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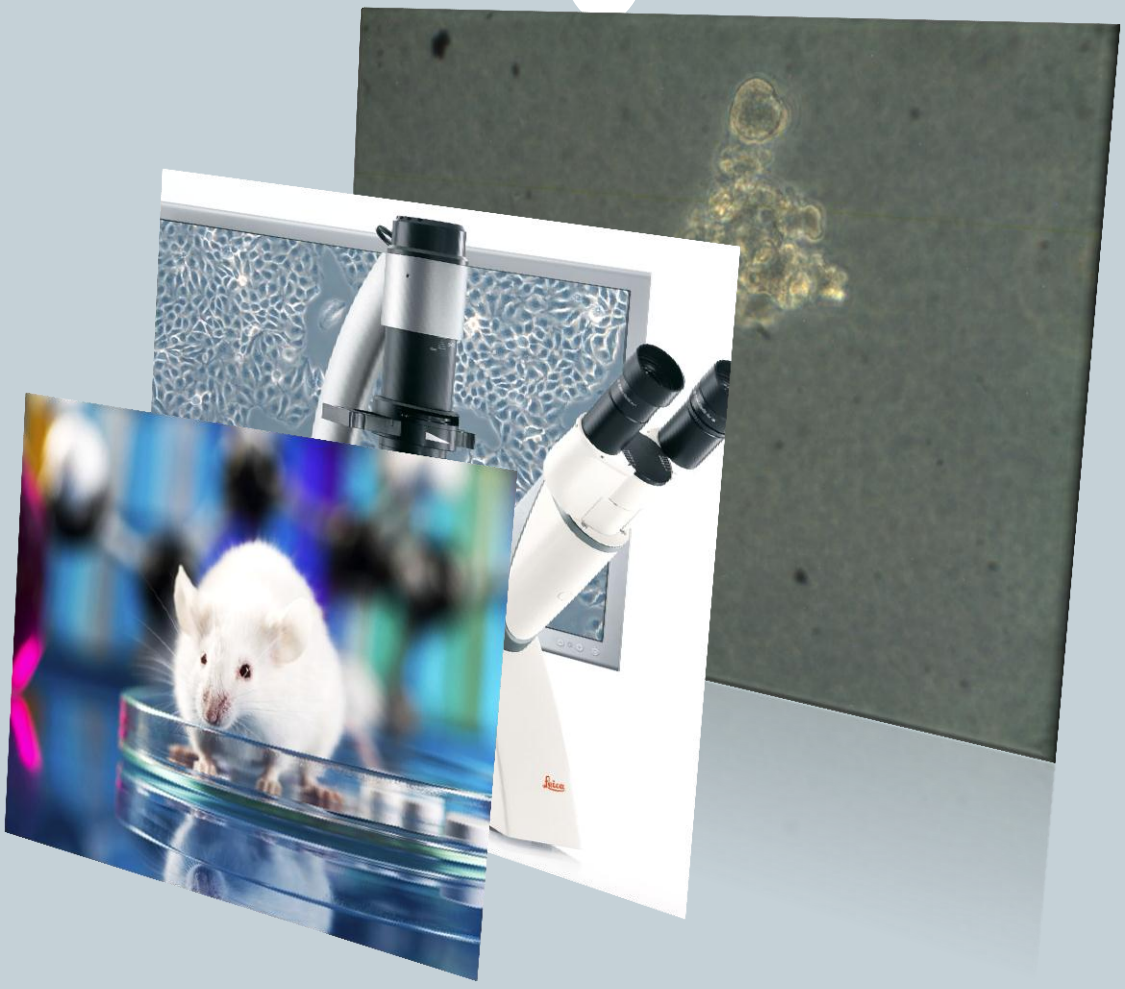
Institute of Experimental Morphology, Pathology and Anthropology with Museum

PROCEEDINGS

OF THE NINTH WORKSHOP ON EXPERIMENTAL MODELS AND METHODS IN BIOMEDICAL RESEARCH WITH INTERNATIONAL PARCIPITATION



JULY 16 – 18, 2018
SOFIA, BULGARIA

ISSN 1314-9091

PROCEEDINGS

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

with international participation

July 16 – 18, 2018

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

Edited by: Dimitar Kadiysky and Radostina Alexandrova

Supported by:

- **Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences**
- **Fund "Scientific Research", Bulgaria, Grant № DFNI Б 02-30/2014, Grant № ДКОСТ 01-16/2017 and Grant № ДКОСТ 01-19/2017**
- **Immunological Society, Bulgarian Union of Scientists**
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"EXPERIMENTAL MODELS AND METHODS IN BIOMEDICAL RESEARCH"

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THE PROGRAM OF THE WORKSHOP

Monday, 16 July 2018

13.30 – 13.45 OPENING CEREMONY

Session A

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

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

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

AO1 13.45 – 14.15	Histochemical expression of increased sialylation in skeletal muscle fibers invaded by *Trichinella spiralis*	K. Todorova , R. Milcheva, B. Nikolov, A. Sultanova, M. Cistjakovs, S. Petkova and R.Russev
AO2 14.15-14.30	Експериментални модели и тестове за изследване на невропатична и ноцицептивна болка	Весела Юлиева Кокова
AO3 14.30-15.00	Checking the field efficacy of some Bulgarian acaricides against *varroa destructor*	D. Salkova[1], K. Gurgulova[2], I. Zhelyazkova[3], V. Popova[4], S. Takova[2]
15.00 – 15.20	**Coffee - break**	
AO4 15.20 – 15.50	Briefly about colorectal cancer and some challenges of targeted antitumor therapy	Radostina Alexandrova
AO5 15.50-16.20	Spheroids as an in vitro model	Radko Sotirov, Milena Kostadinova, Shina Pashova, Snejana Kestendjieva, KameliyaVinketova, Desislava Abadjieva, Elena Stoyanova, Tsvetelina Oreshkova, Elena Kistanova, Milena Mourdjeva
AO6 16.20-16.35	Organ on a chip technology	Бойка Андонова-Лилова
AO7 16.35 – 16.50	New materials for wound dressings – the challenges of cytocompatibility assessment	Radostina Alexandrova, Desislav Dinev, Boyka Andonova-Lilova, Tanya Zhivkova, Lora Dyakova, Abedulkadir Abudalleh, Orlin Alexandrov
16.50 – 17.10	**Discussion**	

Tuesday, 17 July 2018

Session B

Chairpersons:

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

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

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

BO1 13.30 – 14.00	Investigations of the relationship between physical activity and upper respiratory tract infections among Marmara University School of Medicine phase-1 students	Emine Gokce Gumus
BO2 14.00-14.15	Introduce yourself 1	Emine Gokce Gumus
BO3 14.15-14.45	Autophagy and cancer – what do we (not) know?	Radostina Alexandrova, Abedulkadir Abudalleh, Boyka-Andonova-Lilova, Desislav Dinev, Tanya Zhivkova, Lora Dyakova, Zdravka Petrova, Milena Glavcheva, Orlin Alexandrov
BO4 **14.45 – 15.00**	Disulfiram inhibits 2D and 3D growth of virus-transformed chicken hepatoma and rat sarcoma cells in vitro	Desislav Dinev, Lora Dyakova, Boyka-Andonova-Lilova, Tanya Zhivkova, Rosen Spassov, Radostina Alexandrova
15.00 – 15.20	**Coffee - break**	
BO5 15.20 – 15.50	2-carbamido-1,3-indandione – a potential biomarker and an antitumor agent. Quantum-chemical modeling of its complexes with nucleotides of DNA and RNA	N. Stoyanova, N. Markova [1], P.Genova-Kalou[2], I.Philipova[1], V. Enchev[1],
BO6 15.50-16.20	Association of herpesvirus infection with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS)	Evelina Shikova, Antoniya Kumanova, Sevdalina Raleva, Valentina Reshkova
BO7 16.20-16.50	Can human herpes viral infection be a factor in triggering autoimmune	Vera Kolyovska, Katerina Todorova, Rositsa Milcheva, Alina

	thyroiditis?	Sultanova, Maksims Cistjakovs, Zdravka Petrova, Rossen Spasov, Modra Murovska
BO8 16.50 – 17.05	Behavioral and motivational mechanisms of the brain	Antonia Nikolova
17.05 – 17.20	**Discussion**	

Wednesday, 18 July 2018

Session C

Chairpersons:

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

Tanya Zhivkova, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

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

CO1 13.30 – 14.00	Diabetes	Isabel Rocha Miguel and Isabel Hueso Heredia
CO2 14.00-14.15	Introduce yourself 2	Isabel Rocha Miguel
CO3 14.15-14.30	Introduce yourself 3	Isabel Hueso Heredia
CO4 **14.30 – 15.00**	Cancer epigenetics	Radostina Alexandrova
15.00 – 15.20	**Coffee - break**	
CO4 15.20 – 15.50	Detection of gangliosides and anti-ganglioside antibodies in "in vitro", "in vivo" and "in vitro + in vivo" – experimental models	I. Sainova, V. Kolyovska, I. Ivanova-Pandourska, S. Engibarov, R. Eneva, Tz. Markova, D. Maslarov
CO5 15.50-16.05	Ruthenium – one metal with many faces	Zdravka Petrova, Anna Kouncheva, Pencho Beykov, Radostina Alexandrova
CO6 16.05-16.35	Ruthenium (III) complexes with Schiff bases – effect of viability and proliferation of human and rat virus-transformed cells	Zdravka Petrova, Anna Kouncheva, Boyka Andonova-Lilova, Desislav Dinev, Rossen Spasov, Daniela Cristina Culita, Gabriela Marinescu, Luminita Patron, Radostina Alexandrova
CO7 16.35 – 16.50	Zn(II) complexes with Schiff bases as cytotoxic agents	Milena Glavcheva, Desislav Dinev, Boyka Andonova-Lilova, Zdravka Petrova, Rossen Spasov, Daniela Cristina Culita, Gabriela Marinescu, Luminita Patron, Radostina Alexandrova
CO7. 16.50-17.05	Effect of deoxicholic acid and its metal complexes on viability and proliferation of	Lora Dyakova, Tanya Zhivkova, Daniela Cristina Culita, Gabriela

	colon cancer drug sensitive and drug resistant cells	Marinescu, Luminita Patron, Radostina Alexandrova
17.05 – 17.20	**Discussion**	
17.20 – 17.30	**Closing remarks**	

Session A

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

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

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

AO1. HISTOCHEMICAL EVALUATION OF INCREASED SIALYLATION IN SKELETAL MUSCLE FIBERS INVADED BY *TRICHINELLA SPIRALIS*

K. Todorova[1], R. Milcheva[1], B. Nikolov[1], A. Sultanova[2], M. Cistjakovs[2], S. Petkova[1] and R.Russev[1]

1 Institute of Experimental Morphology, Pathology and Antropology with Museum, Bulgarian Academy of Science, 1113 Sofia, Bulgaria

2 Riga Stradins University, A. Kirchenstein Institute of Microbiology and Virology, Riga, Latvia

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Abstract

The infection with the parasitic nematode *Trichinella spiralis* results in encapsulated formation within the infected muscle fibers, where the newborn larvae induce significant morphological, functional and enzymatic changes after penetration. The occupied portion of the muscle fiber transforms into a structure called a "Nurse cell", which is capable of supporting the parasite for a long time, and is accompanied by a complete loss of its contractile properties. All these processes reflect on the cell metabolite pathways and protein expression with impact on the sialylation of glycoproteins. This work focuses on the ultrastructural distribution of the expression of sialylated glycoproteins during the muscle phase of trichinellosis. We found intense gold labeling in the form of conglomerates in Lowicryl and Durcupan embedded specimens, where the underlying cellular morphology was difficult to be observed.

Keywords: Nurse cell, sialylation of glycoproteins, *Trichinella spiralis*, ultrastructural lectincytochemistry

Introduction

Trichinellosis results from an invasion of muscle fibers by a parasitic nematode that belongs to the *Trichinella* genus. The disease starts in the small intestine where nematodes reproduces. The newborn larvae are accommodated only in the skeletal muscle cells where they induce drastic morphological, functional and enzymatic changes after penetration. The occupied portion of the muscle fiber transforms into a structure called a Nurse cell, which is capable of supporting the parasite for years (5). Its formation includes invaded muscle cell response - de-differentiation, cell cycle re-entry and arrest in the G2/M phase, and satellite cell response - activation, proliferation and differentiation (6, 13). The events that occur during the process of nurse cell formation are analogous to the repairing process of muscles cells during muscle cell regeneration/repair (13).

Sialic acids are a diverse family of saccharides and occupy a terminal position in oligosaccharide chains of glycoproteins and glycolipids implicated in almost all known processes of cell adhesion and recognition phenomena (12). Results of different studies have revealed the importance of the sialic acids in skeletal muscle tissue for the functional maintenance, structure and in neuromuscular junctions (3, 10). The biological role of many sialic acids has been studied in the field in many models of skeletal muscle pathology and disfunctions, including ageing (1, 4, 7, 8). In literature, the complex and variable glycoconjugates presented in host-parasite relationship are object of an interest too, and particularly important in these relations.

For their highly specific carbohydrate-binding properties, the lectins are widely useful tools in many immunochemical techniques for recognition and visualization of glycoproteins and glycolipids. Two plant lectins, the *Sambucus nigra* agglutinin (SNA) and the *Maackia amurensis* leukoagglutinin (MAL) specifically recognize α-2-6 and α-2-3 bound sialic acids, respectively.

Increased intracellular accumulation of sialylated glycoproteins within the developing Nurse cell of *T. spiralis* was reported previously (11). The aim of this work was to describe their ultrastructural localization.

Materials and methods

Parasites, invasion, sample collection and processing

The *in vivo* experiments described in this work were performed in compliance with the Institutional Guidelines for Animal Experiments of IEMPAM – the Bulgarian Academy of Sciences and Regulation № 15/ 03.02.2006.

Infective larvae of *Trichinella spiralis* were isolated from previously invaded mice, according to a routine protocol. BALB/C, 6–8 weeks old mice were inoculated with 500 infective larvae of *T. spiralis* per os.

Lectin histochemistry: Portions of murine skeletal muscles were collected at days 0, 10, 14, 17, 24, 35 after the invasion (d.p.i.), fixed in formalin or metacarn, and embedded in paraffin. Parallel tissue sections, 7 μm thin, were stained with H&E for routine histology or were treated with biotinylated SNA and MAL lectins for 1 hour and then with streptavidin-

conjugated HRP for 30 min (Vector Laboratories Ltd, Burlingame, CA, USA). Color reaction was developed with DAB Peroxidase (HRP) Substrate Kit (Vector Laboratories) and the sections were counterstained with hematoxylin. The sections were xamined under light microscope Leica DM 5000B (Leica Camera AG, Wetzlar, Germany) and the intracellular histochemical staining of the muscle fibers was evaluated as negative (-) and positive (+).

***Lectin gold labelling**:* Tissue specimens from days 0, 17 and 35 p.i. were fixed for 1h with 4% paraformaldehyde in 0.1 M phosphate buffer PH 7.4, dehydrated in graded series of methanol and embedded in Lowicryl (2). Ultrathin sections mounted on formvar coated grids were floated in SNA and MAL 30 nm-gold conjugates (EY Laboratories, San Mateo, California, USA) and counterstained with uranyl acetate.

Other specimens were fixed with 2.5% phosphate buffered glutaraldehyde and postfixed in osmium tetroxide (0.5%) (Sigma Aldrich Chemie GmbH, Germany), dehydrated and embedded into Durcupan ACM Fluka epoxy resin, according to standard procedure. Ultrathin sections, 0.1 μm, were made by ultramicrotome Reichert-Jung Ultracut, Austria and double-stained with 2% uranyl acetate and 2% lead citrate (Sigma Aldrich Chemie GmbH, Germany). Samples were viewed on a JEOL 1200 EX transmission electron microscope and accelerating voltage of 80 kV.

Results and discussion

In the present study, we have attempted through the histochemical light microscopy and electron microscopy colloidal gold labeling method using lectin conjugates to elucidate the mechanism of de-differentiation and re-differentiation with loss of contractility and the role of sialic acids in the transformation of muscle cells towards "Nurse cell" (Fig.1). With the ultrastructural investigation of parasite-affected muscle fibers we aimed to determine the morphological state of the loss of contractility in striated muscles and the subsequent destruction of the cells along with visualization of the sialylation processes. Sialic acids play a crucial role in the metabolism of the muscles and their localization is determined mainly in the extracellular interstitial tissue and cell membranes. The presence of sialic acids into the cytosole is not usual. Thus, in healthy skeletal muscle fibers the lectin SNA labelled the blood vessels and the interstitial connective tissue but not the sarcoplasm. The results obtained by us on day 14 after infection revealed only single infected lectin-marked cells. Ultrastucturally, powerful gold-conjugated lectinhistochemical marking covered the entire surface of these individual invaded myofibers (Fig.2). We found that gold marking is in the form of conglomerates under which cellular morphology was difficult to be distinguished in Lowicryl and Durcupan embedded specimens. At a later stage, 35 d.p.i., the morphology of the infected cells was almost lost, only few fragments were positively marked for the presence of sialic acids. At that time the complete immobilization of the muscle fibers is found in order to create a comfortable environment for the developing parasites. These observations were confirmed via light microscopic histochemical study, too. The histochemical study found a positive reaction to occupied sarcoplasm, expressed in brown staining indicating a specific reaction between the SNA lectins and the MAL lectins and molecule residues with α-2-6 and α-2-3 linkages associated to the cell membrane but also of glycoproteins in the inner space of the

sarcoplasm. This process of enhanced sialylation was observed only in the occupied cells and the remaining unaffected cells around them contrasted with a negative reaction and lack of staining (Fig.3).These morphological observations sustain the previous results of the team, particularly the first published new data concerning the sialylation changes in mouse skeletal muscle after invasion by the parasitic nematode *Trichinella spiralis* (11). The conclusion was made that the presence of the parasitic nematode in the cross striated muscle initiates glycosylation changes with increased sialic acid expression within the infected muscle fiber. This affects the satellite cells and the contractility of the muscle in the way to favor the successful accommodation of the parasite in the host cell. The satellite cells actively participate in the process of nurse cell formation (9) and it is likely that the increased sialylation reported is playing a significant role in support of this process. By these experiments we demonstrated that these processes of sialylation play a significant part of the dynamic de-differentiation processes of muscle cell and its morphological and functional transformation during parasitic *Trichinella* invasions.

Acknowledgments:
The work was supported by the National Science Fund of Republic of Bulgaria with grant DN01/16.

Fig.1. Basic histology on mouse skeletal muscles with *Trichinella spiralis* at days 10 (A), 14 (B), 24 (C) and 35 (D) post invasion showing developing (A, B, and C) and mature (D) Nurse cell. H&E.

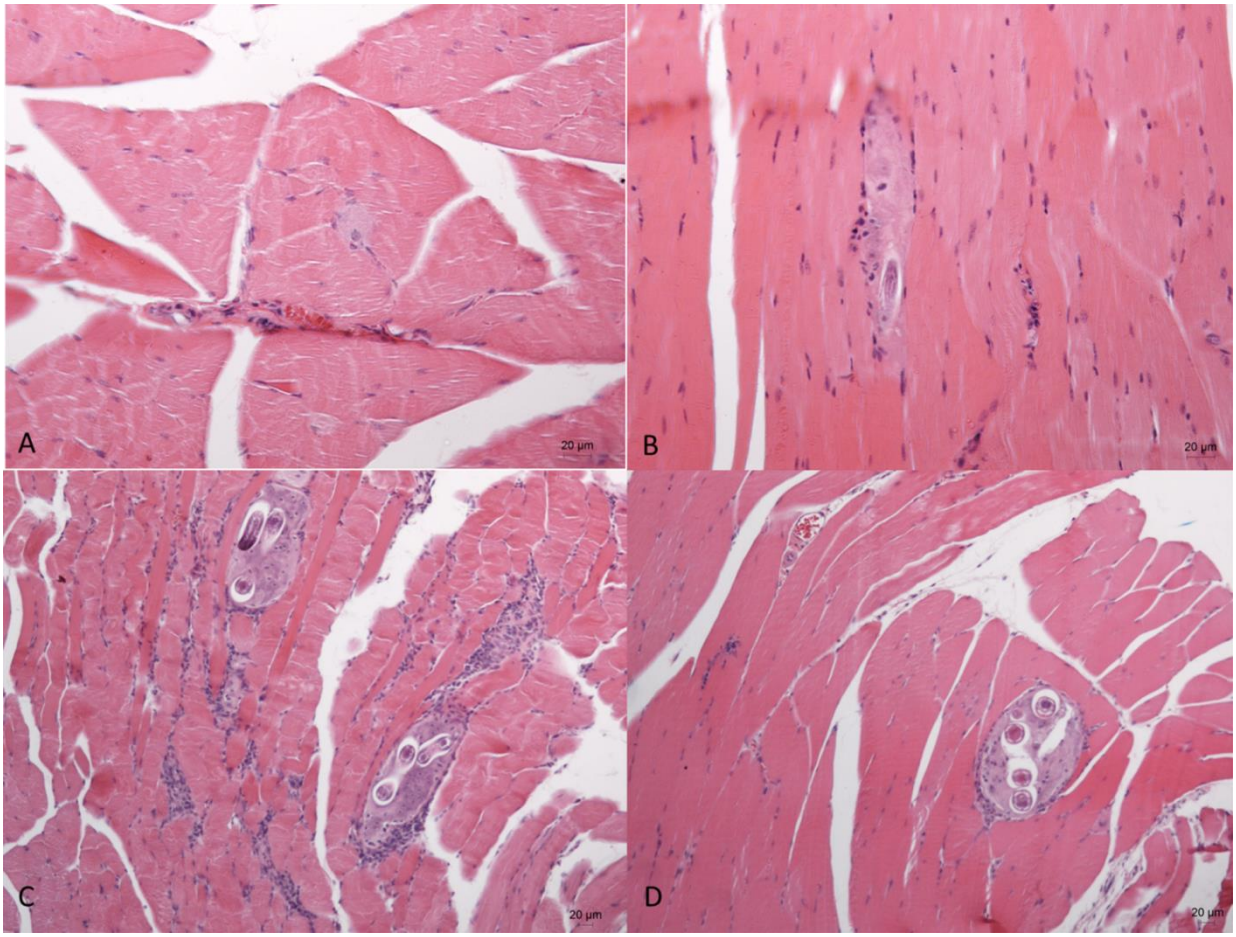


Fig.2. A, B) Non invaded muscle fibres, lacking lectin-gold marking; C) /10 d.p.i. MAL/, D) /17 d.p.i. MAL/, E) /17 d.p.i. SNA/, F) /17 d.p.i/) Positive intracytosolic marking with conglomerates of lectin-gold conugates bonded to α-2-6 or α-2-3 linkages between glycoproteins and sialic residues. The cellular morphology is not well presented and readable in accordance with the ongoing processes of cell transformation and de-differentiation during the parasitic invasion. Only some fragments of microfilaments were distinguished /10 d.p.i./, while mitochondria were not visible (C). In progress the morphology was completely changed at the 17 d.p.i. (D, E, F). Instr. Magnify: A-12700; B-12700; C-22000; D-12700; E-30000; F-12700.

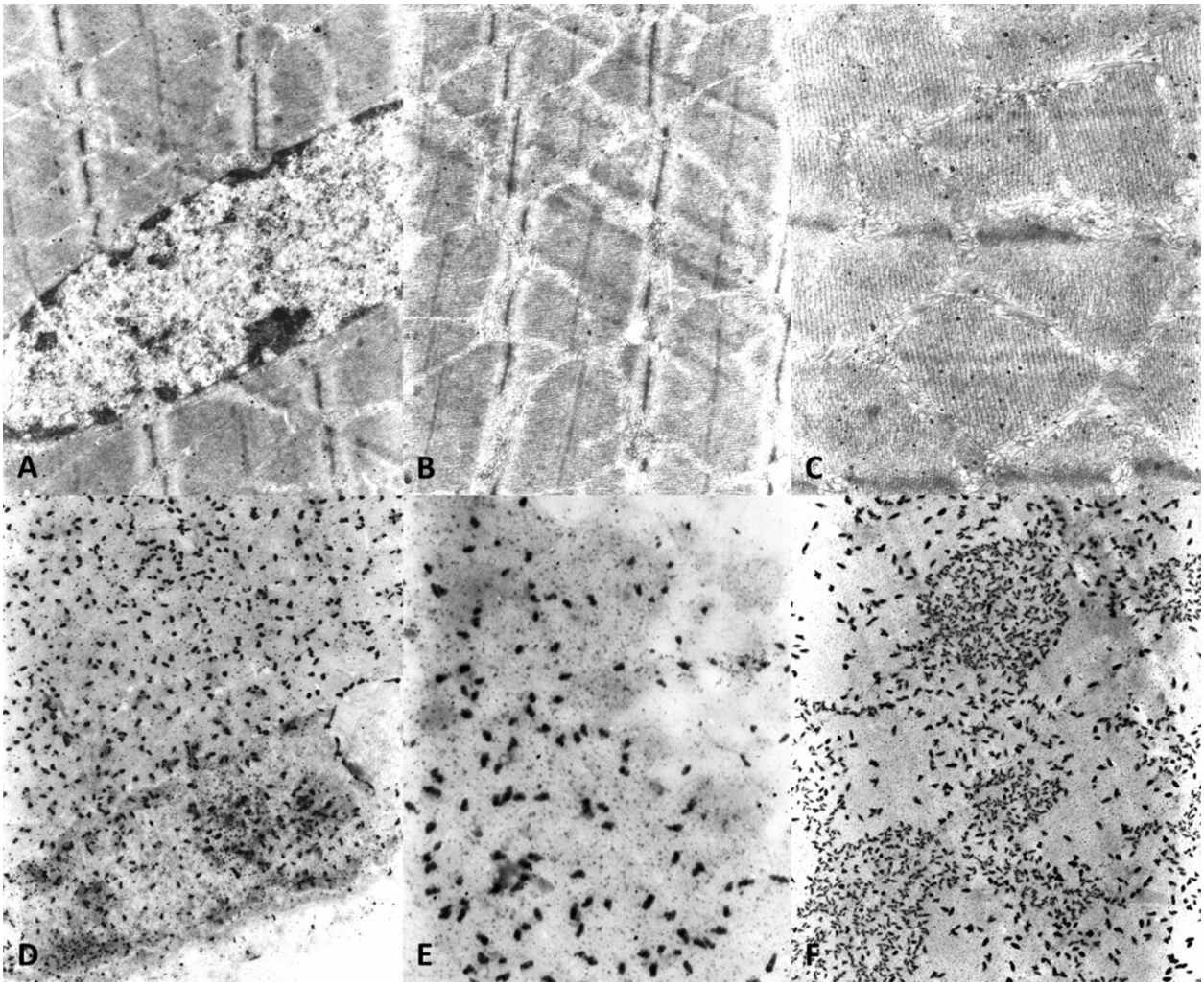
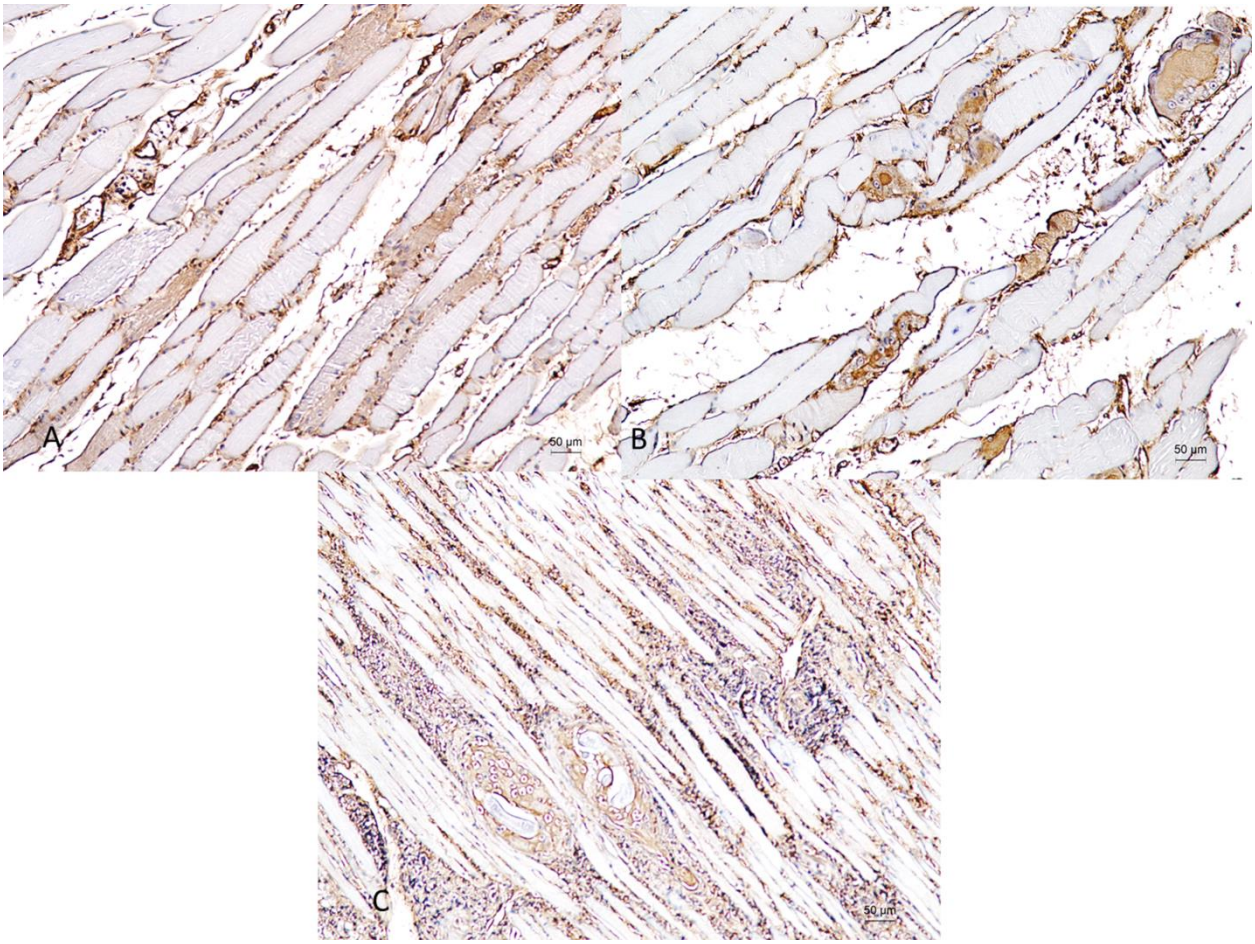


Fig.3. Intracellular expression of α-2,6-sialylated glycoproteins in the developing and mature Nurse cell of *Trichinella spiralis*. Tissue sections from mouse skeletal muscles with *T. spiralis* at days 10 (A), 17 (B) and 35 (C) post invasion were stained with biotinylated lectin SNA, specific for α-2,6-sialic acid. The brown color indicates positive histochemical reaction. Streptavidin-biotin peroxidase, DAB.



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AO2. EXPERIMENTAL MODELS AND TESTS FOR NEUROPATHIC AND NOCICEPTIVE PAIN EVALUATION

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Abstract

Pain is an extremely prevalent symptom. There are many experimental tests and models for pain evaluation, which are used for preclinical characterization of drugs. In this review, the most commonly used animal models and tests to study neuropathic and nociceptive pain are discussed. To study neuropathic pain, diabetic polyneuropathy, chronic constriction injury of n. ischiadicus and chemotherapy-induced painful peripheral neuropathy models in rats are described. Carrageenan-induced hyperalgesia, adjuvant arthritis and formalin assay for inflammatory nociceptive pain examination are summarized. Each animal model has been created with specific methodology and results can vary largely with the slight changes related to methodology.

Keywords: neuropathic pain, nociceptive pain, experimental models, experimental tests

Introduction

Pain, both acute and chronic, remains a significant health problem that has an important impact on function and quality of life. The use of animal models can lead to the discovery of mechanisms, side-effects, and new effective therapeutic modalities for patients suffering from acute and chronic pain syndromes which could alter the clinical outlook we currently have. Numerous animal models exist for the exploration of pain mediators and mechanisms, different pain etiologies and manifestations, and the action of analgesics.

Animal models to investigate neuropathic pain

Ideal models should induce just sensory neurons dysfunction, such as allodynia and hyperalgesia for short periods. The most frequently used animal models include diabetic

polyneuropathy, chronic constriction injury of n. ischiadicus and chemotherapy-induced models of neuropathic pain.

Diabetic neuropathy

A single injection of 75 mg/kg streptozocin intraperitoneally in rats induces diabetic polyneuropathy that initially is characterized by changes in tactile sensitivity (allodynia) followed by decreased thermal nociceptive threshold [14].

Chronic constriction injury (CCI) of n. ischiadicus

Different models of CCI have been described. One of the modifications of CCI is reported by Seltzer et al., 1990 [25]. In this model of partial nerve ligation, nerve injury is created by tying loosely double ligature (the dorsal one third to one half) of the rat's sciatic nerve. Sciatic nerve ligation results in allodynia 12 to 15 days after injury.

Chemotherapy-induced painful peripheral neuropathy

Chemotherapy-induced peripheral neuropathy (CIPN) is most often produced by vinca alkaloids (vinblastine, vincristine), platinum derivatives (cisplatin, oxaliplatin, carboplatin), taxanes (docetaxel, paclitaxel), thalidomide, bortezomib and ixabepilone [9,23]. The toxic attack is directed against the peripheral nerve and the targets of therapy-induced toxicity are the neuronal soma, the axonal microtubules, the axonal transport system, the myelin sheath and glial cells [27]. Abnormalities in mitochondrial structure and function in peripheral sensory fibers that are associated with neuropathic pain induced by chemotherapeutic agents have been reported. The present view is that these anticancer agents interfere with mitochondrial energetics, resulting in energy deficiency that leads to dysfunction of the sodium-potassium pump and axonal depolarization. In the axons of animals treated with paclitaxel oxygen consumption is deficient and decreased amounts of ATP is produced [7,31]. Oxaliplatin has been shown to affect voltage-gated sodium-channels in sensory neurons that leads to sensory hyperexcitability [9,20,21].

Depending on the chemotherapeutic agent used, CIPN can be pure sensory neuropathy (cisplatin, oxaliplatin, carboplatin) or mixed sensorimotor neuropathy with or without autonomic nervous system dysfunction (vincristine, taxanes). The incidence and severity of the neuro-toxicity depend on the type of drug used and are dose-dependent. The rate of CIPN correlates with the cumulative dose delivered and dose per treatment cycle. A wide variety of factors, including treatment schedule, duration of therapy, concomitant medications and co-morbidities, affect the incidence of CIPN. The most common symptoms are burning pain in a “stocking and glove” distribution, allodynia, hyperalgesia, paresthesia, autonomic and motor dysfunctions [18]. Neuropathic pain is an important side effect for many patients and it may occur early in therapy or it can be deferred in time. CIPN can develop even after a single drug application (with oxaliplatin) [1]. Moreover, its psychological aspects in malignant diseases should not be underestimated [11]. Many patients develop anxiety, depression, and suicidal thoughts that affect the perception of pain. It is also reported that the recovery from neuropathic symptoms is often incomplete and a long period of regeneration is required to restore function.

Table 1 presents the most commonly used experimental models causing CIPN in male rats. They consist of injection scheme of an anticancer agent (taxane, vinca alkaloid or platinum derivative).

Table 1. Experimental models of CIPN in male rats.

Anticancer agent causing neuropathy	Experimental design	Time	Application	Cumulative dose (mg/kg b.w./animal)	Reference
Vincristine	0,1 mg/kg/day, 5 days/week	12 days	i.v.	1	[30]
Vincristine	0,075 mg/kg/day	10 days	i.v.	0,75	[2]
Vincristine	0,1 mg/kg/day	14 days	i.v.	1,4	[19]
Vincristine	0,15 mg/kg/2 days	10 days	i.v.	0,75	[4]
Vincristine	0,1 mg/kg/day, 5 days/week	12 days	i.p.	1	[29]
Vincristine	0,05 mg/kg/day	10 days	i.p.	0,5	[26]
Paclitaxel	16 mg/kg/week	5 weeks	i.p.	80	[6]
Paclitaxel	32 mg/kg X 1	1 day	i.p.	32	[6]
Paclitaxel	2 mg/kg/2 days	7 days	i.p.	8	[22]
Paclitaxel	1 mg/kg/day, 5 days/week	2 weeks	i.p.	10	[10]
Docetaxel	10 mg/kg, 1/week	4 weeks	i.v.	40	[24]
Cisplatin	2 or 1 mg/kg/3 days	4 weeks	i.p.	15	[3]
Cisplatin	3 mg/kg, 1/week	5 weeks	i.p.	15	[5]
Cisplatin	2 mg/kg, 1/week	5 weeks	i.p.	10	[28]
Cisplatin	0,5 mg/kg/day	3 days	i.p.	1,5	[8]
Cisplatin	2 mg/kg X 1	5 days	i.v.	2	[12]
Oxaliplatin	2 mg/kg, 2/week	4 weeks	i.v.	16	[16]
Oxaliplatin	6 mg/kg X 1	30 hours	i.p.	6	[15]
Oxaliplatin	2 mg/kg X 1	5 days	i.v.	2	[12]

Experimental tests for neuropathic pain evaluation

Von Frey Hair Test (FHT)

Fibers with different thickness (Von Frey hairs) are used. They apply pressure on the lower surface of the animal's paw. The animal withdraws its paw when the pain threshold is reached.

Heath Plantar Test (HPT)

The method consists of application of a thermal stimulus (infrared ray) on the paw of the animal. When the pain threshold is reached, the animal withdraws its paw. The latency of the withdrawal is measured and used as a criterion for nociception [14].

Experimental models with chemical stimuli, used for inflammatory nociceptive pain examination

Carrageenan-induced hyperalgesia

The subplantar injection of carrageenan solution leads to an acute aseptic

inflammatory reaction. The paw edema and the pain sensitivity are measured using analgesimeter on the 1st, 2nd, 3rd and 24th hour after the injection.

Adjuvant arthritis

Freund's adjuvant is used for subplantar injection. This leads to an autoimmune inflammatory reaction, polyarthritis, and hyperalgesia.

Formalin assay

The animal receives a subcutaneous injection of diluted formalin solution. The animal behavior is studied during the first 5 minutes after the injection (1st phase) and during the period 20th-30th minutes after the injection (2nd phase). Flinching, licking and biting of the paw are used as a sign of the induced pain. The first phase is a result of a direct chemical stimulation of the nociceptive receptors. The second phase is a result of a peripheral inflammation and a subsequent excitation of nociceptive spinal neurons [13,17].

Experimental tests used for nociceptive pain examination

Paw pressure test (Randall–Selitto test)

The test uses an apparatus, known as analgesimeter. A pressure is applied to the hind paw of an animal and when the pain threshold is reached, the animal withdraws its paw.

Incapacitance Test

The animal is positioned in a chamber, a part of an apparatus. Both of its hind paws are placed on different plates, which detect the pressure that the animal applies on every limb. The apparatus measures which part of the body weight of the animal is applied on each limb. Usually, one of the limbs is injured (a model of inflammation or nerve injury). The distribution of the body weight correlates with the level of the pain in the injured paw [14].

Tail-Flick Test (TFT)

The test is similar to the Heath Plantar test (see above), however, the thermal stimulus is applied on the rat's tail.

Hot Plate Test (HP)

The animal is placed in a transparent container. The floor represents a hot plate, which temperature could be adjusted. The pain threshold is measured via the animal behavior. Vocalization, licking of hind paw, or escape attempt are used as end-points in the testing. The cut-off time is set at 30 s in order to avoid thermal injury of the paws.

Conclusion

The study of pain and analgesia is an important area of pharmacological research that has led to a number of significant advances in the treatment of acute and chronic pain. Since pain is multifactorial, different experimental models were developed along the years. Other animal models exist, and many others may be developed in the future.

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AO3. CHECKING THE FIELD EFFICACY OF SOME BULGARIAN ACARICIDES AGAINST *VARROA DESTRUCTOR*

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AO4. BRIEFLY ABOUT COLORECTAL CANCER AND SOME CHALLENGES OF TARGETED ANTITUMOR THERAPY

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AO5. SPHEROIDS AS AN IN VITRO MODEL

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Traditionally, mesenchymal stem cells (MSC) are cultured in 2D conditions where they attached to the surface of the laboratory vessel and are able to make a monolayer. Beside this method in last years 3D culturing emerged as an alternative. In 3D MSC obtained different morphological characteristics compared to the monolayer and enhanced production and secretion of molecules executing their biological properties.
The most common way of 3D culturing is formation of cell structures called spheroids in hanging drops. Spheroids have many advantages compared to 2D culture. These structures are closer to *in vivo* systems. In 3D cultures interactions between different cells types are closer to natural conditions. 3D cultures could be used as models for cancer and cell therapies research, drug testing, etc.
Aim: Comparison of morphology and MSC panel markers expression in 3D and 2D cultures.
Material and methods: Two types MSC: Wharton's jelly mesenchymal stem cells (MSC-WJ) and adipose tissue mesenchymal stem cells (MSC-AT) were cultured in 2D and 3D conditions. Light and confocal microscopies were used for morphology observing. The surface markers (CD29, CD73 and CD90) expression and survival rate of cells were examined by FACS.
Results: Both cell cultures (MSC-WJ and MSC-AT) form spheroid structures about 24 hours after assembling of 25 000 of cells in the drops but there are differences between sizes of structures made from the different MSC cultures. MSC-AT cells formed smaller spheroids (~450μm/ diameter) compared to MSC-WJ which made bigger structures (~600μm/ diameter). Changes in expression of surface markers in 3D conditions compared to 2D monolayers were observed. Cells reduce expression of CD29, CD73 and CD90 by 2-5 folds in 3D conditions. Cell vitality was measured by PI exclusion assay and decrease of 30% in 3D compared to 2D was detected in both cell types.
Conclusion: Our results showed that dimension of spheroids depend of the type of MSC. Changes in cell morphology and expression profile of MSC panel markers in cells when they are cultured in 3D conditions were detected.

Acknowledgments: This work was supported by the National Science Fund, DCOST01/18.

AO6. ORGAN ON A CHIP TECHNOLOGY

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AO7. NEW MATERIALS FOR WOUND DRESSINGS – THE CHALLENGES OF CYTOCOMPATIBILITY ASSESSMENT

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Session B

Chairpersons:

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BO2. INVESTIGATION OF THE RELATIONSHIP BETWEEN PHYSICAL ACTIVITY AND UPPER RESPIRATORY TRACT INFECTIONS AMONG MARMARA UNIVERSITY SCHOOL OF MEDICINE PHASE-1 STUDENTS

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Introduction: Upper respiratory tract infections (URTIs) are one of the most common causes of patients' application to family physicians. Moderate, low-intensity, regular exercise has been shown to improve the immune system in this regard, whereas intense physical exercise has been shown to weaken the immune system. This study was carried out to evaluate the level of physical activity and URTI relationship among the Marmara University School of Medicine Phase-1 students.

Materials and Methods: The research is a cross-sectional research conducted between the MUSM Phase-1 students. The sample has reached 527 people and 419 feedbacks have been received. In the first stage of the distributed questionnaire, the physical activity level of the

participants was evaluated with PAEQ (physical activity evaluation questionnaire) with 16 questions. In the second stage, a questionnaire prepared by the researchers was applied. The second questionnaires were distributed after 15 days to give them time to observe their URTI symptoms. The number of respondents in both surveys is 204. In the evaluation of categorical data, Chi-square and Fisher were used and comparison of the averages was made using the non-parametric Mann-Whitney U Test. The permission was obtained from the MUSM ethics committee and the MUSM Deanery.

Results: 49.5% of the participants (n = 101) were women, and 50.5% (n = 103) were men. When participants were classified according to physical activity levels, it was found that 1.0% (n = 2) had low level, 69.6% (n = 142) had moderate level, and 29.4% (n = 60) high level of physical activity. During the 15-day observation period, 37.3% (n = 76) of the students found to have URTI. There was no significant relationship between physical activity levels and upper respiratory tract infections (p = 0.819). Similarly, there was no significant relationship between the level of physical activity and the number of days with URTI (p = 0.956). When the total amount of time in one week which participants spent during physical activities is evaluated; it was found that the participants who suffered from URTI, on average spent 40.92 minutes (standard deviation 52.49) and the participants who did not suffer from URTI, on average spent 39.33 minutes (standard deviation 47.99) in physical activities (*p=0839).*

Conclusion: It was assessed that the level of physical activity had no effect on URTI exposure ratio and the number of days which participants suffered from URTIs. Although no correlation has been found between physical activity and URTI in our study, the positive effects of exercise are known and some steps could be taken to encourage medical students to take part in physical activities.

Key Words: physical activity, URTI, upper respiratory tract infection, exercise, sports

BO3. INTRODUCE YOURSELF 1

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BO4. AUTOPHAGY AND CANCER – WHAT DO WE (NOT) KNOW

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Autophagy is an evolutionary conservative precisely regulated catabolic process important for maintaining physiological balance at the cellular, tissue and body level. It involves: lysosomal breakdown of mis-structured proteins, damaged organelles and toxic aggregates; reducing oxidative stress and protecting the cell against damage. Autophagy is induced in response to various factors, including: nutrient deficiency, metabolic stress, hypoxia, antitumor agents, radiation effects, and to ensure cell survival.
Autophagy plays an important role in carcinogenesis as it can have both tumor-suppressing and tumor-protective functions. Autophagy promotes tumor growth and progression by facilitating the survival of cancer cells in the tumor microenvironment (hypoxia, acidification) and contributes to the development of resistance to conventional and targeted antineoplastic agents. In addition, autophagy is also associated with protection of the macro-organism from intracellular pathogens. Unproper regulation of autophagy can result in various pathological conditions (neurodegenerative diseases, immunopathologies, etc.).
Numerous studies have shown that exploring the mechanisms of autophagy will contribute to increasing the effectiveness of anti-tumor therapy as well as creating new antimicrobial, immunomodulatory and neuroprotective agents.

Acknowledgements: This work was supported by National Science Fund in Bulgaria – Grant №ДКОСТ 01/19 from 08.12.2017.

BO5. DISULFIRAM INHIBITS 2D AND 3D GROWTH OF VIRUS-TRANSFORMED CHICKEN HEPATOMA AND RAT SARCOMA CELLS IN VITRO

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BO6. 2-CARBAMIDO-1,3-INDANDIONE – A POTENTIAL BIOMARKER AND AN ANTITUMOR AGENT. QUANTUM-CHEMICAL MODELING OF ITS COMPLEXES WITH NUCLEOTIDES OF DNA AND RNA

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The 2-substituted 1-3-indanedione derivatives have a wide application in the field of medicine and biology. 2-Carbamido-1,3-indanedione derivatives have potential as antineoplastic agents, and 2-carboxamide-indan-1,3-dione(CAID) itself as biomarker
From a fluorescence-microscopic analysis of CAID in Balb / c 3T3 cell lines, is established its cytotoxicity, its ability to penetrate through cell membranes and also its cellular localization. Fluorescence microscopy data suggests that CAID penetrates into DNA and RNA-containing cell structures, such as nucleus and nucleoli. The in vitro antitumor activity of the compound is determined on different cell lines, with CAID showing good antineoplastic properties. In order to elucidate the affinity of CAID to nucleic acids, quantum chemical B3LYP/6-31+G (d, p) calculations were made. Two ways of binding CAID to the five nucleotides contained in DNA and RNA are discussed - to the nitrogenous base and to the phosphate group. The interaction energies of the complexes formed are calculated to evaluate the binding priority of CAID to nucleotides. It has been found that 2-carboxamide-indan-1,3-dione binds predominantly to the phosphate group in thymidine monophosphate.

BO7. ASSOCIATION OF HERPESVIRUS INFECTION WITH MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME (ME/CFS)

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Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a serious, chronic, multisystem disease characterized by severe fatigue and other disabling symptoms. ME/CFS is with a considerable social and economic impact and a prevalence rate between 0.2% and 2.6%–(1). There are no data concerning ME/CFS in Bulgaria. So far, the etiology and pathogenesis of ME/CFS are unknown and there are no validated disease biomarkers. Many factors, such as immunological, infection related, metabolic or neurological are associated with the pathogenesis of ME/CFS. Viral infection has been reported as trigger of ME/CFS. Infection with herpesviruses, especially persistent infections with Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesviruses 6 and 7 (HHV-6, HHV-7), have been suspected to be associated with ME/CFS (2-4). It was suggested that these infections may cause chronic activation of the immune system with abnormal regulation of cytokine production leading to ME/CFS. However, the role of these viruses in ME/CFS remains controversial.
As a partner of European network EUROMENE and as member of the Working group on biomarkers, particularly Infection-associated ME/CFS biomarkers, we are involved in characterization of EBV, CMV and HHV-6 and 7 infections in ME/CFS patients in Bulgaria. Studies on prevalence of EBV, CMV and HHV-6 in biological samples obtained from patients with ME/CFS and controls to study the potential role of these viruses in ME/CFS are in progress. Here we present a review of the research on herpesviruses involvement in ME/CFS pathogenesis and also our preliminary original results in this field.
Acknowledgements: This work was supported by the Bulgarian National Science Fund (Grant No ДКОСТ01/9) and by COST Action CA15111

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BO8. CAN HUMAN HERPES VIRAL INFECTION BE A FACTOR IN TRIGGERING AUTOIMMUNE THYROIDITIS?

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ABSTRACT

The diagnosis of Hashimoto's thyroiditis disease (HT) (chronic lymphocytic thyroiditis), is based on the symptoms and blood test results of thyroid hormones and thyroid stimulating hormone (TSH) levels. This is an autoimmune condition where the immune system attacks the thyroid gland under a combination of factors including genes, gender and age. Autoimmune diseases are chronic, not curable condition, where symptoms can only be alleviated by administering immunosuppressants with negative effects and increased susceptibility to viral infections. Risks arise for the emergence of new infections that may complicate the condition of the patient. Inflammation of HT results in an underactivity of the thyroid gland affecting women and rarely men, as well as children. In addition to genetic predisposition, several viruses, including herpesviruses, have been suggested as possible triggers of this condition. Previous results showed that HT patients have an increased cellular immune response directed against HHV-6 U94 protein and increased NK activity against infected thyrocytes, that could effect an inflammatory status in Hashimoto's thyroiditis patients. Microscopic observations of thyroid tissues found morphological evidences of productive viral infection in HT patients' glands.

KEYWORDS: Hashimoto's thyroiditis, autoimmunity, human herpesvirus-6(HHV-6)

Data overview

Hashimoto's thyroiditis (HT) is an autoimmune disease in which the thyroid gland is gradually destroyed. At the early stages there may be no symptoms only a painless enlargement-goiter. Some patients develop hypothyroidism with accompanying of depression, fatigue, weight gain, constipation and joint or general pains [9]. After some years the potential complications may lead to thyroid lymphoma [8].

Diagnosis is made after blood tests for TSH, T4, and anti-thyroid autoantibodies. Other conditions that can produce similar symptoms include Graves' disease and nontoxic nodular goiter [8]. Pathoanatomic diagnosis of the autoimmune HT (stromal variant) is based on the increase of the gland size, its nodular structure and the characteristic large lymphocyte infiltration. HT patients usually develop hypothyroidism, which can contribute to high cholesterol and heart diseases. Rarely severe, untreated hypothyroidism may lead to life-threatening myxedema coma, requiring urgent medical care [5, 8]. Although the several up-to-date management guidelines for subclinical and clinical hypothyroidism, treatment decision-making have to consider the progressive changes in the levels of serum TSH and free thyroxine (T4). Medical doctors have difficulties determining the ideal starting point for thyroid hormone supplementation. Thus, the normal range of TSH depends on patient age and cardiovascular conditions. Individuals may also have a narrow "normal" TSH reference range meaning that there is a risk of adverse health outcomes even within the reference range [20]. Hypothyroidism is treatable with medicines but if left untreated can cause problems with getting pregnant and during pregnancy. Treatment usually depends on the grade of thyroid damage [14].

Since Hakaru Hashimoto first described autoimmune thyroiditis disease (AITD) in 1912, significant progress has been made in our understanding of this chronic inflammatory process [7, 20]. HT is thought to be due to a combination of genetic and environmental risk factors, especially if there is a family history of the condition or another autoimmune disease. Conditions linked to HT include: Addison's disease, a hormonal disorder; autoimmune hepatitis, a disease in which the immune system attacks the liver; celiac disease, a digestive disorder; Lupus, a chronic, or long-term, disorder that can affect many parts of your body; pernicious anemia, a condition caused by a vitamin B12 deficiency; rheumatoid arthritis, a disorder that affects the joints and sometimes other body systems; Sjögren's syndrome, a disease that causes dry eyes and mouth; type 1 diabetes, a disease that occurs when your blood glucose, also called blood sugar, is too high; vitiligo, a condition in which some parts of the skin are not pigmented [5]. Recently, "immunoglobulin G4 (IgG4) thyroiditis" has been added as a unique subtype of HT. characterized by fibrosis, lymphoplasmacytic infiltration, degeneration of follicular cells, progression of hypothyroidism, diffuse low echogenicity, higher antibody levels compared to non-IgG4 thyroiditis, and especially a lower female-to-male ratio.

Along with the particular genetic background, several viruses, including herpesviruses, have been suggested to play a role as possible environmental triggers in autoimmune diseases [10, 19]. Data of some investigations performed showed that HT patients have an increased cellular immune response directed against the HHV-6 U94 protein and increased NK activity directed against HHV-6 infected thyrocytes, which suggested that HHV-6 might contribute to HT development, increasing NK cell secretion of inflammatory cytokines leading to an inflammatory status in these patients [13]. Herpes viruses are not always active, but when they become such they are able to provoke an autoimmune response in the thyroid via their ability to conceal or by involuntary activation. Under stressful conditions stress hormones suppress the immune system and can trigger the transformation of dormant viruses into active ones [3, 5, 8].

Human herpesvirus-6 (HHV-6) is a lymphotropic and neurotropic betaherpesvirus in two variants, variant A and B [1, 15]. The primary targets for HHV-6 are usually lymphocytes [11], in recent years some researchers have reported the presence of HHV-6 sequences in different solid organs, including thyroid gland [2, 4, 16, 17]. HHV-6 is immunomodulating virus and is thought to be involved in several autoimmune disorders: autoimmune hemolytic anemia/neutropenia [20], autoimmune acute hepatitis [6] and MS [12]. Microscopic observations of thyroid lesions of HT patients, especially with elevated number of HHV-6 copies in thyroid glands revealed processes of inflammation and lymphocyte infiltratation and ultrastructuraly virus-like inclusions - unenveloped, only tegumented and enveloped tegumented nucleocapsids in the cytoplasm and Golgi cisternae and cytoplasmic MVB-like membrane structures in thyrocytes, characteristic of herpes infection [18].

In conclusion, the detection of evidences of HHV 6 infection of thyroid gland of HT patients could indicate the important role and participation of the viral infection in the development of its autoimmune disorder.

Acknowledgments: This work was supported by the Bulgarian academy of Sciences under Project. Persistent human herpesvirus infection as a trigger factor in development of autoimmune thyroiditis P10/27.03.2018/ between Bulgaria and Latvia.

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BO8. BEHAVIORAL AND MOTIVATIONAL MECHANISMS OF THE BRAIN

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Session C

Chairpersons:

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

Tanya Zhivkova, PhD

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

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

CO1. INTRODUCE YOURSELF 2

Isabel Rocha Migue

CO2. INTRODUCE YOURSELF 3

Isabel Hueso Heredi

CO3. CANCER EPIGENETICS

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

CO4. DETECTION OF GANGLIOSIDES AND ANTI-GANGLIOSIDE ANTIBODIES IN "IN VITRO-", "IN VIVO-" AND "IN VIVO+IN VITRO" -EXPERIMENTAL MODELS

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Abstract

The values of gangliosides GM3, GM1 and GD1a, but also of antibodies to them were assessed in *in vitro*-, *in vivo*- and "*in vivo*+*in vitro*"-experimental models. So, lysates from laboratory-incubated cultures (*in vitro*-models) of normal mouse embryonic cells, of mouse malignant cells and mixed of both cell types, as well as from rat brain and pancreas were prepared. Separate aliquots from all lysates were passed through GSH-Agarose Columns for selection of molecules with affinity to Glutathione reduced form – GSH (*in vivo*-models). Other aliquots of the extracts from both organs were mixed with isolated protein SCGN from cells, containing additionally-inserted copy of gene *scgn* by transfection with appropriate recombinant DNA-constructs ("*in vivo*+*in vitro*"-models). The values of gangliosides and anti-ganglioside antibodies were estimated by ELISA. In confirmation of literature data, statistically significant frequency of statistically higher values of ganglioside GM3 and anti-GM3 antibodies were observed in the probes from malignant and mixed cell cultures,

compared with these from normal cells. Statistically significant frequency of statistically higher values of this ganglioside and antibodies to it were assessed in the probes of the "*in vivo+in vitro*"-models, compared to these from the *in vivo*-models. The differences could be explained with decreased titers in equivalent volumes of biological material, at the expense of bio-molecules, not possessing affinity. Wide varieties in the values of the other two gangliosides and antibodies to them were assessed. These data were explained with eventual existence of the gangliosides besides in free form, also in various bounded forms with different molecules. The results confirmed the proved possibility for production of antibodies from non-lymphoid cells in appropriate conditions.

Key words: gangliosides, anti-ganglioside antibodies, experimental models, ELISA, antibodies-producing non-lymphoid cells.

Introduction

Gangliosides have been characterized as glycosphingolipids, which are 6% from all lipids in the central nervous system [27]. Their structure is based on the ceramide molecule, bounded to carbohydrates, including sialic residues. These molecules have been determined as components of the oligodendroglia – oligodendrocytes' and/or Schwann cells' myelin envelope. They have been found to participate in the structure of synaptic membrane, but the spectrum of each ganglioside has been found to be different in the separate regions of the nervous system. As such, gangliosides participate in many intra- and extra-cellular inter-molecular interactions in cascade regulatory pathways, responsible for the differences between the cells, as well as living action, specific functions, growth, proliferation and differentiation [3, 8, 11, 12, 17].

The **aim** of the current study was directed to determination the presence and values of gangliosides GM3, GM1 and GD1a and of specific anti-ganglioside antibodies to each one of them. Biological materials from several types of experimental models were used: *in vitro* - from laboratory-incubated cell cultures; *in vivo* - from anatomic organs, and "*in vivo+in vitro*" – from mixtures of organs and cell cultures.

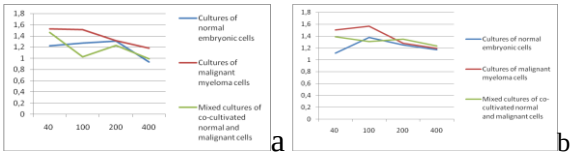
Materials and Methods

The *in vitro*-models were presented by lysates from three types of laboratory-incubated cell culture: of normal mouse embryonic cells 3T3, of mouse malignant myeloma cells, and mixed of co-cultivated cells from both types. The *in vivo*-models were presented by extracts of rat brain and pancreas. Separate aliquots from the so prepared total extracts (control probes), containing the full composition of bio-molecules, were passed through GSH-Agarose Columns, for separation of "selected" molecules with affinity to GSH. This tri-peptide has proved as a key molecule in the control of cell growth and proliferation [9, 11]. The experimental models "*in vivo+in vitro*" were presented by lysates from the same anatomic organs, determined as containing molecules with affinity to hormone-like protein Secretagogin (SCGN) after passing through GSH-Agarose Columns. These probes were prepared by mixing of separate aliquots from the lysates of both organs with isolated SCGN from cells, containing previously transferred tumor-suppressor gene *scgn* by transfection with appropriate recombinant DNA-constructs. Besides the established action of this protein as a tumor suppressor [12], its protective role in endocrinology and neurodegenerative disorders has also been shown [2, 17]. The values of the three gangliosides, as well as of antibodies to each one of them in all tested probes, were determined by slight modifications of the ELISA-

method of Mizutamari et al. (1994) [18]. In determination the values of anti-GM3 antibodies, 1000 ng of the ganglioside (Sigma) in 100 ml of methanol were pipetted into micro-titre plate wells, containing the respective probes. In determination the values of GM3, instead of ganglioside solution, sera, previously proved to contain specific anti-GM3 antibodies, were added the tested probes. After air drying, the wells were blocked with BSA-PBS (Sigma) (1% bovine serum albumin in phosphate-buffered saline) for 1 hour. After six-fold washing with PBS, 100 ml of each one of the prepared lysates from the cell cultures, described above, diluted 1:20 to 1:5000 in PBS, were added to each well and incubated overnight. Subsequently, the plates were washed six-fold with PBS. Binding was detected by following 2 hours incubation period with BSA-PBS (Sigma) diluted (1/3200) peroxidase-conjugated goat anti-human IgG antibodies (Bul Bio Ltd., NCIPD, Sofia) and with BSA-PBS (Sigma) diluted (1/4800) peroxidise-conjugated goat anti-human IgM antibodies (Bul Bio Ltd., NCIPD, Sofia). All the incubations were performed at 4^{o}C. After six-fold washing with PBS, colour development was achieved in a substrate solution, containing 15mM O-phenilendiamine and 0.015% H_2O_2 in 0.1M sodium acetate buffer (0,2 M CH_3COONa/ 0,2 M CH_3COOH; pH 5.0) at 20ºC. The reaction was stopped after 30 minutes by addition of 50 ml of 1N H_2SO_4, and the optical density (OD) was read spectrometrically at 490 nm on ELISA-reader (TECAN TM, Sunrise, Austria). Non-specific antibody binding (OD value in a well not containing the specific molecule in the respective probe) was subtracted for each measurement. Probes were considered strongly positive only if the mean OD exceeded 2 ± SD (standard deviation) of the controls. Determinations were carried out in triplicate.

Results and Discussion

Statistically significant frequency of statistically higher titers of both ganglioside GM3 and anti-GM3 antibodies were observed in the probes from the neo-plastic cells and mixed cultures, compared with these in the lysate from normal cells (Fig. 1 – a, b).

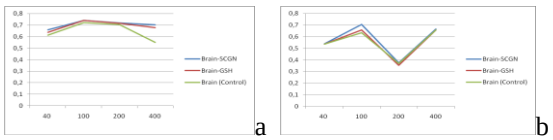


a b

Figure 1: Values of ganglioside GM3 (a) and of anti-GM3 antibodies (b) in lysates from *in vitro*-cultures of normal mouse embryonic cells, mouse malignant myeloma cells, and mixed of both cell types, OD – optical density.

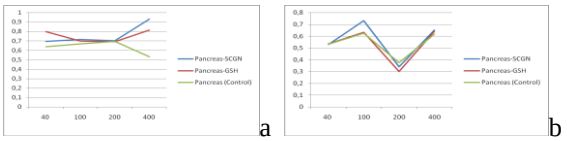
These results were in agreement with the literature findings about the proved role of these molecules as markers of malignancy [19]. A protective function of the increased values of this ganglioside in malignant cells has been proposed on the basis its accumulation during their apoptosis [26]. It can act as a regulator of membrane-transmitted signals and by modulation of the functions of tumor suppressors [5].

Statistically significant frequency of statistically higher values of the same ganglioside and antibodies to it were established in the lysates containing molecules with affinity to SCGN and to GSH, respectively, compared to the control extracts, non-passed through columns and containing the full composition of biological molecules (Figs. 2, 3).



a b

Figure 2: Values of ganglioside GM3 (a) and of anti-GM3 antibodies (b) in lysate from rat brain: control probe, containing the full composition of bio-molecules; containing selected molecules with affinity to GSH; containing selected bio-molecules with affinity to protein SCGN, OD – optical density.

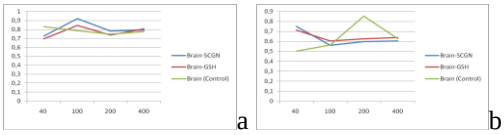


a b

Figure 3: Values of ganglioside GM3 (a) and of anti-GM3 antibodies (b) in lysate from rat pancreas: control probe, containing the full composition of bio-molecules; containing selected molecules with affinity to GSH; containing selected bio-molecules with affinity to protein SCGN, OD – optical density.

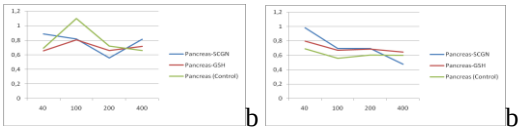
These differences could be explained with probably decreased titers in equivalent volumes of the respective biological material, at the expense of the presence of other bio-molecules, not possessing such affinity. These data confirmed the literature findings about the importance of this ganglioside for the insulin signaling pathways [13, 14] in the control of diabetes [10]. These results were also in agreement with the neuro-protective function of this ganglioside GM3 [28], supporting its role in the control of neurodegenerative processes by the proved relationship of disorder symptoms and mutations, connected with lack of enzyme GM3 synthase [24, 29].

Wide varieties in the values of the other two gangliosides, as well as of the antibodies to them, were assessed in the three types of experimental models (Figs. 4-7). The observed variations suggested the eventual existence of each one ganglioside besides in free form, also in different bounded forms with different bio-molecules, depending of the respective functions, in which it participates, as well as with different localization, accumulation and distribution of gangliosides and specific to them antibodies in separate regions of the various cells, tissues and organs [6, 18].



a b

Figure 4: Values of ganglioside GM1 (a) and of anti-GM1 antibodies (b) in lysate from rat brain: control probe, containing the full composition of bio-molecules; containing selected molecules with affinity to GSH; containing selected bio-molecules with affinity to protein SCGN, OD – optical density.



b b

Figure 5: Values of ganglioside GM1 (a) and of anti-GM1 antibodies (b) in lysate from rat pancreas: control probe, containing the full composition of bio-molecules; containing selected molecules with affinity to GSH; containing selected bio-molecules with affinity to protein SCGN, OD – optical density.

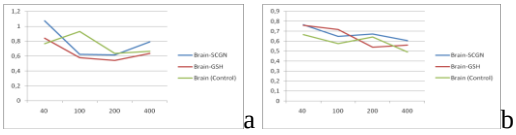


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Figure 6: Values of ganglioside GD1a (a) and of anti-GD1a antibodies (b) in lysate from rat brain: control probe, containing the full composition of bio-molecules; containing selected molecules with affinity to GSH; containing selected bio-molecules with affinity to protein SCGN, OD – optical density.

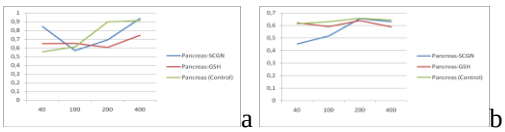

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Figure 7: Values of ganglioside GD1a (a) and of anti-GD1a antibodies (b) in lysate from rat pancreas: control probe, containing the full composition of bio-molecules; containing selected molecules with affinity to GSH; containing selected bio-molecules with affinity to protein SCGN, OD – optical density.

The current results were in agreement with literature data for antagonistic actions of ganglioside GM3 with gangliosides GM1 [6] and GD1a [20]. On the other hand, the data obtained supported the established variety in the role of GM1 and GD1a in the pathogenesis and control of malignancies, diabetes and neurodegenerative disorders, depending of the substances, to which bounds each one of both gangliosides in the concrete cell types [7, 22, 23, 25]. These data were also in confirmation of the ageing changes in the values of the three gangliosides and of antibodies to them in people and rats [15, 16, 30]. Also, these data confirmed the proved ability of many types non-lymphoid cells, tissues and organs [1, 21], including malignant cell types [3], to produce immunoglobulins (antibodies). As another explanation, in particular for the *in vitro*-incubated cells, could be accepted the proposed possibility for lymphoid differentiation direction of incubated in appropriate laboratory conditions immature embryonic cells [4].

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CO5. RUTHENIUM – ONE METAL WITH MANY FACES

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CO6. RUTHENIUM (III) COMPLEXES WITH SCHIFF BASES – EFFECT OF VIABILITY AND PROLIFERATION OF HUMAN AND RAT VIRUS-TRANSFORMED CELLS

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CO7. ZN(II) COMPLEXES WITH SCHIFF BASES AS CYTOTOXIC AGENTS

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CO8. EFFECT OF DEOXICHOLIC ACID AND ITS METAL COMPLEXES ON VIABILITY AND PROLIFERATION OF COLON CANCER DRUG SENSITIVE AND DRUG RESISTANT CELLS

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