Barr virus (EBV), is a virus of the Herpesviridae family which affects the lymphocytes and epithelial cells in humans. The first site of replication is the nasopharynx, after which the B cells are infected. An EBV infection is usually more common and asymptomatic in children, with a higher frequency of shedding from the oropharynx in patients with immunodeficiency (patients treated with immunosuppressants, HIV-positive individuals, etc.). Infants become susceptible as soon as maternal antibody protection disappears. EBV infects 50% of children before age 5. Transmission usually occurs by kissing, but is also possible by blood transfusion. Early childhood transmission is more common among lower socioeconomic groups; in developed countries most people do not become infected until later in their lifetime. Adolescents and adults develop more distinguishable symptoms – in such cases, EBV causes infectious mononucleosis 35% to 50% of the time. The Epstein-Barr virus has been implicated in several cancers, specifically Burkitt's lymphoma, Hodgkin's lymphoma, and nasopharyngeal carcinoma. It causes an abnormal growth in the infected B cells, which can turn into cancer cells.

Another aspect in the etiology of such lymphomas is that viruses act together with genetic mutations to produce the disease. In Burkitt's lymphoma the cellular oncogene Myc (c-Myc) from the long arm of the eighth chromosome is translocated to a new active enhancer in the long arm of the fourteenth chromosome – t(8;14), starting the synthesis of carcinogenic

proteins. Another such protein is the Epstein-Barr nuclear antigen 1 (EBNA-1) which is expressed by EBV and found in Burkitt's lymphoma. This protein is required for viral DNA replication. An experiment conducted with an EBNA-1 derivative, which inhibits the original one by binding to its site on the EBV DNA, shows that the derivative prevents the EBV infected cells from proliferating and induces apoptosis, hence showing the important role EBNA-1 plays in developing Burkitt's lymphoma. It is not, however, the sole cause for the disease.

Today, Burkitt's lymphoma is considered a tumor whose onset and progression depend on several factors. A possible three-step pathway eventually leading to carcinogenesis would initially start with an Epstein-Barr virus infection, leading to lymphocytic proliferation, which would be further boosted in the second step by the Plasmodium infection. This may also activate the latent form of the virus and increase its shedding. All these processes make the B cells predisposed to chromosomal translocation – the third step, as a result of which the c-Myc gene is placed in a new active site of DNA, leading to uncontrolled cell division.

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CO5. МАЛАРИЯТА – ВЧЕРА, ДНЕС... А УТРЕ?

Ивона Пламенова Иванова

Медицински факултет, СУ „Св. К. Охридски", София, България

CO6. КАКВО (НЕ) ЗНАЕМ ЗА ТЕНИИ

Венсан Велчев

Медицински факултет, СУ „Св. К. Охридски", София, България

СО7. СТРАННИ ПАРАЗИТИ

Али Хади Зрейк

Медицински факултет, СУ „Св. К. Охридски”, София, България

CP1. PHARMACO-TOXICOLOGICAL METHODS FOR TESTING ON SOME ANTIHELMINTICS

E. Arnaudova, M. Gabrashanska, D. Salkova, V. Nanev, N. Tzocheva-Gaitandhjieva, D. Hrusanov, I. Vladov, B. Arnaudova, B. Georgiev*

Institute of Experimental Morphology, Pathology and Anthropology with museum (IEMPAM)
**University of Forestry*
E-mail: elvirabg@hotmail.com

In our in vitro studies we have been testing (including comparatively) Earthworms (as a model) and nematodes (ascaris of pigs and poultry), which were clinically extracted, and have been setting them on Engelman's shift in temperature-controlled physiological fluid and recording their spontaneous or toxic induced movements (from convulsions to death) recorded at kymograph.

For the pharmacodynamic analysis arecoline hydrobromide and sulfate atropinic - were used. [1][our unpublished data - N. Donev and B. Georgiev]. For trematodes this method was difficult to apply (partially for the *Fasciola hepatica).* For paramfistomi the tracking of the impact of the antitrematods by registering toxic changes on the breathing of the paramfistomite until death from trematodocid was a better method. It turned out that paramfistomum are still alive (breath) after 24 - 48 h, placed in a new environment.[2]. Interestingly, only after an approximate study was conducted (N. Donev and B. Gr. Georgiev - unpublished data) with a bathtub, designed by Professor D. Daskalov, it concludes that we can register the waves of the liquid in the bathtub under the influence of the movement of parasites, including dynamically and aggregated. From our experience the last two methods are the most physiologically realistic and credible.

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24 April 2012 (Tuesday)

Session D. Molecular Biology, Biophysics, Biochemistry

Chairpersons:

Assoc. Prof. Georgi Miloshev, PhD
Institute of Molecular Biology, Bulgarian Academy of Sciences

Assist. Prof. Roumiana Todorova, PhD
Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences

Secretary: Zina Ivanova
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

DO1.ФИЗИКА НА ЖИВАТА МАТЕРИЯ

Александър Г. Петров

ИФТТ-БАН, София 1784

Живата материя е мека материя. Меката материя е вид кондензирано състояние на веществото, което е частично подредено и междинно на твърдите тела и течностите. Живата материя е състояние на материята, което се самоорганизира, самоподдържа се и се самовъзпроизвежда. Характерните особености на живата материя следва да бъдат описани и разбрани на нивото на молекулната й организация и междумолекулните взаимодействия в нея.

В настоящата лекция ще бъдат разгледани следните въпроси:

- Течнокристален тип мека материя. Лиотропни течни кристали.
- Основни типове биомолекули. Хидрофобен ефект.
- Живата материя като мека материя: течнокристален нанокомпозит.
- Теоретично описание на живата материя: модел на обобщената молекулна асиметрия.

DO2. DOES THE BUDDING YEAST LINKER HISTONE Hho1p INFLUENCE SURVIVAL AND MORPHOLOGY OF CHRONOLOGICALLY AGING CELLS?

K. Uzunova, M. Georgieva and G. Miloshev

Yeast Molecular Genetics Laboratory, Institute of Molecular Biology "Acad. R. Tsanev", Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

e-mail for correspondence: miloshev@bio21.bas.bg

Over the last decades the budding yeast *Saccharomyces cerevisiae* has been proved as a model for studying of aging. Its ease of genetic manipulation, well annotated genome, and short generation time were the usual reasons in using these cells for detailed investigation of the process. Two different types of aging have been discovered in yeast - replicative and chronological both used as useful models for studying of dividing and non-dividing post mitotic eukaryotic cells, respectively.

The linker histone of yeast cells, Hho1p is one of the key players in chromatin organization and its knock-out leads to altered higher-order chromatin structure. We followed survival of the mutant cells in a time course of 20 days. Cellular morphology and survival of the mutant has been accordingly checked during the time course of the experiment and compared with the wild type progenitor cells. Some intriguing morphological and physiological features of yeast *hho1Δ* mutants were found. Mutant cells were bigger in size and possessed abnormally enlarged vacuole in comparison with the wild type cells. Taken together our results lead to the noteworthy conclusion that the yeast linker histone has a significant role in cellular aging.

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DO3. ANTIOXIDANT EFFECTS OF BULGARIAN PROPOLIS AND ITS COMPONENTS CAPE AND CHRYSIN ON Ty1 TRANSPOSITION IN YEAST *SACCHAROMYCES CEREVISIAE*

M. Georgieva[1], O. Krastanova[1], V. Bankova[2], M. Pesheva[1]

[1]Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of Genetics, 8 Dragan Tsankov Blvd, Sofia, Bulgaria
[2]Institute of Organic Chemistry with Centre of Phytochemistry, Sofia, Bulgaria

E-mail: mdimitrov83@gmail.com, mpesheva2000@yahoo.com

We examined the antioxidant effects of Bulgarian propolis and two of its components, CAPE and chrysin, by reducing Ty1 transposition frequency and ROS levels induced by different agents.

Retrotransposons are a class of genetic mobile elements that replicate through an RNA intermediate. Five families of retrotransposons (*Ty1–Ty5*) have been identified in the genome of the yeast *Saccharomyces cerevisiae*, *Ty1* being the most abundant. *Ty1* is transposed spontaneously with a frequency rate of about $1*10^{-7}$element/generation. Certain stress conditions, chemical mutagens and carcinogens cause an increase in retrotransposition frequency.

Many activators of *Ty1* mobility are powerful inducers of oxidative stress. Reactive oxygen species (ROS) play a key role in many physiological and pathogenetic processes. ROS are generated through both endogenous and exogenous routes. H_2O_2, O_2^- and OH· are the best-known ROS. At increased levels, however, ROS can not be detoxified sufficiently and may have different deleterious effects on cells, cause a variety of DNA lesions.

It was found recently that increased ROS levels and Ty1 transposition could be reduced by antioxidants.

Propolis is a beekeeping resinous and complex product, collected and transformed by honey bees, *Apis mellifera*. Different propolis types can present diverse chemical and pharmacological properties including antimicrobial, antifungal, antiviral, immunomodulating effects, hepatoprotective and anti-inflammatory properties. Propolis is the most powerful antioxidant bee product. This effect is mainly due to the high concentration of phenolics.

Caffeic Acid Phenethyl Ester (CAPE), lipophilic derivatives of caffeic acid and a phenolic antioxidant, is one of the most active components extracted from propolis. Chrysin is

a naturally occurring flavone chemically extracted from the blue passion flower (*Passiflora caerulea*), different herbs and propolis. CAPE and chrysin exhibit different pharmacological properties such as anti-inflammatory, anti-mitogenic, antibacterial, anti-carcinogenic, antiviral and immuno-modulatory properties.

It is provide evidence for a dependence of carcinogen–induced *Ty1* retrotransposition on increased production of ROS that instigated us to test propolis, CAPE and chrysin for their ROS scavenging activities by reducing the frequency of *Ty1* retrotransposition in yeast *S. cerevisiae*.

We used MMS, Cr VI and H_2O_2 as inducers of *Ty1* mobility and O_2^- formation. Each successful transposition event of the marked *Ty1* strain 551 requires transcription, splicing the artificial *AI* intron, reverse transcription and insertion of the resulting *Ty1* cDNA into a new location in the genome, which gives rise to one histidine prototrophic colony on SC-histidine medium. Thus, the number of His^+ transformants is a quantitative measure for the frequency of retrotransposition of the marked *Ty1*.

We used an assay for superoxide anions (O_2^-) determination adapted to S. cerevisiae cells. The assay is based on the reduction of tetrazolium dye XTT and allows determination of the quantity of O_2^- per live cell.

We tested different concentrations of propolis, CAPE and chrysin to determine their antioxidant effects. It was established that all three tested substances showed concentration dependence of antioxidant activity. Propolis, CAPE and chrysin decreased ROS levels and Ty1 transposition induced by all three agents, MMS, Cr VI and H_2O_2. We established that antioxidant activity decreases in order as follows CAPE > chrysin > propolis. We also established that CAPE is 12 times more active than propolis and 4 times than chrysin. Chrysin is 2 times more active than propolis.

Acknowledgements: This research was supported by SU Research Found, grant 84 given to assoc. prof. M Pesheva.

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DO4. DETERMINATION OF ANTIOXIDANT ACTIVITY OF HONEYBEE PRODUCTS (HONEY AND ROYAL JELLY) FROM BULGARIAN ORIGIN, BY A NEW TEST

Dimitrov M., Pesheva M.

University of Sofia "St. K. Ohridski", Faculty of Biology, Department of Genetics

Reactive Oxygen Species (ROS) are induced by laboratory carcinogens CrVI and MMS (methylmethansulphonate). All experiments include several variants: negative control with DMSO (used as a solvent of royal jelly (RJ)) or water (as a solvent of honey), positive control with CrVI or MMS (as ROS inductors). The experimental variants, which number were determined by the quantity of antioxidant (honey or RJ), used in antiROS determination.

Cell culture of each variant was divided into two parts. One part for measure of ROS levels, other for determine Ty1 transposition frequency in Saccharomyces cerevisiae.

Right-proportional dependence is proven between ROS levels measured after treatment with CrVI or MMS and Ty1 transposition frequency (double increased ROS levels, lead to double increased Ty1 transposition frequency).

We determined that 50% decreasing of ROS levels is reached with 250μg/ml honey by our cell-based assay. Presented results are illustrated by logarithmic dependence with two ordinates. When the concentration of honey is increased, Ty1 transposition frequency and ROS levels are decreased and the points described right-line dependence. It is calculated 50% inhibition compared to negative control without honey by this way.

After literature reference, we suppose that our method is different to others. The dependence between ROS levels, to the concentration of antioxidant is exponential in metric coordinate system. This dependence in the same system is linear obtained by other antiROS tests.

All other tests are criticized, about determination of antiROS-activity in nonphysiological conditions (out of cell) and present a chemical reaction between ROS and antioxidant, where indicate linear dependence. These chemical reactions determine all ROS in the cell independently they are physiology active or not.

We measure anti-ROS by contribution of ROS in physiological process in one living cell, not by chemical reaction. Increased ROS levels increase Ty1 transposition process in S. cerevisiae. Therefore, our test is the one, measuring physiological active ROS till now.

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DO5. ESTIMATION OF THE INTRINSIC STRUCTURAL DISORDER OF NATIVE EWS AND ITS REPORTED FUSION ONCOGENIC PROTEINS WITH ANALYSIS OF THE FUNCTIONAL REGIONS

Roumiana Todorova

IBFBMI-BAS, Acad G Bonchev Str, Bl 21, 1113 Sofia, Bulgaria,

E-mail: todorova@bio21.bas.bg

Ewing's Sarcoma Oncogene (EWS) on chromosome 22q12 is encoding a RNA binding protein that is target of tumor-specific chromosomal translocations in Ewing sarcoma tumor, Myxoid liposarcoma, Malignant melanoma of soft parts, Desmoplastic small round cell

tumor, Peripheral neurectodermal tumour, Angiomatoid fibrous histiocytoma, Extra-skeletal myxoid chondrosarcomas, Rhabdomyosarcoma, Locally destructive tumour, Myoepithelioma tumours of soft tissue, Hidradenoma or eccrine acrospiroma, Mucoepidermoid carcinoma, Neuroblastoma, Olfactory neuroblastoma, Solid pseudopapillary tumour of the pancreas and Acute myeloid leukemia. Around 85% of Ewing tumours carry the EWSR1/FLI-1 fusion.

An attempt was made to estimate the Intrinsic structural disorder (ISD) of EWS and its reported fusion oncogenic proteins by different methods of prediction. On the bases of the prediction results, an analysis was made of the EWS sequence and its functional regions with increased ISD that could be used to design antitumor agents against the corresponding malignances. Small molecules that disable EWS-FLI1 function with minimal toxicity could potentially provide a therapy for patients with ESFT and other related sarcomas.

Comparing, the EWS oncogenic fusions show similar ID in the NTD (AA 1-264, EAD). The CTD disorder of fusions differs from that of native protein and between the fusion proteins. A strong relationship was found between the structure and estimated ID of EWS and its oncogenic fusions. Finally, the oncogenic function is related to a decreased IPD in the CTD, due to the fused partner, a TF. This is consistent with the finding that the particular AA composition of the EAD creates an enabling structure with several critical Tyr residues dispersed in a polar/neutral environment, favoring hydrogen bonding interactions and flexibility.

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DO6. LUNG MATURITY ASSESSMENT BY *IN VITRO* ANALYSES OF GASTRIC ASPIRATES FROM NEWBORN INFANTS

Maya Bangyozova[1], Albena Jordanova[2], Asya Tsanova[3], Jordan Doumanov[1], Emilia Christova[4], Zdravko Lalchev[1]

[1] Faculty of Biology, St. Kl. Ohridski University of Sofia, Sofia, Bulgaria

[2] Institute of Biophysics and biomedical engineering, Bulgarian Academy of Sciences, Sofia

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

[4] Faculty of Public Health, Medical University of Sofia, Sofia, Bulgaria

E-mail: maya_bangyozova@mail.bg

Alveolar surfactant (AS) is a lipoprotein complex that covers alveoli at the air/water interface in the lung. The main function of alveolar surfactant *in vivo* is decreasing surface tension (γ, mN/m) during breathing, i.e. ensuring alveolar stability. Deficiency in AS, mainly in prematurely newborn infants, leads to different disorders the most common of which is neonatal respiratory distress syndrome (NRDS). The clinical therapy currently includes the application of various native or synthetic exogenous surfactant preparations which substitute human AS. Analysis of the composition and the properties of the alveolar surfactant are crucial for the assessment of lung maturity in premature newborns and may prove the need of surfactant therapy application.

The aim of the present study was to estimate the lung surfactant maturity by analysis of gastric aspirates from prematurely born and full term infants. In this regard we investigated the surface properties of gastric aspirates (GA) from 41 babies: 3 with NRDS, 15 prematurely born and 23 normally born and healthy. A biochemical analysis of the protein and lipid content in GA from the groups tested were made. Individual phospholipid components in GA samples from prematurely born and full term children were detected by thin-layer chromatography. In addition, surface characteristics (equilibrium, maximal and minimal surface tension values, γ, mN/m) were determined by using the pending drop method.

Our results showed an increase in the phospholipid and the protein concentrations in GA during pregnancy progress as well as significant differences in the individual phospholipid profiles of the aspirates from prematurely born and full term children.

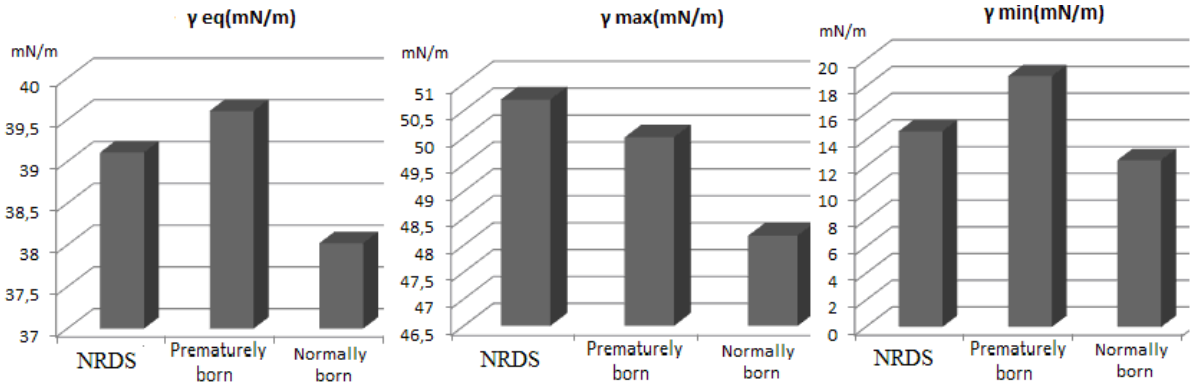


Fig. 1. Values of the surface characteristics ($\gamma_{equilibrium}$, γ_{max} and γ_{min}) of the gastric aspirates from newborn infants

In the case of surface characteristics a highest difference was observed for the minimal surface tension values (γ_{min}, m/Nm); while in the full term children a lower surface tension was determined, in the prematurely born children significant higher values were reached, which define γ_{min} as the most informative from the parameters studied (Fig.1).

Our results could find application into the clinical practice for fast surfactant maturity diagnostics in prematurely born children regarding lifesaving therapy with exogenous surfactants administration.

Acknowledgements: This work is supported by Bulgarian Science Fund, Ministry of Education and Science, grant DO 02 107/08.

DO7. ВЪЗСТАНОВЯВАНЕ НА РАНА – ФИЗИОЛОГИЯ И МОЛЕКУЛЯРНИ МЕХАНИЗМИ НА РЕГУЛАЦИЯ

Александър Тасев

Биологически факултет „СУ. Св. Климент Охридски”, Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

DP1. СЪЩНОСТ НА РЕКОМБИНАНТНИТЕ ВАКСИНИ

Б. Трайкова

Биологически факултет, СУ.” Св. Климент Охридски”, София, България

DP2. ГМО – ЗАЩО НЕ?

Г. Донкова

Биологически факултет, СУ.” Св. Климент Охридски”, София, България

DP3. ХИПОТЕЗИ И ТЕОРИИ ЗА ПРОИЗХОДА НА ЖИВОТА

Амар Фесток

Медицински факултет, СУ.” Св. Климент Охридски”, София, България

Session E. Virology

Chairpersons:

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

Assist. Prof. Petia Genova, PhD
*Department of Virology, National Centre of Infectious and Parasitic Diseases***,**

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

EO1. HIV-1 POL GENE SEQUENCING AND PHYLOGENETIC ANALYSIS OF THE HIV-1 EPIDEMIC IN BULGARIA REVIEWED PRESENCE OF A BOUQUET OF VARIOUS SUBTYPES AND COMPLEX RECOMBINANT FORMS

Ivailo Alexiev[1], D. Beshkov[1], V. Georgieva[1], L. Karamacheva[1], I. Elenkov[2]

[1]National HIV Confirmatory Laboratory, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria.
[2]Hospital of Infectious Diseases, Sofia, Bulgaria

Corresponding author: ivoalexiev@yahoo.com

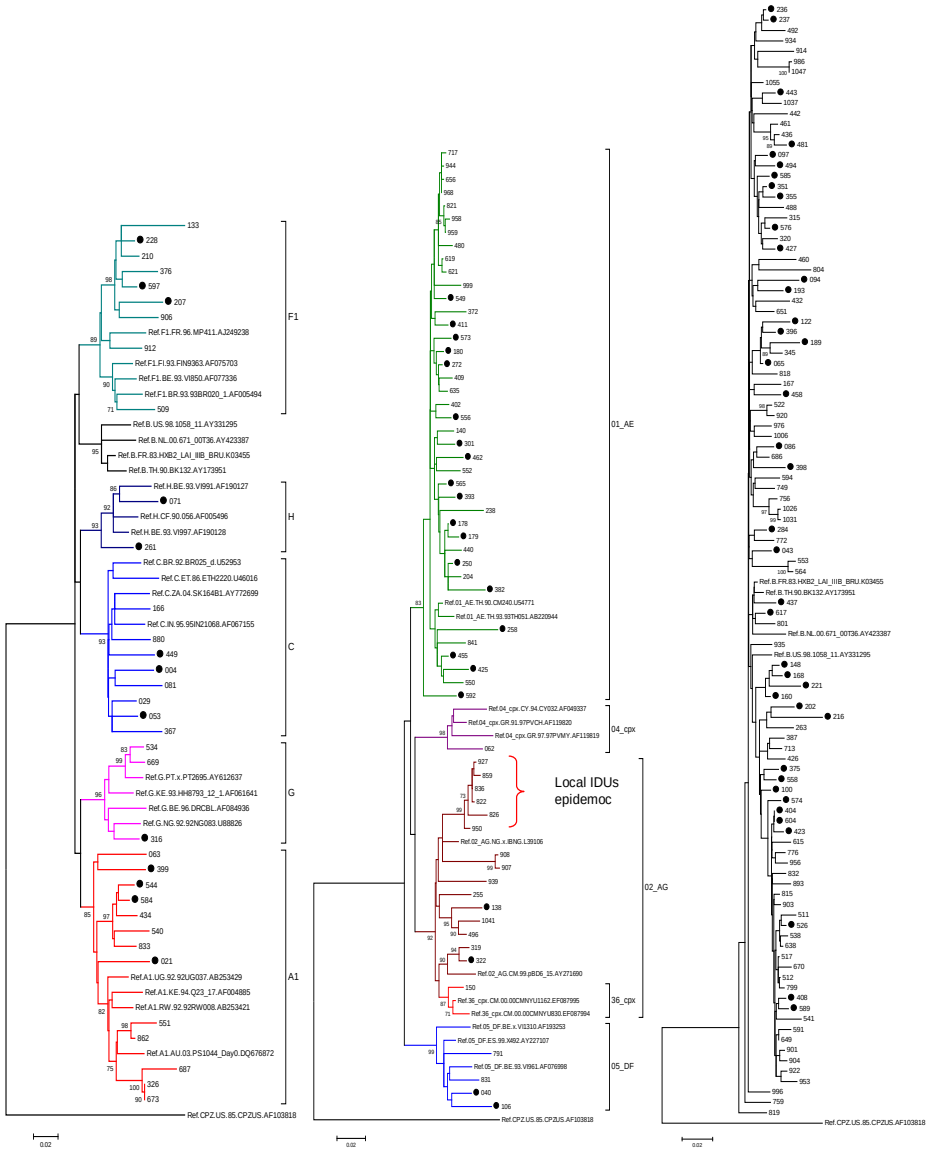
Background

The aim of the present study was to determine HIV-1 genetic diversity among different risk groups in Bulgaria, by sequencing and phylogenetic characterization of 202 (18.36%) plasma samples from 1100 cumulative seropositive individuals diagnosed within 1986-2009. Our study showed the presence and spread six different major HIV-1 subtypes and eight different circulating or unique recombinant forms.

Materials and Methods

We successfully performed HIV-1 genotyping on 202 out of 1100 cumulative seropositive individuals diagnosed within 1986-2009. Sequencing and genotyping of the Protease (PR) and Reverse Transcriptase (RT) of HIV-1 pol gene was performed with Applied Biosystems Viroseq HIV-1 Genotyping System (Abbott Wiesbaden Germany) and/or TruGene DNA Sequencing System, OpenGene, Visible Genetics, Siemens. Bulgarian sequences, referent sequences from Los Alamos and the most similar GenBank sequences for each subtype and CRF were aligned using Bioedit. A maximum likelihood (ML) tree was then inferred with MEGA5.0 software. A probabilistic approach for detection of genomic recombinations in HIV-1 using jpHMM (jumping profile Hidden Markov Model) by jpHMM web server at GOBICS was performed.

Figure 1 (A, B and C)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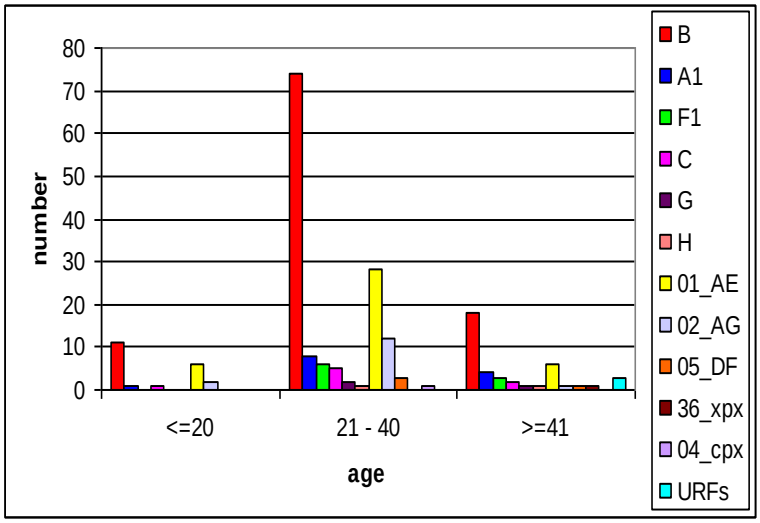
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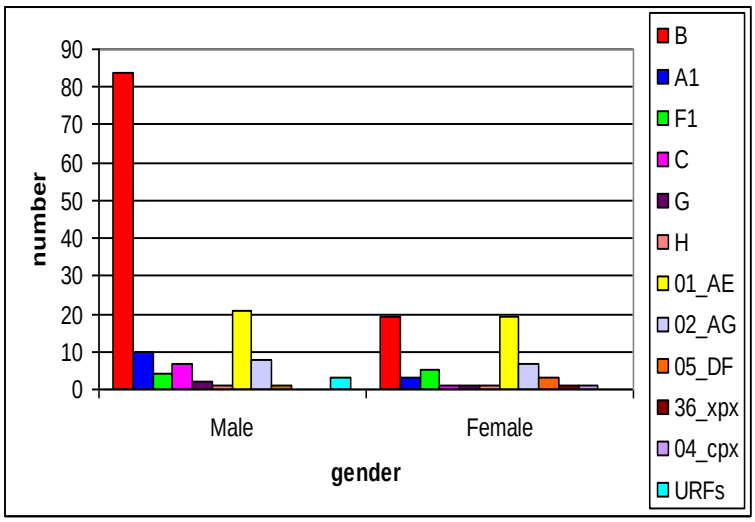
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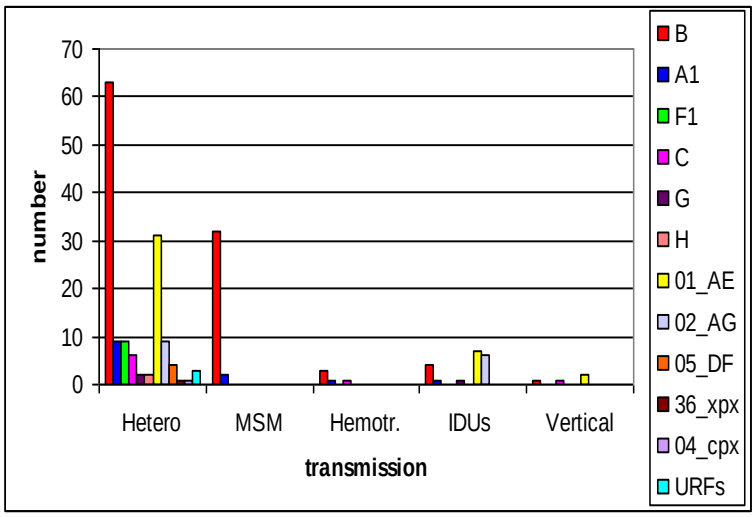
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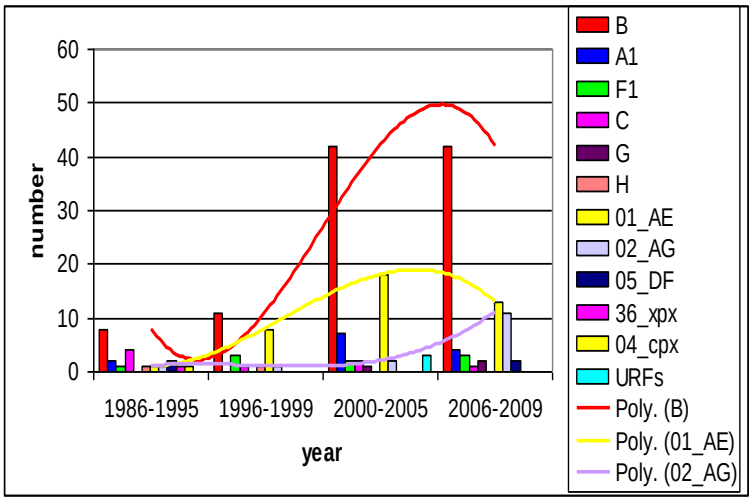

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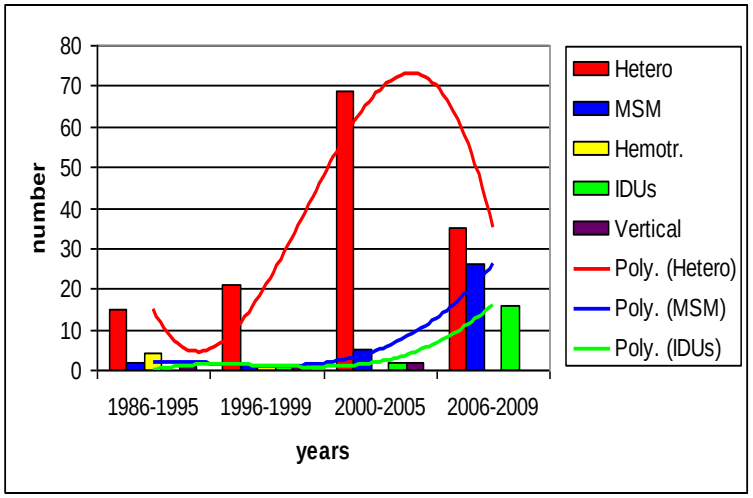

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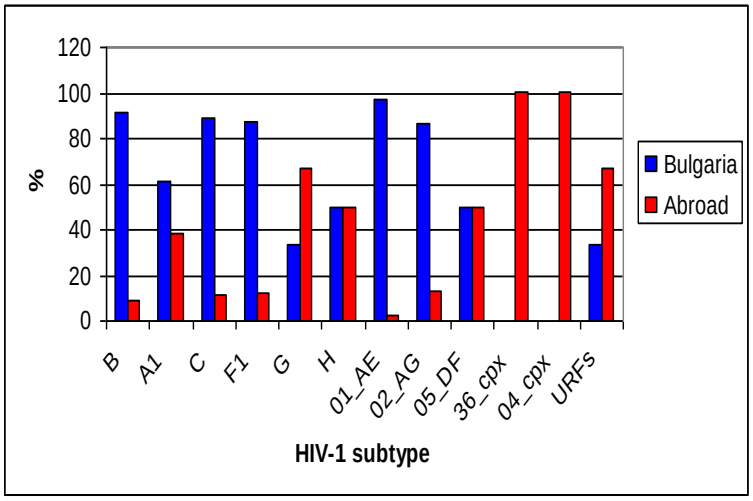

Figure 2 (A, B, C, D, E and F)

Figure 1. Inferred phylogenetic trees of Bulgarian HIV-1 polymerase (*pol*) sequences: (A) *pol* sequences of "pure subtypes A1, C, F1, G and H"; (B) *pol* sequences of CRFs; (C) *pol* sequences of subtype B only. Black dots indicate sequences from our previous study (40).

Figure 2. Characterization of trends of HIV-1 subtypes and epidemiological characteristics in Bulgaria. (A) prevalence of different HIV-1 genotypes by age group; (B) prevalence of different HIV-1 genotypes by gender; (C) distribution of HIV-1 genotypes among infected individuals from different vulnerable groups; (D) dynamic changes of circulating HIV-1 subtypes overtime; (E) transmission route of HIV-1 infection by time period; (F) rates of HIV-1 subtypes and complex circulating forms (CRFs) according to the origin of infection within or outside of Bulgaria. The sum of the percentages of the two possible locations of infection for each subtype is 100%.

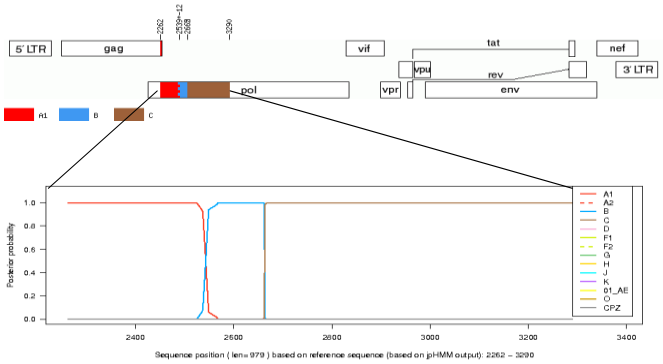

Figure 3.

Figure 3. The jpHMM reviews a fragment of the HIV-1 sequenced genome on the viral genome map and the points of recombination.

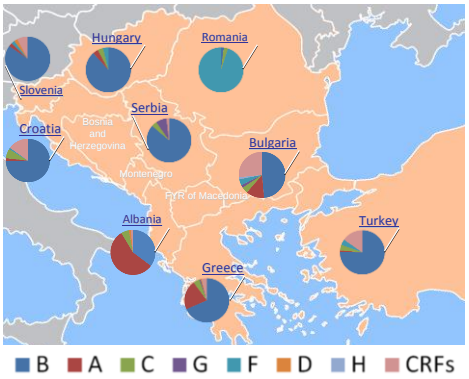

Figure 4

Figure 4. Geographic distribution of HIV-1 subtypes on the Balkans

(AIDS Review, VOLUME 14 - NUMBER 1 / January - March 2012).

Results

Our phylogenetic analysis of *pol* sequences classified 138 (68.32%) of the 202 Bulgarian HIV-1 samples to six different major subtypes: 103 (50.99%) were subtype B, 13 (6.44%) subtype A1, 9 (4.46%) subtype F1, 8 (3.96%) subtype C, three (1.49%) subtype G, and two (0.99%) were subtype H. In addition, 64 (31.68%) sequences were classified as eight different circulating or unique recombinant forms: 40 (19.8%) CRF 01_AE, 15 (7.43%) CRF 02_AG, four (1.98%) CRF 05_DF, one (0.5%) 04_cpx, one (0.5%) as 36_cpx, and three (1.49%) sequences were found to be either unique recombinant forms or were unclassified.

Conclusions

We found high genetic diversity in Bulgaria, represented by at least 11 different HIV-1 clades. Three of the genetic forms we found are extremely rare in Europe: CRF 04_cpx, 05_DF and 36_cpx. Various subtypes and CRFs have been introduced in the country emphasize the need for detailed surveillance and control of HIV-1 infection in Bulgaria.

Acknowledgments: This study was funded in part by the Bulgarian Ministry of Health Directorate "Management of Specialized Donor-funded Programs" and by the European Commission sixth framework supported programs EuropeHIVResistance, grant LSHPCT-2006-518211.

EO2. NANOTECHNOLOGICAL APPROACH FOR THERAPY OF HIV/AIDS BY DESTRUCTION OF THE VIRUS NAVIGATION SYTEM (HIGINS)

Evgeni Gabev

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Acad. Georgy Bontchev Str., block 25, Sofia 1113, Bulgaria
E-mail: egabev@bas.bg

Further to our research studies for development of novel approaches for HIV/AIDS treatment we recently have created a theoretical formula of a 2 component nano preparation (Julihivir) based on our hypothesis of the existence of HIV-1 inner gimbal navigation system (HIGINS). Navigation is widely available in animal world and is indispensable for their migration, surviving and reproduction. Due to its much smaller size (100 thousand time less) over the average diameter blood vessel, limited extracellular half-life (several hours) and ability to infect just 1-2 susceptible cells per 100 thousand it is hard to believe that HIV will lack some sort of navigation when after the transmission in the blood circulation it is looking to find the target CD4+ cells. It is hypothesized the existence of a feedback controlled navigation system used by the cell free HIV-1 virion when floating in the blood circulation and looking for susceptible hosting cell(s). It is further assumed that the latter is driven by an inner discrete (quantised) gimbal like mechanism which in turn is defined by Euler rotational angles geometrical constraints and the availability of axes of rotational symmetry inherent for the HIV-1 icosahedral symmetry capsid. A pharmacological aided way for destruction of HIV-1 navigation system (HIGINS) which will render the virus incapable to find and respectively to infect the immune cell(s) is proposed. The latter will lead to abolishment of its reproduction. One potent candidate for HIGINS destruction is our experimental anti HIV/AIDS preparation Julihivir. Such destruction of the above navigation system (which is the mechanism of action of Julihivir) will eliminate the virus and in the same time will prevent the non infected cells from being HIV affected.

EO3. OUR EXPERIENCE IN USING NANOTECHNOLOGIES FOR IMPROVING THE THERAPY OF HIV/AIDS

Evgeni Gabev

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Acad. Georgy Bontchev street, block 25, Sofia 1113, Bulgaria
E-mail: egabev@bas.bg

The Acquired Immunodeficiency Syndrome (AIDS) is the final stage of one of the most severe and devastating infectious diseases caused by a retrovirus named Human

Immunodeficiency Virus (HIV-1 and HIV-2 types). The major hallmark of the HIV infection is slow and insidious destruction of the immune system. HIV-1 primary infects both the peripheral blood and the gastrointestinal tract CD4+ T cells, but targets are also monocytes/macrophages, dendritic cells, microglia in the brain and a subset of natural killer (NK) cells that express CD4 and the HIV-1 coreceptors CCR5 and CXCR4 as well. The disease is clinically manifested by developing of associated relentless life-threatening opportunistic infections, malignant tumours, dementia, cachexia (wasting syndrome) and eventually an inevitable death. There are more than 60 million people affected up to now with half already not alive. Unfortunately, despite the intensive research efforts for more than 30 years, there is no effective therapy giving full cure. The subject of the present work is a novel nanotechnological approach for treatment of HIV/AIDS. It is based on blocking of the polyphosphoinositide transmembrane signaling between the virus and the target cell combined with antiretroviral action. The preparation named Lipohivir contains Lithium ions as a blocker of the virus to cell signal transduction and AZT as an antiretroviral agent both encapsulated in liposmes (artificial lipid nano vesicles). Animal and clinical trials on 10 AIDS suffering subjects reveals that the preparation is not toxic and also it is more effective than the standard therapy (HAART). While the results in the literature show that the standard therapy will theoretically eradicate the virus for at least 75 years under treatment, our estimation is for 2 to 5 years depending of the stage of the disease. Very recently we have developed theoretical formula of a 2 component nano preparation (Julihivir) based on our hypothesis of the existence of a navigation system (HIGINS) for HIV being extracellular (in the blood circulation) and looking for binding the target cells. Destruction of the above navigation system (which is the mechanism of action of Julihivir) will eliminate the virus and in the same time will prevent the non infected cells from being HIV affected.

EO4. DETERMINATION OF HEPATITIS C VIRAL LOAD BY TAQMAN PCR IN HIV-HCV COINFECTED PERSONS

E. Golkocheva-Markova[1], I. Alexiev[2], A. Kevorkyan[3], P. Teoharov[1]

[1] NRL of Viral hepatitis, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria

[2] National HIV Confirmatory Laboratory, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria

[3] Department of Hygiene, Ecology and Epidemiology, Medical University, Plovdiv, Bulgaria

E-mail: golkochev@abv.bg

As a result of shared modes of transmission, hepatitis C infection is common in HIV infected person and as a result the prevalence of HCV in HIV infected persons is higher than in the general population. Diagnosis of acute HCV is difficult due to high proportion of asymptomatic cases and absence of specific IgM based serological test. Antibody testing is the main screening method for HCV infection in HIV infected individuals. In the same time the HIV coinfection is associated with higher HCV RNA level and a more rapid progression of HCV-related liver disease. In the present study we examined HCV RNA plasma levels in cohort of HIV-HCV coinfected patients. All patients were HCV antibody positive. HCV RNA was detected by TaqMan PCR in 87% of sera samples and 13% were negative. The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test demonstrated a >6-log dynamic range of 43–6.90E+7IU/mL, a sensitivity of at least 15IU/mL for HCV WHO standard and a comparable

quantification of genotypes 1–6. The use of TaqMan PCR is reliable tool in determination of HCV viral load in blood sera of HIV-HCV coinfected individuals.

ЕО5. ДИФЕРЕНЦИАЛНО-ДИАГНОСТИЧНИ ПОДХОДИ ПРИ ВИРУСНИТЕ ИНФЕКЦИИ

Ст. Иванова[1], А. Тошев[2], З. Михнева[1]

[1]НРЛ "Морбили, паротит, рубеола", НЦЗПБ

[2] Катедра Биология, МУ-София

Точната диагноза при вирусните инфекции може да бъде трудно начинание. Причина за това е неспецифичната проява при някои от тях и атипичното протичане след прилагане на специфична профилактика. Ето защо клиницистите трябва да се противопоставят на общата тенденция за преждевременно заключение и да генерират всеобхватна диференциална диагноза, като се вземе под внимание комплексната симптоматика на сходните нозологични единици. Тя е свързана основно с фокусиране върху историята на заболяването и търсене на ключовите му клинични прояви. Всичко това налага разработването на бързи, лесно приложими и сравнително евтини методи за вирусологична диагностика.

Първата стъпка при провеждането на вирусологична диагностика е вземането на подходящи клинични материали/проби – смивове от носоглътката, фекалии, урина, кръв, везикуларни течности и др. Като общо правило пробите се вземат по време на ранните етапи на заболяването, тъй като най-често тогава се отделя вирус. Друг важен/критичен момент е начинът на събиране на пробите и транспортирането им.

Конвенционалните методи за вирусологична диагностика са изолиране на вируса в клетъчни култури и серологични изследвания. Изолирането на вирусите в **клетъчни култури** има редица предимства и недостатъци. Той е високо чуствителен диагностичен метод, при който вирусният материал може да бъде замразен и запазен. При него могат да бъдат открити неочаквани вирусни антигени, не само тези за които е предназначено тестването. От друга страна, той е бавен и скъп метод, изискващ специализирани лаборатории, високи технически умения, обучен персонал, както и поддържането на определени клетъчни култури.

Серологичните методи се основават на високата специфичност на реакцията „антиген-антитяло", чрез която може както да се идентифицира вируса причинител или неговите съставни части (протеини/антигени, нуклеинови киселини), така и да се определят специфичните антитела: от класовете M и G, служещи за доказване етиологичната роля на причинителя и прекараната инфекция, т.нар. имунен статус. Методите биват:

- ***Вируснеутрализираща реакция*** – откриване на типово специфични антигени при вируси, които показват цитопатичен ефект.

- ***Имунофлуоресцентен метод*** – откриване на вирусен антиген посредством моноклонално антитяло, конюгирано с флуоресцентна боя.

- ***Реакция за свързване на комплемента*** – взаимодействието на вирусния антиген с антитялото предизвиква свързване на комплемента, което води до лизиране на мембрани.

- ***Реакция задръжка на хемаглутинацията*** - основава се на блокирането на антивирусни антитела с хемаглутинационна способност.

- ***Имунодифузионен тест*** – антигенът и антитялото дифундират един към друг в полутвърда среда като агар. При срещата им се образува преципитационна линия. Именно тази линия показва дали има специфична реакция между антигена и антитялото.

- ***Имуноелектронна микроскопия*** – комплексите вирус-антитяло се откриват с по-голяма вероятност от индивидуалните вирусни частици.

- ***Радиоимунологичен тест*** – бърз и точен метод за доказване наличието на IgM. При него се използва радиоактивно белязан антиген (най-често с йод^{125}). Резултатите се отчитат чрез гама-брояч.

Съвременните тенденции за откриване и доказване на вирусните компоненти са насочени към развитието на **молекулярнобиологични и имуноензимни методи.** Спецификата на вирусните частици, насочва диагностицирането им към търсенето на вирусни компоненти, вместо на цели вируси с инфекциозни качества. Търсят се вирусни нуклеинови киселини, структурни белтъци или вирус-индуцирани ензими.

- ***Имунопероксидазни методи*** – реакцията антиген-антитяло се открива посредством цветна реакция, наблюдавана чрез светлинен микроскоп.

- ***Имуноензимни методи (EIA/ELISA)*** – най-масово практикувания скринингов метод за директно и индиректно откриване на вирусна инфекция. Използва се високопречистен вирус или вирусен антиген. ELISA се прилага за откриване на специфични вирусни IgM (признак за прясна инфекция или активиране на хронична) или IgG (наличие на имунитет) антитела.

- ***Western blot (WB)*** – потвърдителен метод за доказване на антитела. Серумната проба се инкубира с нитроцелулозни мембрани, върху които предварително са пренесени специфични вирусни протеини, разделени чрез гел електрофореза. При наличие на антивирусни антитела, те се свързват със съответния антиген. Образуванияj комплекс се визуализира на мястото на съответния антиген върху мембраната.

- ***Молекулярна хибридизация*** – анализ на вирусни ДНК и РНК и изучаване на взаимодействията между тях. Хибридизацията може да бъде извършена с комплементарни вериги ДНК или РНК, като възникват ДНК-ДНК, ДНК-РНК, РНК-ДНК хибриди. Това е метод за откриване и намножаване (амплифициране) на целева ДНК последователност до количества, необходими за анализ.

Сходната симптоматика на някои от вирусните инфекции, както и неправилната интерпретация на получените резултати, налага използването на набор от методики за изследване: ELISA, Western blot, PCR анализ, уреен тест за авидност на IgG антитела и др. Всичко това, в съпоставка с клиничната картина, подпомага ясната и правилна диагноза и в последствие адекватното лечение. Изгражда се съвременна модел-система за изучаване на биологичните характеристики на вирусите. Посредством редица лабораторни изследвания се обогатяват познанията върху епидемиологията и

патогенезата на вирусните заболявания, което от своя страна е предпоставка за разработването на ваксиналните препарати.

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EO6. ANIMAL PAPILLOMAVIRUSES AS MODELS FOR TESTING OF HPV VACCINES

Evelina Shikova, Zina Ivanova

IEMPAM-BAS, evelina_sh@abv.bg

Cervical cancer is the second most common cancer of women worldwide. It is induced by a virus, the human papillomavirus (HPV). Two recently developed prophylactic cervical cancer vaccines (Cervarix and Gardasil), based on virus-like particles (VLP) technology, have the potential to prevent a large proportion of cervical cancer associated with HPV infection.

Since HPV are species-restricted and does not cause disease in animals, it is difficult to conduct the animal research needed for vaccine development. Animal studies have therefore involved naturally occurring mammalian papillomaviruses. Three animal models have been used to examine the ability of VLPs to protect against experimental animal papillomavirus infection: cotton-tail rabbit papillomavirus (CRPV) infection of domestic rabbits in a cutaneous disease model and bovine papillomaviruses (BPV) and canine oral papillomavirus (COPV) in oral mucosal models in cattle and dogs, respectively. Passive transfer of IgG antibody from L1 VLP-immunized to unimmunized dogs was able to induce protection against COPV and live CRPV could be neutralized by pre-incubation with antibodies generated by immunization of rabbits with CRPV-based L1 VLPs. The antibodies responsible for the protective effect were shown to be IgG acting by directly neutralizing the infectivity of the virus and the conformational integrity of the VLPs was required for the generation of high-titre protective antibodies.

Studies using BPV in cows and CRPV in domestic rabbits established the principle that antibodies elicited by intramuscular injection of intact virions protect against experimental infection by the homologous virus type. Passive transfer of antibodies into naive animals protected against subsequent infection but was unable to induce regression of established lesions.

In conclusion, animal model systems have been invaluable in the evaluating the efficacy and safety of new HPV prophylactic and therapeutic vaccines.

EO7. HUMAN CYTOMEGALOVIRUS (HCMV) AND EPSTEIN-BARR VIRUS (EBV) INFECTIONS LEAD TO INCREASED RISK OF BREAST CANCER

P. Genova-Kalou[1*], J. Ivanova[2]

[1]National Centre of Infectious and Parasitic Diseases, Department of Virology, National Laboratory of Influenza, 44A "Gen. Stoletov" Blvd., 1233 Sofia;

[2]Sofia Medical University, Medical Faculty, Department of Biology, 1 "Zdrave" Str., Sofia,

[*]E-mail: petia.d.genova@abv.bg

Tumor diseases are one of the biggest challenges facing modern medical sciences. Despite the undoubted successes in early diagnosis and treatment of some malignancy, cancer remains the leading cause of death or permanent disability. According to WHO every fourth person of the planet is in danger of cancer. As the number of cancer victims had taken second position, immediately after cardiovascular diseases. From all cancers breast cancer is the most frequently diagnosed malignancy of women in many populations. Breast cancer affects one in eight women during their lives. Worldwide, it comprises 22.9% of invasive cancers in woman and 16% of all female cancers. No one knows why some women get breast cancer, but there are a number of risk factors, included ages, genetic susceptibility, affecting of producing and circulating of sex hormones and other personal factors. Breast cancer is a multistep disease in which a virus could play a role. Viruses have been implicated in the development of various cancers, but those routes of breast cancer pathogenesis are important to consider. Epstein-Barr virus (EBV) may be a cofactor in the development of different malignancies, including several types of carcinomas, including African Burkitt lymphoma, AIDS-associated non-Hodgkin, nasal NK/T-cell lymphomas, post-transplant lymphoproliferative disorder, nasopharyngeal carcinoma (NPC), lymphoepithelioma-like squamous cell malignancies in the salivary glands, lung and thymus, gastric adenocarcinoma and leiomyosarcoma. Human cytomegalovirus (HCMV) causes severe and often fatal disease in immunocompromised individuals including recipients of organ transplants and AIDS patients. Monocytes may be an important reservoir for latent HCMV; however, the primary reservoir may be a more primitive cell from the myeloid lineage. Reactivation may result from cellular differentiation or inflammation. Although HCMV is generally not regarded to be oncogenic virus, HCMV infection has been implicated in malignant diseases from different cancer entities. It plays a role of oncomodulator, which means that HCMV infects tumor cells and increases their malignancy. Some case-control study found an increased risk of breast cancer with later exposure to HCMV and EBV infectious and geographical distribution of this neoplasia. Existed several indirect proofs that could be associated with EBV and breast cancer: 1) EBV is present in breast tissue, where it is detected in breast milk in some women; 2) transfection of EBV DNA stimulates growth of human breast milk cells; 3) some EBV-associated lymphomas occur in the breast; 4) breast cancer has epidemiological similarities to Hodgkin lymphoma; 5) EBV has been identified in benign breast tumors in immunosuppressed women; and 6) in vitro, breast epithelial cells can be infected by direct contact with EBV-bearing lymphoblastoid cell lines. HCMV could be associated with breast cancer because it is a ubiquitous virus that is shed in breast milk, as well as in saliva, urine, cervical secretions, and semen, which implies that CMV persistently infects epithelial cells. In that presentation we would like to described the link between EBV and HCMV infections with breast cancer etiology and progression. Consequently, identification of this relation could give a new light

for detection strategies that are sensitive and specific for EBV and HCMV and able to localize these viruses to particular benign or malignant cells within the tissue. If patients with EBV- and HCMV-related breast cancers prove to have more advanced disease, viral detection could be relevant to planning the course of treatment.

EO8. MuLV - BASED ANIMAL MODELS TO STUDY RETROVIRUS - INDUCED NEUROLOGIC DISEASE

Evelina Shikova

IEMPAM-BAS, evelina_sh@abv.bg

Viral invasion of the nervous system and the development of neurological symptoms is characteristic of many retroviruses including human immunodeficiency virus (HIV). The mechanisms underlying the neurological dysfunction are unclear and many hypotheses have been proposed. Murine retroviruses have provided useful models for the neuropathology of retroviruses and they are the most extensive studied group of retroviral infections of the nervous system. The spongiform lesions induced by murine leukemia viruses (MuLV) are similar to those of the vacuolar myelopathy found in some HIV-infected patients. So far data about several neurovirulent MuLVs are available in the literature: Wild mouse MuLV isolated from the brain of a paralyzed mouse trapped at Lake Casitas, Temperatuse-sensitive (ts) mutants of Moloney MuLV, clones of rat-passaged Friend MuLV. The isolated in Bulgaria MuLV named Ly/Ya induces lymphoma and/or hindlimb paralyses in mice.

The incidence, severity and progression of the disease in the MuLV-induced neurodegeneration are mostly dependent on the viral genotype and the strains of mice used. The mouse models are economical and convenient and have been well characterized in terms of viral determinants of pathogenicity. For example, most of the determinants for neuropathogenicity have been localized to the *env* gene. The virulence can be manipulated through the use of different strains and dose of inocula and cells can be obtained *ex vivo* for determination of phenotype of infected cells. In many respects, the pathways leading to neuronal degeneration in the MuLV models are very similar to those proposed for HIV infection. Continued research with murine models will provide information regarding neuropathogenesis that can have relevance to human diseases.

EP1. COMBINED APPLICATION OF POLYPHENOL EXTRACT FROM *GERANIUM SANGUINEUM* L. AND PROTEASE INHIBITOR AS A NEW APPROACH FOR TREATMENT OF LETHAL EXPERIMENTAL INFLUENZA INFECTION IN MICE

Toshkova R.*, L. Yossifova*, E. Gardeva*, J. Serkedjieva, S. Apostolova***

** Institute of Experimental Morphology, Pathology and Anthropology with Museum - BAS, Acad. G. Bonchev str., bl. 25, Sofia 1113, Bulgaria*

**** Institute of Microbiology – BAS, Acad. G. Bonchev str., bl 26, Sofia 1113, Bulgaria**

E mail: reneta_toshkova@yahoo.com

Influenza virus infections remain an important cause of morbidity and mortality. Current vaccination strategies and antiviral drugs provide limited protection, therefore, new strategies are needed. The application of natural products with antiviral activity in appropriate therapeutic combinations and designs is a promising approach for the treatment of influenza. Here we demonstrate that combined treatment with polyphenol-rich extract from *Geranium sanguineum* L. (PC) and protease inhibitor (PI) is promising for control of influenza virus infection.

In *in vivo* experiments, mice with lethal experimental influenza infection were treated individually or in combination with a protease inhibitor (PI) and plant polyphenol complex (PC). The infection was induced intranasally (i.n.) under light ether anaesthesia with A/Aichi/2/68 (H3N2) influenza strain, adapted to murine lungs.

In *in vitro* experiments, the effect of the therapy was studied on 2, 6 and 8th day post infection. As activation of the immune system is a possible therapeutic approach we investigated the effect of a combination of plant polyphenol extract and protease inhibitor on the functions of alveolar macrophages (aMa), peritoneal macrophages (pMa) and spleen lymphocytes, isolated from PC, PI and PC+PI-treated and untreated healthy and influenza virus infected mice and in this way to provide evidence for the implication of its immunomodulatory potential for the overall protective effect in the lethal experimental influenza virus infection. The results obtained, clearly showed that macrophage functional activity (spreading and phagocytosis) was stimulated in different degree, and the strongest effect was observed in the group with combined therapy (PI + PC) and in group treated only with PC. It was established a beneficial effect of combination of both compounds (PC + PI) on the spontaneous NO production and myeloperoxidase activity in macrophages and strong stimulation of the proliferative activity of spleen lymphocytes. Based on these results we could suggest that the combination of polyphenol-rich extract from Geranium sanguineum and standard protease inhibitor is a promising therapeutic approach, useful for the treatment of influenza-virus infection and overcoming the immunosuppression, which occurs during it. Further studies are warranted to elucidate the biological effects of its immunopotentiating activity.

Keywords: plant extract, *Geranium sanguineum*, influenza, macrophages

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Session F. Medicine

Chairpersons:

Assoc. Prof. Reneta Toshkova, MD, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Assist. Prof. Albena Alexandrova, PhD
Institute of Neurobiology, Bulgarian Academy of Sciences

Assist. Prof. Milena Georgieva, PhD
Institute of Molecular Biology, Bulgarian Academy of Sciences

FO1. IN VITRO SYSTEMS FOR STUDYING DRUG METABOLISM: ORGAN SLICES

Albena Alexandrova

Institute of Neurobiology, Bulgarian Academy of Sciences, Acad. G. Bontchev Str., Bl.11, 1113 Sofia, Bulgaria

E-mail:a_alexandrova_bas@yahoo.com

Metabolism of xenobiotics occurs mainly in the liver, but in addition, the lungs and kidneys may contribute considerably. The process can be studied with different preparations ranging from the intact organ in vivo, through perfusion system, slices, isolated hepatocytes (in case of liver), homogenates and membrane fractions, to purified enzymes. Every model has its special advantages and disadvantages.

Tissue slices are an attractive in vitro system for the study of drug biotransformation and organ specific toxicity. The major advantages of precision-cut tissue slices are: (1) A maintenance of a higher level of biological organization which may better reflect the response of the target organ; (2) Maintenance of a differentiated state which is favored in tissue slices based on cell-cell and cell-matrix interaction; (3) The functional heterogeneity of the cultured tissue which may be better preserved in tissue slices; (4) The lack of a requirement for proteolytic enzymes normally employed in cell isolation which avoids digestion of important cell surface proteins; (5) Maintenance of intermediary metabolic control over xenobiotic metabolism which may better reflect in vivo.

Tissue slices retain the biochemical capacity and the functional heterogeneity of the whole organ, allowing for the assessment of total metabolism of a compound under conditions assumed to be most similar to the in vivo situation. Additionally, various cell types within an organ can participate in the biotransformation of the compound and contribute to cell injury, increasing the likelihood that the tissue slice system will be a good predictor for in vivo. The slicing procedure is flexible in that slices are prepared in a similar manner regardless of the species or organ, providing a means to investigate biotransformation of a compound in various species as well as in different organs. Furthermore, slice preparation is an efficient use of tissue. Any tissue remaining after the production of slices can be used for the preparation of microsomes, S9 fractions and cytosol and thereby further aid in the elucidation of metabolic routes and involvement of isozymes of cytochrome P450.

Nowadays the precision-cut tissue slices have become a reliable, affordable model for toxicological, pharmacological, and metabolic studies and the number of laboratories using tissue slices continuously expands. Therefore a caution must be taken to ensure that universal guidelines are established if data generated from different laboratories are to be compared.

FO2. AN EXPERIMENTAL MODEL OF SODIUM NITRITE-INDUCED HYPOXIA

E. Petrova[1], V. Ormandzhieva[1], Y. Gluhcheva[1], V. Atanasov[2], E. Pavlova[1], St. Dimitrova[1], B. Eremieva[1], M. Dimitrova[1], D. Kadiysky[1]

[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. G. Bontchev Str., Bl. 25, 1113 Sofia, Bulgaria

[2]Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski", 1 James Bourchier Blvd., 1164 Sofia, Bulgaria

E-mail: emiliapetrova@abv.bg

Background. Oxygen is essential for the life of the most living beings. Insufficient oxygen or hypoxia induces great physiological stress leading to cellular responses that result in deleterious effects of certain tissues. Models of hypoxia are useful for exploring these effects and understanding the involved pathogenic mechanisms.

Sodium nitrite ($NaNO_2$) can be used for induction of hemic hypoxia in experimental animal models. Hemic hypoxia refers to a reduction in haemoglobin's ability to transport oxygen. Sodium nitrite converts haemoglobin to methemoglobin and, unlike the ferrous form of haemoglobin, methemoglobin does not bind oxygen strongly. Thus the oxygen-carrying capacity of blood is reduced. The primary acute effect of $NaNO_2$ is methaemoglobinaemia.

Experimental design. We have developed a model of hemic hypoxia based on sodium nitrite administration. The model is characterized by the following features:

1) Mature rats and mice are injected (i.p.) with a single high dose of sodium nitrite
2) Treated animals are sacrificed at different time intervals: 1 h, 5 h, 24 h, 48 h, 5-, 10- and 20 days following the administration
3) Morphological (cytological, histological, enzyme histochemical, morphometrical) investigations on blood smears and tissue samples from brain, liver, spleen and testes at both - light and electron microscopic level
4) Hematological and biochemical analyses of blood samples

The rate of methemoglobin formation varies between species suggesting that exposure to $NaNO_2$ would have specific impact on the morphological characteristics and the biochemical parameters in different species. To our knowledge, there is no comparative study on the effect of sodium nitrite-induced hypoxia in mice and rats. The assessment of the morphological and biochemical changes would be beneficial for prevention mechanisms and therapeutic approaches for $NaNO_2$ exposure in humans.

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FO3. REVIEW: ADRENERGIC RECEPTORS AND CARDIAC MYOCYTE APOPTOSIS

Mina Vapireva

Faculty of Medicine, Sofia University St. Kliment Ohridski, Kozyak 1 Str., Sofia 1407, Bulgaria

E-mail: m_vapireva@abv.bg

In recent years heart failure has become one of the leading causes of mortality in many parts of the world. The molecular mechanisms involved in this condition reveal promising approaches, carrying the possibility to positively influence related cases.

Myocardial apoptosis plays crucial role in this serious condition. Apoptosis, the mechanism by which organisms eliminate cells without inflammatory response, is being strictly regulated on a molecular level. In a healthy system, a balance exists between apoptotic and anti-apoptotic signals. Pathological predominance of one of them leads to tissue damage.

The adrenergic receptors, also known as adrenoceptors, play central role in myocardial apoptosis. In response to agonist catecholamines, released by the sympathetic nervous system, the β-adrenergic receptor (βAR) signals cascade serves mainly to regulate contractility and heart rate. Nevertheless, chronic failure of the heart, which is also known as the "beta-adrenergic organ", results from increased sympathetic outflow. Although initially being compensatory, in the long term, chronic βAR stimulation leads to worsening of the pathophysiology. Therefore, chronic exposure to catecholamines is known to be toxic to cardiac myocytes. Also, regulatory phenomena – severe changes in certain neurotransmitter and hormone receptors, are observed in heart failure. Examples are redistribution of β_2-AR, selective activation of the β_2-AR-Gi pathway and changes in the compartmentalization of cAMP. The role of β_3-AR and β_4-AR in heart pathology is to be evaluated further.

In the search of treatment of heart failure on the molecular level, an antiadrenergic therapy should be explored in details. Taking into consideration the molecular nature of the process, apoptosis in cardiac myocytes should be subject to gene therapy.

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FO4. ЕЛЕКТРОПОРАЦИЯ. ПРИЛОЖЕНИЕ В МЕДИЦИНАТА И МОЛЕКУЛЯРНАТА БИОЛОГИЯ.

Д. Мартинов [1], Е. Стоилкова[2]

Биологически факултет, СУ „Св. Климент Охридски”

Електропермеабилизацията е универсално явление – наблюдава се както при биологични мембрани (всички типове клетки), така и при изкуствени мембрани (плоски бислойни мембрани и липозоми).

Когато върху клетки или липозоми се приложи електрично поле, върху мембраните се създава напрежение, което би могло да доведе до електричен пробив.

Клетките могат да бъдат третирани с постоянно електрично поле или променливо електрично поле. Вследствие на приложеното външно електрично поле възниква поляризация. Генерира се трансмембранен потенциал в зависимост от интензитета на полето. Настъпват структурни изменения в мемраната, тя загубва бариерните си свойства и става проницаема за неорганични йони и молекули с различни размери - явление наречено електропорация или електропермеабилизация.

В днешно време промяната на мембранната проницаемост чрез електрични импулси е широко използван метод при сливане на клетки, въвеждане на гени, белтъци и лекарства както в единични клетки, така и в интактни тъкани.

Могат да се посочат няколко направления при използване на електропорацията:

“Електрохимиотерапия” на тумори, генна терапия на моногенетични и други заболявания, изясняване на генната регулация, функция и експресия чрез електрогенетичен пренос и др.

FO5. PLATELET AGGREGATION INHIBITORY EFFECT OF PHOSPHOLIPASE A_2 FROM *VIPERA AMMODYTES MERIDIONALIS* VENOM

Y. Goranova[1], S. Stoykova[1], V. Atanasov[1,2], D. Danchev[3], S. Petrova[4]

[1] *Department of Analytical Chemistry, Faculty of Chemistry and Pharmacy, Sofia University, 1 J. Bourchier Blvd., 1164 Sofia, Bulgaria.*

[2] *Clinic of Urgent Toxicology, Military Medical Academy, 3 G. Sofiiski Str., 1606 Sofia, Bulgaria.*

[3] *Unit of Hemostasis, Central Clinical Laboratory, Military Medical Academy, 3 G. Sofiiski Str., 1606 Sofia, Bulgaria.*

[4] *Department of Biochemistry, Faculty of Biology, Sofia University, 8 Dr. Tzankov Blvd., 1164 Sofia, Bulgaria.*

E-mail: yana_goranova@abv.bg

Platelets have a major role in blood coagulation process and control of hemostasis. Platelet aggregation is a part of the sequence of events, including important adhesion and activation processes. Reagents such as adenosine diphosphate and collagen bind to specific receptors in the platelet membrane, activating the platelet and initiate platelet aggregation. Some of the snake venom enzymes as phospholipase A_2 (PLA_2) induce or inhibit platelet aggregation.

In this work for the first time, we describe the ability of PLA_2 toxin isolated from *Vipera ammodytes meridionalis* to influence the platelet aggregation, induced by different agonists (adenosine diphoshate (ADP), collagen and arachidonic acid).

Turbidimetric method of Born (Born, G.V.R. Quantitative investigations into the aggregation of blood platelets, *J.Physiol*, 1962) using a chronolog dual channel aggregometer was used to measure the platelet aggregation. Human platelet rich plasma (PRP) was preincubated with PLA_2 toxin (15, 25, 35, 45 μg/mL final concentration) before adding the agonist ADP (10μM), collagen (2μg/mL) and arachidonic acid (0.5mM).

The tested PLA_2 inhibited the platelet aggregation in all cases of tested inductors. When arachidonic acid and collagen were used as inductors, the PLA_2 toxin inhibited platelet aggregation in a dose-depended manner. Almost 100% inhibition of platelet aggregation occured at high concentrations of PLA_2 (45 μg/mL) in the presence of induction of arachidonic acid and collagen. In contrast, when ADP as agonist was applied, PLA_2 inhibitory effect was less pronounced. Different mechanism of platelet aggregation inhibition by PLA_2 toxin could be discussed depending on the agonist applied.

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FO6. IDENTIFICATION OF CRYSTALS IN RHEUMATOLOGY

[1]A. Ivanov,[2]M. Geneva, [1]L. Simeonov

[1]Medical University of Plovdiv, 15A Vassil Aprilov Blvd.

[2]Department of Rheumatology, Medical University of Plovdiv, 15A Vassil Aprilov Blvd.

E-mail: genevapopova@yahoo.com

lyudmilsimeonov@yahoo.com

Key words: rheumatology, crystals, polarising microscope, gouty and psoriatic arthritis

Gouty and psoriatic arthritis are diseases which are well known in the area of Rheumatology. These conditions are characterized with the presence of crystals in the synovial fluid. Accumulation of monosodium urates and calcium pyrophosphate dihydrate crystals is not rarely observed. They are the cause for an inflammatory response. A polarised light microscope is used for the identification of the crystals. Polarised light is generated using a polarized lens over a visible light source . An additional help to crystal identification is the use of dyes- staining with red alizarin, Sudan Black, Congo red and Gram staining. Often the polarized light microscopy is the only way for confirming the diagnosis gouty and psoriatic arthritis.

FO7. ANTI-INFLAMMATORY EFFECT OF ROSEMARY OIL IN MODEL SYSTEM

Milena Draganova-Filipova[1], Plamen Zagorchev[2]

[1]Department of Biology, Faculty of Medicine
[2]Department of Medical Physics, Biophysics and Mathematics, Faculty of Pharmacy, Medical University of Plovdiv, 15A V. Aprilov Blvd., Plovdiv, 4000, Bulgaria

E-mail: milena_draganovafilipova@abv.bg

Inflammation is the complex process induced by different endo- and exogenous factors. Different anti-inflammatory drugs are used in medical practice. To avoid the negative effects of acute inflammatory reactions the researches are focused on new substances that can reduce inflammation without any sides effects (1). Most of the natural products have similar potential. One of the essential oils, rich in biological active substances with different properties is rosemary oil. It has analgesic, antimicrobial, antiseptic, anti-inflammatory and antioxidant effects (2,3).

The aim of this study is to investigate the anti-inflammatory effect of rosemary oil, comparing two experimental models – paw rat oedema and photometric detection of cyclooxygenase inhibitory activity.

The mail laboratory Wistar rats were treated subplantar with histamine for oedema induction. The oedema volume was observed in time (4). The control group of rats wasn't treated with anti-inflammatory drugs. The second group was intraperitoneal treated with rosemary oil in concentration 70mg/kg, and the third group was treated with 10 mg/kg Indomethacin. The paw rat oedema was studied on special plethismograph "PLZ Graph 012", which have software for detection of paw volume alteration in real time.

The cyclooxygenase inhibitory activity and potential to affect on paw oedema was investigated for rosemary oil and Indomethacin. The process was studied in time and measurements at 5; 7; 15; 20; 25; 30; 60 and 120 min were done. In the first phase of the induced inflammation were detected significant differences between process of oedema reduction after rosemary oil and Indomethacin administration. The inhibitory activity of the essential oil was statistically higher. In the first and second measurement on the 5^{th} and 7^{th} minute from the beginning of reaction was registered 75% oedem reduction from rosemary oil in comparing with 5% reduction after Indomethacin treatment. In the second phase of both of inflammation process the differences wasn't detected.

The results of prostaglandin synthase activity estimated by photometric detection method demonstrated inhibitory ability of both of investigated products. They have 100% inhibitory effect but inhibitory concentration of 50% (IC50) for rosemary oil was 4,7 mg/l, and for Indomethacin – IC50 was 0,3 mg/l. After results analysis we have reason to define the anti-inflammatory effect of rosemary oil comparing with effect of the standard prostaglandin synthase inhibitor.

In our investigation the reaction of inflammation was observed using modification of standard method. We construct the high-sensitive, reliable interface system, which have ability for relative analyzing of an oedema reduction. With this system the process can be investigated by means of different experimental and statistical methods in real time.

The observed differences in progress of both of inflammation processes were completely in their initial phase. According to experimental protocol we suggest, that this differences were as a result of rosemary oil`s activity, which is similar to effect of the antihistamine products. In the second phase of process of inflammation, the absence of differences is a reason to conclude that anti-inflammatory effect of rosemary is due to the similar action as an unspecific COX – inhibitors (5).

Rosemary oil completely inhibits activities of prostaglandin synthase in dose-dependent manner. For effects, equivalent of the influence of Indomethacin it is necessary to use 18 times higher concentration of rosemary oil but it is known that rosemary have not toxic effect both in vitro and in vivo in concentrations lower than 1% (6).

The differences in the initial phase of inflammation after rosemary oil influence in comparison with effects of the standard prostaglandin synthase inhibitor suggest ability of the natural product to affect H1- histamine receptors.

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FO8. DIETARY EXPERIMENTAL MODELS FOR THE STUDY OF THE DIFFERENT STAGES OF OBESITY AND METABOLIC SYNDROME IN RATS

P. Angelova, N. Boyadjiev

Department of Physiology, Medical Faculty, Medical University, Plovdiv, 15A, Vasil Aprilov Blvd., 4002 Plovdiv, Bulgaria

E-mail: angelovapepa@abv.bg

The Metabolic Syndrome (MetS), obesity and all obesity related diseases are of considerable importance for contemporary medicine. Their etiology is multi-factor; it is attributed mainly to changes in dietary habits and reduced physical activity. When studying the pathogenesis of MetS and obesity, it is appropriate to use diet-induced animal models. The main diet-induced models in rats are: high-fat diet (HFD), high-carbohydrate diet (HCD), combined high-fat and high-carbohydrate diet (HFCD), and dietary regimens simulating dietary habits in people.

High-Fat Diet

With the use of HFD and a very high-fat diet (VHFD) development of obesity is guaranteed. The effect of diet on the body mass depends on the total amount of fats ingested (1). HFD enables monitoring of the connection between body mass, quantity of consumed fats and the effect from the use of different types of fats. In addition, HFD is easy to introduce. High variability of glucose tolerance, insulin sensitivity and triglyceride levels in the plasma

were reported as disadvantages of HFD (2). Moreover, it takes more than 15 weeks to achieve MetS by that method. J. Y. Kim *et al.* assumed that the application of HFD in rats led to a state equivalent to MetS in humans. He upholds the hypothesis that increased content of triglycerides in muscles is responsible for the insulin resistance of rodents fed with HFD as well as in people with reduced insulin sensitivity (3).

High-Carbohydrate Diet

The use of refined carbohydrates is associated with the increase of body mass, rise of the level of circulating triglycerides, and development of insulin resistance in humans and in animals (4). It is considered that the increased fructose intake is one of the prime factors for the development of the MetS and obesity.

One advantage of the mono- and di-saccharide models applied is the rapidly achieved insulin resistance and hypertriglyceridemia. However, the development of obesity could be achieved only after prolonged application of the diet. R. Kanarek and N. Orthen-Gambil published a study on the various effects of sucrose, fructose and glucose in rats. The authors found that the rats in the groups, which received sucrose and fructose solutions increased substantially their body mass and had reduced glucose tolerance. The animals receiving granulated sugar had the biggest growth per consumed kilocalories and had significantly increased retroperitoneal fatty tissue (5). The application of high-fructose and high-sucrose diets allows for studies on muscle and liver changes in the course of insulin resistance (6).

Combined High-Fat and High-Carbohydrate Diet

MetS inducing high-fat and high-carbohydrate models have various advantages. A lot of studies use prolonged HFCD for investigating the changes in metabolism and the development of cardio-vascular diseases. The results indicate significant lipid accumulation in the myocardium, left ventricular hypertrophy and morphological liver damage (7). This allows for research of morphological, biochemical and functional characteristics of cardio-vascular disorders in addition to metabolic changes.

Diets Imitating Human Dietary Habits

These dietary manipulations offer great opportunities for studing the biochemical, genetic and physiological mechanisms of obesity and its related diseases. In rats, the so-called "Western diet" which is characterized by increased intake of saturated fatty acids, cholesterol, sugar and NaCl, affects the glucose homeostasis, fat profile and adipocyte hormones (8). S. Brante *et al.* found that the so-called cafeteria diets (containing crushed biscuits, waffles, snacks, etc.) represent an effective model of a metabolic syndrome causing obesity, deteriorated glucose tolerance, and inflammatory condition (9).

Rats are the most common laboratory animals used in experimental models for the study of metabolism. They are convenient for investigating the adaptive changes in different functional, morphological and biochemical indicators. When it is necessary to monitor changes in metabolism, markers of inflammatory and thrombotic status, functional indicators, morphologic and molecular alterations at different stages of obesity inducement and metabolic syndrome, it is appropriate to use dietary experimental models in rats.

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FO9. CERTAIN DETERMINANTS OF THE IRRELEVANCE BETWEEN CLINICAL AND EXPERIMENTAL SEPSIS

D. Popov

Department of Physiology, Medical Faculty, Medical University, 15A, Vasil Aprilov Blvd., 4002 Plovdiv, Bulgaria
E-mail: dobrinpopov@gmail.com

Research of sepsis in humans is difficult because of complexity of pathological processes, heterogeneity of the affected population, lack of firmly established diagnostic markers, and restrictions of methodological and ethical nature (2). Due to such difficulties, sepsis models with animals have been created which represent an accessible and valuable means to clarify the mechanisms of disease, as well as outline the opportunities and specific approaches of therapeutic impact. Unfortunately, none of them is capable of wholly reproducing the very complex picture of human sepsis. Moreover, certain therapeutic means which show promising results in experimental research turn out to be ineffective in clinical

practice (11). Thus, the question of relevance of experimental to clinical sepsis appears. There exist factors of indisputable evidence that determine certain irrelevances between them.

The method of inducing sepsis is one of the essential factors with impact on clinical relevance, and thence the opportunity to relate the experimental data to sepsis in humans. Models with application of endotoxin are technically easy and reproducible, however they do not simulate the characteristic features of sepsis. The case rather refers to endotoxicosis (4). Models with intravascular application of pathogens allow controlled introduction of one species, or a combination of microorganisms. Disadvantages there include the rarely observed in patients massive bacteremia and lack of septic focus (11). Models induced by cecal ligation and puncture (CLP), insertion of stent in the wall of colon ascendens, and implantation of infected clot in the peritoneal cavity are relatively realistic, however they demand significant surgical intervention. Thus, under the influence of preceding non-septic impact, cytokine production is stimulated what may modify the follow-up response to the infection (4). Leaving behind certain limitations of CLP model, it is most relevant to clinical sepsis.

Choice of animals is undoubtedly one of the most important precondition to establish a state which resembles to the greatest extent clinical sepsis. To this end, such animals should be selected which are closest to human in biologic terms. Use of such animals, for example primates, relates to ethical and financial limitations. Most commonly used experimental animals include rodents mainly because of their easy breeding and low cost. Nevertheless they have the biologic features of mammals, in rodents we observe certain physiologic distinctions which explain irrelevances in manifestations of sepsis (4).

Another factor with significance for the relevance of experimental to clinical sepsis is age. Adults predominantly suffer with a greater potential to develop complications and have lethal end (8, 9). In sepsis models analogous data of impact of age have been found. In adult animals reduced immune reactivity is found, as well as enhanced sensitivity to endotoxin and higher lethality rate (10). Almost in every case, however, young animals aged less than 18 years in human equivalence are used in modeling of sepsis. The reason is the higher price cost of adult animals.

Sex is also a factor which could be taken into account in sepsis models since significance of hormonal differences has been experimentally proven. It has been found that estrogens influence the functions of immune system and accordingly have protective effect. Female animals in proestrus show higher resistance to infections and higher rate of survivability (1). Likewise, female patients tend to fare better than male patients with sepsis. As is known from clinical practice, nearly 50% of patients are women (8), however male animals are most commonly used in experiments.

Current clinical guidelines emphasize the importance of aggressive and standardized critical care support when treating patients with sepsis. It is based on the early application of appropriate antibiotics and purposeful hemodynamic and respiratory resuscitation, analgesia and metabolic control (3). In modeling of sepsis therapy is consistent with both the aim of particular experiment and laboratory equipment. That makes some of the reasons for lack of standardized treatment (4, 7). In animals, in most cases, certain elements of the therapy applied in clinical practice are implemented one-time or not systematic which is an important factor for distinctions in the elapse of sepsis therein. Proper therapeutic approach with animals is also hampered by the inability to monitor continuously a number of parameters which demand correction.

Comorbidity is a factor which substantially determines the irrelevance of models in animals and clinical reality. Epidemiologic data show that diseases, such as diabetes,

hypertonic disease, immune-deficient conditions, renal and hepatic disorders, as well as malignant diseases, are common in patients with sepsis and have serious effect on the elapse thereof (6, 8). On the contrary, mainly healthy animals are used in experimental research (5). To establish more successful models of clinical sepsis it is appropriate to use laboratory animals where some of the most common concomitant diseases have been induced.

It is obvious that the existing models do not simulate the complex picture of sepsis in its entirety. Taking into account the determinants of the irrelevances between clinical and experimental sepsis will enhance the applicability of models in the process of scientific research.

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FO10. EXHALED NITRIC OXIDE - MEASUREMENT AND CLINICAL APPLICATION

B. Atanasova[1], D.Vasilev[1], M.Sirakova[2], S. Mandadzhieva[2]

[1]Medical students, III year, Medical University of Plovdiv

[2]*Department of Pathophysiology, Medical University of Plovdiv, 15a V. Aprilov Blvd. 4002 Plovdiv, Bulgaria*

E-mail: smandadzhieva@pathophysiology.info, msirakova@pathophysiology.info

NO is a widely distributed endogenous regulatory molecule in the body synthesized from L-arginine and L-citrulline by the enzyme NO synthase (NOS). NO is produced by the human lung and is elevated in asthma patients.

There are two validated methods for assessment of eNO - chemiluminesence-based frequently used in the near past and electrochemical, integrated in portable hand-held NO-analysers, used recently. Studies comparing the performance of both analyzers have shown excellent agreement between them.

The use of FeNO in clinical practice complements conventional pulmonary function testing in the assessment of patients with non-specific respiratory symptoms. FeNO is a good marker for eosinophilic airway inflammation and high FeNO levels (>50 ppb) may be used to distinguish eosinophilic from non-eosinophilic lung pathologies. On the other hand there is an important relationship between eosinophilic airway inflammation and steroid responsiveness.

The use of FeNO for diagnosis applies different principle in contrast to monitoring airway inflammation. It is also important to identify patients who do/do not require treatment with inhaled steroids. Cut-points rather than reference values for interpretation of FeNO levels are preferred in the course of the clinical decision making.

In conclusion, measurement of fractional exhaled nitric oxide (FeNO) is a noninvasive, simple, and safe method of measuring airway eosinophilic inflammation that provides a complementary tool to other ways of assessing airways diseases.

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FO11. EXPERIMENTAL MODEL OF A COMPOUND LIVING SKIN EQUIVALENT OF NEONATAL CELLS

K. Tokmakova[1], P. Molchovski[1], A. Stanchev[1], W.Y.Ip[2], G. Tipoe[3]

[1]*Department of Orthopaedics and Traumatology, Medical University – Plovdiv, 66 Peshtersko Shose Blvd., Plovdiv,Bulgaria, E-mail: ketitoo@yahoo.com*
[2]*Department of Orthopaedics, Queen Mary Hospital, Hong Kong University*
[3]*Department of Anatomy, Hong Kong University, Hong Kong*

Background: There is a need for development of a safe and effective skin replacement. Skin autograft is the ideal choice but it is not always possible or enough and there are a number of early and late complications associated with it.

Objectives: To use compound living skin equivalents of neonatal cells for coverage of experimental skin defect on adult S-D rats and evaluate the quality of the graft as a skin substitute and alternative to autografting.

Materials and Method: Fifteen adult and 14 neonate S-D rats were used in this study. Cell populations from neonatal S-D rat skin were amplified through cell culture techniques for the production of compound living skin equivalent. It was used for coverage of experimental skin defect on adult S-D rats. The clinical follow-up, wound healing, and microscopic findings are presented. The restoration of surgically appropriate phenotype is investigated using panels of cytokeratin monoclonal antibodies against regionally variable epithelial differentiation markers.

Results: The results showed good wound healing with minimum contraction and no graft rejection. The establishment and maintenance of the appropriate graft phenotype were accessed.

Discussion: With the advance of tissue culture techniques it is now possible to grow and amplify defined cell populations for construction of compound tissue equivalent consisting both of epidermal and dermal layer. Neonatal cells as rapidly proliferating and less immunogenic are extremely suitable for this purpose.

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FO12. ПРИЛОЖНАТА КИНЕЗИОЛОГИЯ – ИНТЕГРАТИВЕН МЕТОД ЗА ОЦЕНКА НА ЗДРАВЕТО

Е. Тодорова

Медицински факултет, СУ „Св. К. Охридски”, София, България

FO13. EXPERIMENTALLY INDUCED DIABETES MELLITUS AND ITS EFFECTS ON ARGININE-VASOPRESSIN AND ANGIOTENSIN II - ELICITED MYOMETRIAL CONTRACTILITY

T. Georgiev[1], P. Hadzhibozheva[1], R. Kalfin[2], A. Tolekova[1]

[1]Dept. of Physiology, Pathophysiology and Pharmacology, Medical Faculty, Trakia University, Stara Zagora, Bulgaria
[2]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
E-mail: phript@gmail.com

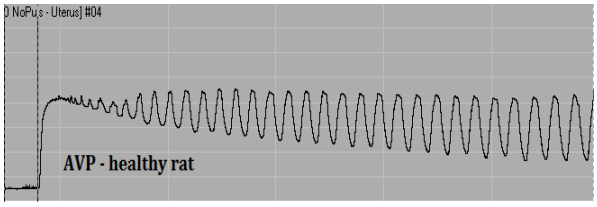
Diabetes mellitus is one of the most common diseases with a progressive and chronic tendency. The insulin deficiency concerns the whole organism and leads to a number of complications particularly in cardiovascular, nervous and excretory systems. The reproductive system is also affected. In man, impotence and premature ejaculation are observed, while in women - reduced fertility.

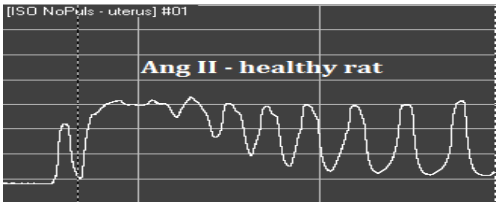
As Angiotensin II (Ang II) and Arginine - Vasopressin (AVP) are important regulators of the smooth muscle tone, it is interesting how diabetes changes the myometrial response to these peptides.

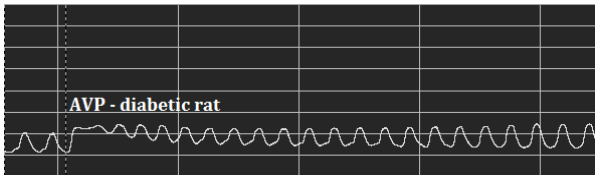
For this purpose we used female Wistar rats, in which diabetes was induced by a single intraperitoneal injection of 60 mg/kg Streptozotocine. Only those animals, which had more than 16 mmol/l blood glucose levels on the 72-hour of the injection, were considered to be diabetic. Six weeks after the diabetes induction, smooth muscle preparations of the myometrium were prepared and examined by the isolated tissues method. For a control healthy female rats of the same age were used. The smooth muscle preparations we influenced by Ang II and AVP in dose of 1 μmol.

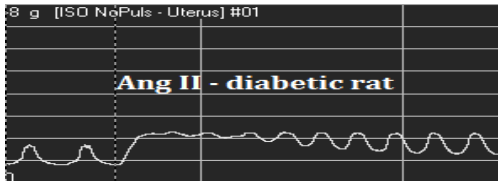
The resulting responses were compared by measuring the amplitude and the integral force of the contraction. Peptide-mediated contractions of the healthy myometrial preparations were with significantly higher amplitudes and several times larger integral force than the diabetic ones.

In conclusion, the experiment shows that diabetes mellitus considerably affects the uterine muscle by suppressing its response to humoral regulators.









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FO14. GENDER DIFFERENCES IN SUSCEPTIBILITY TO TYPE 2 DIABETES IN RAT EXPERIMENTAL MODELS

M. Yakovlieva[1], S. Mihaylova[1], A. Anastasov[2], T. Tacheva[2,] T. Vlaykova[2], K. Trifonova[1], A. Tolekova[1]

[1]Dept. of Physiology, Pathophysiology and Pharmacology, Medical Faculty, Trakia University, 11 Armeiska Str., 6000 Stara Zagora, Bulgaria
[2]Dept. of Chemistry and Biochemistry, Medical Faculty, Trakia University,11 Armeiska Str., 6000 Stara Zagora, Bulgaria.

E-mail: mariayakovlieva@abv.bg

Background: In humans the prevalence of diabetes in premenopausal women is lower than in man and postmenopausal women. In ovariectomized rats estrogen replacement lowered serum glucose and AGE products. This suggests antidiabetic action of estrogens. In another study 17β-estradiol (E2) and the synthetic G protein-coupled receptor 30 (GPR30) ligand G-1 have antiapoptotic actions in mouse pancreatic islets. This is confirmed also in vivo after single dose of 150 mg/kg STZ in mice, in females normal islet architecture, b-cell numbers, and pancreas insulin concentration remained normal, while males exhibited a loss of b-cells and pancreas insulin concentration.

Aim: The purpose of our study is to investigate the streptozotocin action at low dose – 40 mg/kg and the development of diabetes type2 in both male and female rats. This low dose STZ causes only particular destruction of pancreatic β- cells and consequently diabetes type 2.

Material and methods: Twenty healthy rats, ten male and ten female at the age of four months, were divided in four groups of five animals each. Group one – male controls which receive only 0.2 ml citrate buffer i.p.; group two – female controls which received 0.2 ml citrate buffer i.p.; group three - male rats which received one single dose of 40 mg/kg STZ i.p, freshly dissolved in citrate buffer; group four – female rats with one single dose of 40 mg/kg STZ i.p., freshly dissolved in citrate buffer. The animals were kept under standard condition of 12/12 dark-light cycle, they received normal chow and water ad libitum. After period of acclimatization animals of group three and four were injected with 40 mg/kg STZ i.p. Blood sugar was measured on day 0 with glucometer from tail vein; all animals were with normal levels of blood glucose. The diabetes was evaluated on third day after STZ injection. On the 7^{th} day blood sugar was measured with glucometer from tail vein, and blood samples were collected under anaesthesia via cardiac puncture. We also measured FRAP and the level of thiol groups to evaluate the status of antioxidant defence.

Results: Male rats treated with 40 mg/kg STZ i.p. develop hyperglycemia at 72 h (controls - 5.0±0.06 mmol/l vs. 14.16±0.36 mmol/l), while blood glucose in female rats, treated with STZ, stays normal at 72 h (controls - 6.5±0.25 mmol/l vs. 6.32±0.38 mmol/l).

Discussions and conclusions: Streptozotocin is glucosamin-nitrosurea agent, like other alkylate agents in the nitrosurea class it causes DNA damage and is particularly toxic to the β-cells. Although initially female rats were with higher blood glucose than male at baseline, after single i.p. injection of 40 mg/kg STZ only male rats develop diabetes and hyperglycemia in short period of time. This confirms previous observations that female animals are more protected from pancreatic damages.

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FP1. ИНСУЛИНОМНИ КЛЕТЪЧНИ ЛИНИИ

Радостина Александрова[1], Бойка Андонова-Лилова[1], Таня Живкова[1], Лора Дякова[2], А. Толекова[3], Р. Калфин[2]

[1]Институт по експериментална морфология, патология и антропология с музей- БАН;
[2]Институт по невробиология – БАН;

[3]Катедра по физиология, патофизиология и фармакология, Медицински факултет, Тракийски университет, Стара Загора

Без съмнение, създаването на постоянни клетъчни линии от бета клетките на панкреаса е привлекателна, но, за съжаление, нелесна задача. Особено трудно е получаването на инсулин-секретиращи клетъчни линии, при които да е запазена нормалната регулация на инсулиновата секреция и да не са настъпили генетични изменения. През последните 3 десетилетия са използвани различни подходи за преодоляване на т. нар. репликативно стареене, включително индукция на тумори в панкреаса чрез въздействие с биологични (вируси SV40, EBV, HPV) и физични (лъчение) фактори, обезсмъртяване на бета-клетки в in vitro условия и др. Известни са т.нар. RIN (RINr, RINm, RIN5mF) клетъчни линии, получени от тумори на панкреаса, предизвикани с висока доза рентгеново облъчване. Това са инсулин-секретиращи клетки, които съдържат соматостатин и глюкагон. Интерес представляват и клетъчните линии, създадени от трансгенни лабораторни животни (мишки и плъхове). В повечето от линиите секрецията на хормона инсулин и способността на клетките да „отговарят" при въздействие с глюкоза са високи в началните пасажи, но намаляват с течение на времето с напредването на пасажите.

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Session G. Cancer Research

Chairpersons:

Prof. Dimitar Kadiysky, MD, PhD, DSc
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Assist. Prof. Yordanka Gluhcheva, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Assist. Prof. Emilia Petrova, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

GO1. A NEW APPROACH FOR LOCAL TREATMENT OF SOLID TUMORS, INVOLVING GOLD NANOPARTICLES

Yossifova L.*, E. Gardeva*, R.Toshkova*, N. Nedyalkov, M. Alexandrov*,P. Atanasov****

** Institute of Experimental Morphology, Pathology and Anthropology with Museum - BAS, Acad. G. Bonchev Str., bl. 25, 1113 Sofia, Bulgaria*
*** Academician Emil Djankov Institute of Electronics – BAS, 72, Tzarigradsko chaussee blvd, 1784-Sofia, Bulgaria*

E mail: lilyayosifova@gmail.com; reneta_toshkova@yahoo.com

Summary: Local application of heat is a well-known concept in therapeutic medicine that has been explored extensively for the treatment of cancer and other conditions. This study has been designed to determine the photothermal properties of plasmonically heated gold nanoparticles (GNPs) *in vivo*, using experimental animal model – solid myeloid Graffi tumor in hamsters. Combining cytochemical, biochemical and histopathological methods we found that combination of GNPs (40 nm and 100 nm) and laser treatment with different characteristics of the laser beam resulted in localized heating and causing local destruction of the tumor tissue, prolonged survival rate and mean survival time of the tumor bearing animals. This study demonstrates that GNPs are a novel class of photothermal agents which cause cell injury and death through conversion of absorbed light to thermal energy.

Introduction: The revolution in cancer therapy has taken place by emerging use of laser light to achieve controlled and confined thermal damage in the tumor tissue. Laser is an optical source that emits photons in a coherent and narrow beam [1]. Noble metal nanoparticles have become very useful as agents for photothermal therapy of their enhanced absorption cross sections, which are four to five orders of magnitude larger than those offered by conventional photoabsorbing dyes. This strong absorption ensures effective laser therapy at relatively lower energies rendering the therapy method minimally invasive. Irradiation with short laser pulses has been shown to lead to rapid heating of the particles and vaporization of thin layer of fluid surrounding each particle, producing microscopic explosions and bubble formation [2-4, 5, 6, 7]. Clusters formed by the assembly of gold nanoparticles enhance the bubble formation, causing more efficient cancer cell killing [5].

Aim: The aim of the present study is to elucidate the effects of local application of gold nanoparticles in combination with laser beam irradiation on parameters of the tumor growth and histopathological evaluation of the tumor tissue damage.

Materials and Methods: Golden Syrian hamsters, 2–4 months old, weighing approximately 100 g were purchased from a breeding base Oncology Center, Sofia. The animals were divided into experimental groups and were kept under standard conditions in individual plastic cages with free access to food and water. All studies were performed in accordance with the Guide for Care and Use of Laboratory Animals, as proposed by the Committee on Care Laboratory Animal Resources, Commission on Life Sciences and National Research Council. An experimental Graffi myeloid tumor was created and maintained monthly *in vivo* by subcutaneous transplantation of live tumor cells by method described by Toskova et al., 2008[8]. Spontaneous regression in this experimental tumor model was not observed. The tumors were irradiated using Nd–YAG laser at λ=532 nm, pulse duration τ p= 15 ns and repetition rate 1 Hz. Nanoparticles with diameters of 40 nm and 100 nm (BBInternational, Cardiff, UK) were used as colloid solutions without surfactants, stabilizers or enhancers. Samples of tumor tissue were selected for histopathological studies. They were obtained from animals from each experimental group and were processed and stained with haematoxylin–eosin according to the standard histological technique. All the data were expressed as mean±standard deviation (SD). The statistical significance between the treatments was evaluated by one-way ANOVA and with Bonforroni's post hoc test using GraphPAD InStat, Software, USA.

Results: In *in vivo* experiments locally combined treatment on hamsters with well-formed subcutaneous tumors (1-1.5 cm in diameter) was applied. It was found that the therapy with nanoparticles (40 nm or 100 nm) and laser irradiation with the density of the laser beam 80mJ/cm^2 effectively suppressed the growth of *Graffi* tumor in hamsters, increased the average survival time of animals and reduced mortality rates of treated animals.

Histopathological studies clearly showed areas of nano-thermolysis of the tumor tissue and well defined zones of laser impact. The most significant disintegration of the tumor tissue was observed when a combination of gold nanoparticles with size 100 nm and energy of the laser beam 80mJ/cm^2 was applied.

The results obtained showed that application of plasmonically activated gold nanoparticles for the treatment of *Graffi* tumor in hamsters *in vivo* have effective anti-tumor effect and have a potential to be used for local treatment of small solid tumors.

Keywords: gold nanoparticles, photothermal therapy, *Graffi* tumor

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GP1. НАНОТЕХНОЛОГИИ В ЛЕЧЕНИЕТО НА РАКОВИТЕ ЗАБОЛЯВАНИЯ

Ж, Коюмджян-Иванова[1], Р. Александрова[1], Т. Живкова[1], Л. Дякова[2], Б. Андонова-Лилова[1], М. Симеонова[3], С. Пашевич[4], А. Денисов[4], В. Кульчицкий[4]

[1]*Институт по експериментална морфология, патология и антропология с музей- БАН;*

[2]*Институт по невробиология – БАН;*

[3]Химикотехнологичен и металургичен университет, София

[4]Институт по физиология, Национална академия на науките на Беларус

Нанотехнологиите представляват "създаване, характеризиране, производство и приложение на материали, структури, устройства и системи, чиито размери са в границите 1 – 100 нм)". Размерите на наноматериалите са сходни с тези на биологичните молекули и системи, което ги прави потенциално интересни за нуждите на медицината.

С термина „наномедицина» се означава използването на нанотехнологиите в полза на човешкото здраве и благосъстояние. Тя се основава на употребата на наночастици в диагностиката и доставянето на лекарствени препарати в организма. Нанотехнологиите правят възможно провеждане на лечение на молекулярно ниво и улесняват изучаването на патогенезата на заболяванията (Surendiran et al., 2009; Betty, 2010).

Създаване на терапевтични препарати с помощта на нанотехнологиите

Наночастиците имат потенциално приложение в почти всички клонове на съвременната медицина, включително онкология, кардиология, имунология, неврология, ендокринология, офталмология, травматология, ортопедия, стоматология...
Проучване, проведено от Европейската комисия за наука и технологии през 2006 г., показа, че:

- > 150 компании работят върху създаването на терапевтични препарати с помощта на нанотехнологии;
- 24 създадени на основа на нанотехнологии терапевтични продукта са били одобрени за клинична употреба, като общата стойност на продажбите надхвърля 5.4 милиарда щатски долара;

- Приложението на нанотехнологиите за контрол на раковите заболявания (т.нар. наноонкология) в момента е най-важната и бързо развиваща се област на наномедицината. В момента с помощта на нанотехнологиите се разработват около 150 антитуморни препарата;

- В 35 държави са регистрираните патенти в областта на нанотехнологиите. Нанофармацефтичните патенти са съсредоточени предимно в областта на незаразните заболявания, като вниманието е насочено основно към неоплазиите, следвани от хепатита. Голяма част от патентите с терапевтична насоченост са свързани със създаване на системи за доставяне на лекарства (du Toit et al., 2007; Jain, 2010; Betty, 2010).

Доставяне на лекарства с наночастици

Наночастици (НЧ) за първи път са разработени преди 35 години като носители на ваксини и средства за химиотерапия. Те са стабилни, твърди колоидни частици, състоящи се от биоразградим полимер или липиден материал. Лекарствата могат да се абсорбират върху повърхността на частиците, да се включат в полимер/липид, да бъдат разтворени в матрикса на частиците. Свойствата на НЧ варират в зависимост от използваните по време на производството им полимери, стабилизатори и повърхностноактивни вещества. Различни фактори могат да окажат въздействие върху бионаличността на лекарството в клетката/тъканта, както и върху стабилността на препарата в плазмата.
Трябва да се подчертае, че НП имат редица предимства като системи за доставяне на лекарства:

- Подобрява се разтворимостта на слабо разтворими във вода вещества;
- Удължава се времето на полуживот на лекарстветия препарат чрез намаляване на имуногенността му;

- Намалява честотата на приложение;
- Насоченото доставяне до прицелни тъкани и органи води до отслабване на страничните ефекти;
- Възможно е едновременното доставяне на два или повече лекарствени препарата за провеждане на комбинирана терапия с цел постигане на синергичен ефект и предотвратяване възникването на лекарствена устойчивост;
- Могат да бъдат използвани и с диагностични цели.

Използването на биоразградими материали за изготвяне на наночастици позволява продължително освобождаване на лекарствения препарат в прицелното място в продължение на дни и дори седмици. Едно от предимствата на наночастиците е, че могат да осъществят постъпването в мозъка на лекарства, които обикновено не преминават през кръвно-мозъчната бариера. Нещо повече, НП могат да бъдат химически «програмирани» по начин, който да повишава афинитета им към туморните клетки (например, посредством свързване с моноклонални антитела, които разпознават специфични рецептори по повърхността на раковите клетки). С цел осигуряване на по-висока ефективност, новите НП са програмирани да променят структурата и свойствата си по време на процеса на доставяне на лекарството. Тези промени може да се постигнат чрез включването на молекулни сензори, които са в състояние да отговорят на физични или биологични стимули, включително промени в pH, окислително-редукционния потенциал, ензими (Ringe et al., 2004; Kingsley et al., 2006; Kairemo et al., 2008; Surendiran et al., 2009; Betty, 2010).

Предизвикателства пред наномедицината

Преди наночастиците да навлязат повсеместно в клиничната практика и да бъдат възприети като напълно реалистичен метод за доставка на лекарствени препарати, те трябва да отговорят на редица условия: да са стабилни в кръвта, да не са токсични, да не благоприятстват образуването на тромби, да не са имуногенни, да не предизвикват възпалителна реакция, да не активират действието на неутрофилите, да са биоразградими, да избягват ретикулоендотелната система (РЕС), да бъдат приложими към различни молекули (малки молекули, протеини, пептиди, нуклеинови киселини), производственият им процес да е с разумна цена (Ringe et al., 2004; Kingsley et al., 2006; Kairemo et al., 2008).

Рутинното навлизане на нанобиотехнологиите в клиничната онкология изисква преодоляването на редица предизвикателства. Така например, все още не е изяснен напълно въпроса за потенциалната токсичност на наночастиците. Необходимо е провеждането на задълбочени и мащабни проучвания, които да дадат отговор на този въпрос. Все още не е постигнат консенсус за оценяване на биологичния риск при наноматериалите. Затрудненията в тази област идват от недостатъчните данни, комплексната природа на тези материали, трудности при измерването, липса на общоприета схема за оценка на опасността от приложението им (Jain, 2010; Wesselinova, 2011).

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GP2. IN VITRO EFFECTS OF NANO-STRUCTURED HYBRID MATERIALS CONTAINING QUATERNIZED CHITOSAN AND GOSSYPOL ON HELA HUMAN CARCINOMA CELL LINE

Toshkova R.*, L. Yossifova*, E. Gardeva*, M. Ignatova, M. Alexandrov*, N. Manolova**, I. Rashkov****

* *Institute of Experimental Morphology, Pathology and Anthropology* with *Museum* - BAS, *Acad. G. Bonchev str., bl. 25, Sofia 1113, Bulgaria*
***Laboratory of Bioactive Polymers, Institute of Polymers - BAS, Acad. G. Bonchev str. bl. 103A, Sofia 1113, Bulgaria*

E mail: reneta_toshkova@yahoo.com

The use of electrospun micro- and nanofibrous materials as antitumor drug carriers is a promising approach for the targeting delivery of the antitumor drugs, because they have numerous advantages, such as improved therapeutic effect, reduced toxicity and convenience. It is well known that natural polysaccharide chitosan and its derivatives possess good antitumor activity, as well as high antimicrobial and antimycotic properties. The combination of the advantageous properties of the antitumor drug Gossypol (GOS) and the biological properties of quaternized Chitosan (QCh) is a promising strategy for the preparation of hybrid nanofibrous materials suitable for local tumor treatment.

Nanofibrous mats containing poly (l-lactide-co-d,l-lactide) (coPLA) or poly (l-lactide-co-d,l-lactide)/polyethylene glycol (coPLA/PEG) and GOS, and then coated with a thin film of QCh were successfully prepared. *In vitro* cell viability studies revealed that the QCh-coated and uncoated nanofibrous mats containing GOS showed a higher antiproliferative activity against HeLa tumor cells than that of free GOS. Furthermore, the cytotoxicity of nanofibrous mats containing a combination of both GOS and QCh was significantly higher than that of the mats contained only GOS or mats covered only with QCh. This fact can be explained by the synergy action of QCh and GOS. The observed effect was mainly due to induction of apoptosis in the tumor cells which is confirmed by fluorescence microscopic observations of AO and EtBr double-stained cells. Therefore these nanofibrous materials have excellent potential for the treatment of cervical tumor, which remains a critical public health problem.

Keywords: electospun nanofibers, quaternized chitosan, Gossypol, HeLa

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GO2. ЦИТОТОКСИЧЕН И АНТИПРОЛИФЕРАТИВЕН ЕФЕКТ НА ПОЛИБУТИЛЦИАНОАКРИЛАТНИ НАНОЧАСТИЦИ IN VITRO.

М. Петкова[1], Р. Скробанска[1], Г. Йорданов[2]

[1]*Биологически факултет, СУ „Св. Климент Охридски”, бул. Драган Цанков 8, София, България*

[2]*Факултет по химия и фармация, СУ „Св. Климент Охридски”, бул. Джеймс Баучър 1, София, България*

E-mail: [1]*Milena_kp@yahoo.com*

С напредването на технологиите наночастиците намират все по-широко приложение в биологията и медицината. Използването им като лекарствени носители, с цел локализиране на лекарствените вещества в различни части на организма, е иновативен метод за борба с рака. За да бъдат носители на противоракови медикаменти, наночастиците трябва да отговарят на някои условия, а именно да бъдат биосъвместими, биоразградими и нетоксични за организма.

Едни от най-широко изследваните наноносители, с цел използването им в медицината за лечение на тумори, са полибутилцианоакрилатните наночастици. Нови изследвания показват, че тяхното приложение като лекарствени носители е възможно, защото полибутилцианоакрилатът (PBCA) е биосъвместим, биоразградим синтетичен полимер, чиито мономер – бутилцианоакрилата, се използва в хирургията като тъканно лепило. Като синтетичен полимер обаче, PBCA не се явява естествен фактор в клетъчната среда.

Нашите експерименти имат за цел да бъде изследван in vitro цитотоксичния ефект на PBCA наноносителите при различни концентрации чрез MTT-тест върху две епителни клетъчни линии – цервикален карцином (HeLa) и здрав роговичен епител (SIRC). Като колоиден стабилизатор за полибутилцианоакрилатните наноносители сме използвали амфифилен съ-полимер (Pluronic F-68), в концентрации под критичната точка на мицелообразуване. Допълнително бе отчетен ефекта на самия детергент върху степентта на жизненост на изследваните клетъчни линни. Биохимично сме анализирали промяната в пролиферацията на клетките, при концентрации на PBCA, при които сме отчели повишена цитотоксичност. Това е постигнато чрез изследване на ERK сигналната каскада - основен път за регулиране на нивото на експресия на циклин Д1. Направена е корелация между силата на цитотоксичния ефект на полибутилцианоакрилатните носители и темпа на нарастване на културите, чрез построяване на растежните им криви и тяхното съпостяване с данните от MTT тестът и биохимичните резултати.

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GO3. IN VITRO STUDIES ABOUT THE INHIBITION OF THE PROLIFERATION ACTIVITIES OF SOME GASTROPODAN HEMOCYANINS

P. Genova-Kalou[1], Y. Raynova[2], K. Idakieva[2]

[1]National Centre of Infectious and Parasitic Diseases, Department of Virology, National Laboratory of Influenza and Laboratory of Cell cultures,44A "Gen. Stoletov" Blvd., 1233 Sofia, Bulgaria

[2]Bulgarian Academy of Sciences, Institute of Organic Chemistry with Centre of Phytochemistry, Acad. G. Bontchev Str., Bl. 9, 1113 Sofia, Bulgaria

E-mail: petia.d.genova@abv.bg

The hemocyanins (Hcs) are extracellular type-3 copper proteins whose function is the transport of oxygen in a number of mollusk and arthropod species. Hcs have a number of applications in immunology, immunochemistry and biotechnology, since they are potent immunogens for inducing the synthesis of a variety of antibodies and also for T-specific lymphocytes. Thus, it has been reported that keyhole limpet hemocyanin (KLH), isolated from marine gastropod *Megathura crenulata*, has significant anti-proliferative effect *in vitro* against esophageal squamous cell carcinoma, breast, pancreas, and prostate cancers [1]. KLH has been used as a form of therapy for years for patients in both the United States and Europe with diagnoses superficial bladder cancer, who have failed or are intolerant to the current therapy [2]. Recently, it has been shown that the Hc, isolated from marine gastropod *Rapana thomasiana* (RtH), has strong adjuvant immunostimulatory effect as well as it is possible to use it as a protein carrier and vaccine adjuvant [3]. Based on these studies, we hypothesized that RtH and *Helix aspersa maxima* Hc (HaH) may also have considerable possibilities for the treatment against other epithelial-derived carcinomas. Native RtH was isolated from freshly obtained hemolymph of marine snails *Rapana thomasiana* by ultracentrifugation at 180 000 *g* for 4 hours at 4^{o}C and stored in the presence of 20 % sucrose at -20^{o}C until used. RtH was further purified by gel filtration chromatography on a Sepharose 4B column, equilibrated and eluted with 50 mM PBS, pH 7.2. The purity of the isolated Hc was controlled by SDS- and native PAGE as described previously [4]. Protein concentration was determined

spectrophotometrically using the absorption coefficient $A_{278}^{0.1\%}$ = 1.36 mg^{-1} ml cm^{-1}. The HaH was obtained in similar way by preparative ultracentrifugation at 180 000 *g* for 4 hours at 4°C of the hemolymph collected from snails *Helix aspersa maxima*. By this treatment, the total Hc is sedimented while the α-macroglobulin, which is also present in the hemolymph at low concentration, mainly remains in the supernatant [5]. HaH was purified additionally by gel filtration chromatography on a Sepharose 4B column. Absorption coefficient $A_{278}^{0.1\%}$ = 1.413 mg^{-1} ml cm^{-1} was used for determination of the protein concentration. Purified sterile RtH and HaH were used for further study of their anti-tumour activity. Multiple human cancer cell lines were tested, including rhabdomyosarcoma (RD 64), larynx adenocarcinoma (Hep-2), ovarian carcinoma (CaOV) and estrogen-dependent breast cancer cell line (MCF-7). Following RtH and HaH treatment cell viability was evaluated at 24h and 48h by MTT assay at an absorbance of 540 nm. Dose-response curves were performed beginning with concentrations of 15 225 μg/ml of RtH and 24 09 0μg/ml of HaH respectively and ending at a concentration of 0,01 μg/ml. Each experiment was done in triplicate. CD_{50} values were determined from the compound concentrations that induced a 50% reduction in light absorbance. The growth of the all tested cell lines were inhibited in a dose-dependent manner and time-dependent. Significant cancer cell growth was observed in CaOV cell line tested at both time treatment intervals. All the HaH concentrations tested in all cell lines exhibited significant anti-proliferative effects compared to controls, with the exception of 1 μg/ml, 0,1 μg/ml and 0,01 μg/ml. The ovarian cancer cells had a mean growth inhibition of 43% of HaH at 48h and 72h, whereas treated CaOV cells with RtH had 56% at the almost same concentrations. There was no increase in growth inhibition after 24h incubation with both tested hemocyanins on rhabdomyosarcoma cells.

In summary, we have study for the first time anti-proliferative effect in ovarian, breast and larynx cancer cells in response to treatment with two gastropodan hemocyanins – one from marine organism and other from land representative. Promising results from the use of RtH and HaH in inhibiting growth of ovarian cancer cells prompt us to continue our investigation in further *in vivo* anti-cancer experiments.

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GO4. PLANT EXTRACTS WITH ANTITUMOR ACTIVITY

B. Petkova[1], V. Moskova-Doumanova[2], M.Dimitrova[1], T. Topouzova-Hristova[2], V. Kapchina[1]

[1] Department of Plant Physiology, Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria

[2]Department of Cytology, Histology and Embryology, Faculty of Biology, Sofia University "St. Kl. Ohridski", 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria

E-mail: bela.petkova@gmail.com, moskova@biofac.uni-sofia.bg, madimitrova@yahoo.com, topouzova@biofac.uni-sofia.bg, veneta@biofac.uni-sofia.bg

Cancer is one of the leading causes of death worldwide. Despite many years of research and advances in cancer therapy there is still no change in the overall mortality statistics [1, 2]. A potential new trend is using chemopreventive agents with natural origin. Plants used in traditional and folk medicine with already prooved pharmacological value are of great interest for this alternative treatment as they have fewer side effects and lower toxicity levels [3].

Several clinical drugs based on plant-derived agents have been designed. Between them paclitaxel which is isolated from the bark of Pacific Yew Tree [2] is one of the most successful chemopreventive agents with antitumor activity against lung, ovarium, breast, head and neck cancers. Other natural occurring substances like epigallocattechin gallate (green tea) , (6) – gingerol (ginger), myricetin (red wine, grape, vegetables and fruit) have also expressed activity against different cancer types [2].

A commonly used preclinical approach for studying the anticancer activity of different plant-derived extracts is conducting screening tests using different cell cultures [4].

Investigations of the anticancer effects of the *Phyllanthus* species on human lung (A549) and breast (MCF-7) cancer cell lines revealed IC_{50} values ranging from 50–180 mg/ml and 65–470 mg/ml for methanolic and aqueous extracts respectively. In comparison, the same extracts have lower toxicity on normal cells (human bronchus epithelium line NL20 and breast epithelium 184B5) with the cell viability percentage remaining above 50% when treated up to 1000 mg/ml for both extracts [3]. *P. emblica* has demonstrated growth inhibitory activity on A549 and HepG2 (liver carcinoma) [5], while the toxicity of *P. polyphyllus* on MCF-7, HT-29 (colon adenocarcinoma), and HepG2 was reported [6]. In another study, *Phyllanthus* was demonstrated to inhibit the growth of PC-3 (prostate adenocarcinoma) and MeWo (melanoma) via cell cycle arrest and apoptosis induction [7]. The antimetastatic activity of *Phyllantus* is probably due to the presence of flavonoids, phenolic acids or ellagitannins [3].

The anticancer effects of abnormal Savda Munziq (composed of 10 kinds of Chinese herbs) and its potential mechanism of action have been studied on HepG2 cells, Hela cells, Caco-2, T- lymphoma and breast cancer cells. The growth inhibitory effect of extract on Caco-2 is concentration and time dependent with the IC_{50} at 48h and 72 h of 5.99mg/mL and 3.02 mg/mL, respectively [1]. Induction of cell death is possibly mediated through an

apoptotic pathway. The acting ingredients in the extract that exerted the anticancer effect may include polyphenols such as flavonoids, which are abundant in this extract [8].

Wide spectrum investigation of the anticancer activity of several Cameroonian plants on 12 cancer cell lines showed great inhibitory effects of two compounds - xanthone V1 and 2-acetylfuro-1,4- naphthoquinone. These compounds exert their greatest inhibitory effects on breast MCF-7, cervix HeLa and Ca Ski, leukemia PF-382 and melanoma colo-38 cell lines [9].

Some Bulgarian species used in the traditional and folk medicine could also possess anticancer effects. Among them is *Lamium album* L. (white dead nettle), a medicinal plant from the *Lamiaceae* family. It possesses a variety of activities like: anti-inflammatory, astringent, antiseptic, antibiotic, antispasmodic, antioxidant and anti-proliferative, which is related to the variety of biologically active substances which could be found in that plant: flavonoids, iridoids, phenolic acids, polysaccharides, triterpenes, saponins, phytoecdysteroids, amines, essential oils, tannins and mucilage [4].

We studied the effects of methanol and chloroform leaf extracts from *in vivo* and *in vitro* propagated *Lamium album* L. on normal and cancer cell lines. We used as model systems A549 (lung cancer), HeLa and MDCK II (normal kidney) cell lines. In our investigation, cells were treated with different concentrations and combinations of extracts and cell viability was evaluated after 24h and 48h by MTT-assay. All extracts showed inhibitory effect in time and concentration dependent manner.

Thus, we consider that methanol and chloroform leaf extracts from *Lamium album* L. possess potential cytotoxic effect that needs further evaluation in respect to its anticancer activity.

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GP3. ТРАНСФОРМИРАНИ С ПАПИЛОМАВИРУСИ ЧОВЕШКИ ТУМОРНИ КЛЕТКИ И ТЯХНОТО ПРИЛОЖЕНИЕ ПРИ ПРОУЧВАНИЯ ВЪРХУ АНТИТУМОРНАТА АКТИВНОСТ НА НОВОСИНТЕЗИРАНИ МЕТАЛНИ СЪЕДИНЕНИЯ

[1]А. Абудаллех, [2]Ж. Коюмджян-Иванова, [2]Т. Живкова, [3]Л. Дякова, [2]Б. Андонова-Лилова, [2]М. Александров, [4]Г. Милошев, [4]М. Георгиева, [3]Р. Калфин, [5]Г. Маринеску, [5]Д. Кулита , [5]Л. Патрон, [2]Р. Александрова

[1]Софийски университет „Св.Климент Охридски”, Биологически факултет;
[2]Институт по експериментална морфология, патология и антропология с музей, Българска академия на науките;
[3]Институт по невробиология, Българска академия на науките;
[4]Институт по молекулярна биология, Българска академия на науките;
[5]Институт по физикохимия „Илие Мургулеску”, Румънска академия на науките, Румъния
E-mail: rialexandrova@hotmail.com

Човешки папиломни вируси и причинявани от тях неоплазии

По своята честота ракът на шийката на матката е на второ място при жените, веднага след рака на гърдата и е отговорен за 10% от онкологичните заболявания при тях. Всяка година се регистрират по 471 000 нови случая, а 215 000 завършват със смърт. През 1996 г. СЗО и Европейската организация за проучвания върху гениталните инфекции и неоплазиите признаха човешкия папиломен вирус (HPV) като важна причина за рака на маточната шийка (Shikova, Alexandrova, 2005).

Човешките папиломни вируси причиняват най-често срещаните полово предавани инфекции в света и са отговорни за почти всички случаи на рак на шийка на матката (Juckett, 2010). Идентифицирането на HPV като основни участници в етиологията и патогенезата на рака на шийката на матката е безспорно постижение с огромно значение за науката и практиката. За заслуженото му оценяване говори и фактът, че през 2008 г. Харалд цур Хаузен (Harald zur Hausen) беше удостоен с Нобелова награда в областта на физиологията или медицината именно за откриването и изолирането на ДНК на човешкия папиломен вирус (HPV) тип 16 и 18 от рак на маточната шийка (Stanley, 2010).

До момента са идентифицирани около 30 типа HPV вируси, които инфектират маточната шийка. Въз основа на участието им в етиологията и патогенезата на цервикалния карцином те се делят на две групи (Shikova, Alexandrova, 2005):

- Онкогенни (Високорискови) – HPV типове 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 и 70;

- Неонкогенни (Нискорискови) – HPV типове 6, 11, 42, 43, 44.

Онкогенният потенциал на човешките папиломни вируси се свързва с продуктите на гените E6 и E7, които инактивират действието на тумор-супресорните гени p53 и кодиращия ретинобластомния протеин pRB. В допълнение, E6 допринася и за активирането на ензима теломераза. (Yugawa, Kiyono, 2009)

Заразяването с HPV при жените става още при първите сексуални контакти. В 80% от случаите обаче, инфекциите са преходни. До развитие на CIN (цервикална интраепителна неоплазия) не се стига, а вирусът се „изчиства" за 6-8 месеца. В малка част (около 20%) от първоначално заразените жени обаче, вирусът не се елиминира. Инфекцията става персистентна, което води до развитие и прогресия на CIN от стадий I (най-лекия) до стадий III (най-напредналия преканцерозен стадий), което в някои случаи се следва от инвазивен карцином (Shikova, Alexandrova, 2005, Juckett, Hartman-Adams, 2010).

HPV 16 се открива в 50 – 70% от случаите на рак на маточната шийка, докато HPV 18 се доказва в 7 – 20% от жените с тази диагноза. HPV е свързан не само с рака на шийката на матката, но предразполага и към развитие на други неоплазии – на вулвата, вагината, пениса, ануса, в областта на главата и шията (Stanley, 2010).

Линията HeLa

Линията HeLa е получена от карцином на шийката на матката. Това е първата създадена постоянна клетъчна линия. Наречена е на името на Хенриета Лакс – пациентката, от която е взет (на 8 февруари 1951 г.) изходният материал за получаването й. Въпреки проведеното лечение, Х. Лакс умира на 4 октомври 1951 г. Линията HeLa обаче вече повече от 60 години е сред основните експериментални модели в хиляди лаборатории по целия свят (Scherer, 1953, Rahbari, 2009).

Клетките от линия HeLa съдържат копия HPV18 – един от високо онкогенните щамове човешки папиломен вирус, инфекцията с когото е свързана с висок риск от развитие на рак на шийката матката и други неоплазии (Mougin et al., 2009; Ruschoff et al., 2011). Неслучайно HPV18 е една от мишените на създадените неотдавна профилактични и терапевтични ваксини срещу рака на маточната шийка (Albers et al., 2010; Lu et al., 2011).

Клетките й са доста устойчиви и с висока пролиферативна способност, поради което, при неспазване на правилата за работа, често се откриват като замърсители в други клетъчни линии (Capes-Davis, 2010, Wats, 2010). Клетките имат по 82 хромозоми, като хромозома 12 е представена с 4 копия, а хромозоми 6,7 и 8 – в по 3 копия (Macville, 1999)

Изпитване на цитотоксичната и антипролиферативната активност на новосинтезирани метални съединения върху клетки от линия HeLa

При проведените от нас експерименти линия HeLa е използвана успешно за проучвания върху потенциалната антитуморна активност на осем новосинтезирани

комплекса на цинк, сребро и злато с лиганди - производни на 2,6-диформил крезол (diald) (означени като Zn-ampy, Zn-aepy, Zn-dmen). Изследванията бяха проведени чрез MTT тест, метод за включване на неутрално червено, оцветяване с кристалвиолет, колонии-формиращ метод, електрофореза на единични клетки в агарозен гел при неутрално pH, комбинирано оцветяване с акридин оранж/пропидиев йодид, както и комбинирано оцветяване по Папенхайм.

Получените резултати показаха, че приложени в определени концентрационни граници (1-100 μg/ml) за 24-72 часа, изследваните вещества намаляват преживяемостта и пролиферативната активност на използваните като експериментални модели туморни клетки от линия HeLa, предизвиквайки в тях характерни цитопатологични изменения. Ефектът на веществата нараства с увеличаване на концентрацията и времето на въздействие. Като най-ефективни изпъкнаха съединенията на Zn/Au. Основният лиганд Diald проявява по-силно изявени цитотоксични и антипролиферативни свойства в сравнение със съответните лиганди на отделните подгрупи съединения (Zn-ampy, Zn-aepy, Zn-dmen).

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GO5. РАКОВИ ЗАБОЛЯВАНИЯ И СИНДРОМ НА ДАУН

Тим Владимиров

Медицински факултет, СУ „Св. Климент Охридски”