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Glycosylation changes in different developmental stages of *Trichinella*

Rositsa S. Milcheva[1,3], Svetlozara L. Petkova[1], Pavol Dubinský[2], Zuzana Hurniková[2] & Pavel Babál[3]*

[1]Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., block 25,
1113 Sofia, Bulgaria
[2]Parasitological Institute, Slovak Academy of Sciences, Hlinkova 3, SK-04001 Košice, Slovakia
[3]Department of Pathology, Faculty of Medicine, Comenius University, Sasinkova 4, SK-81372 Bratislava, Slovakia; e-mail:
pavel.babal@fmed.uniba.sk

Abstract: The in situ identification of carbohydrate structures in Trichinella spiralis intestinal larvae, adults and L1 muscular larvae was carried out by lectin histochemistry, with emphasis on the O-linked glycans. The absence of reactivity with two lectins-TML and MAL indicated thatTrichinella spiralis does not synthesize sialic acid. Reactivity with HPA, VVL-B4, PNA and UEA-I staining suggested that T. spiralis synthesizes and expresses on its cuticle O-linked glycans analogous to Tn-antigen (GalNAc-*α*-Ser/Thr), T-antigen (Gal-*β*1,3–GalNAc-*α*-Ser/Thr) and also structures analogous to A-blood group antigens (GalNAc-*α*1,3-Gal-*β*1,3(4)-(Fuc-*α*1,2-)-R). Expression of the saccharidic moieties is stage-specific. Blood group-A and T-antigen structures were identified on the cuticle of the intestinal and muscular larvae. The Tn-antigen structure was missing in the intestinal larvae. Appropriate ligands for WGA were not identified in the adult individuals. The obtained results may contribute to a better understanding of the glycobiology of this parasitic nematode in relation to occupation of its intracellular niche. The presence of saccharidic structures analogous to some of those expressed on the intestinal epithelial cells may serve as a protective shield on the surface of the parasite.

Key words: Trichinella spiralis; lectin histochemistry; T-antigen; Tn-antigen; O-glycans

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Apoptosis as the adaptation mechanism in survival of *Trichinella spiralis* in the host

Pavel Babal & Rosica Milcheva & Svetlozara Petkova & Pavol Janega & Zuzana Hurnikova

Abstract: The study evaluates the role of apoptosisinducing factor (AIF) in the process of striated muscle cell transformation after occupation by *Trichinella spiralis*. Its relationship with other apoptosis-related factors [apoptotic protease-activating factor 1, Bcl-2 associated protein X (BAX), Bcl-2, caspase 3, survivin, poly (ADP-ribose) polymerase-1 (PARP-1), and endothelial and inducible (iNOS) nitric oxide synthase] was evaluated by immunohistochemistry. In the context of low BAX and caspase 3 expression and strong distribution of AIF in the sarcoplasm and nucleus at the very early stage of infection, we suppose that AIF-mediated signaling is involved in the apoptosis activation in the area of *Trichinella* occupation. In the time course of nurse cell formation, survivin and caspase 3 migrated into the enlarged nuclei with strong PARP-1 expression. In the end of encapsulation of *Trichinella*, expression of all proapoptotic factors ceased and only survivin in the nuclei and Bcl-2 positivity in the cytoplasm persisted in the formed nurse cell. The expression of sarcoplasmic iNOS was absent during the process of muscle cell de-differentiation and reappeared within the nurse cell. It seems that upregulation and downregulation of factors of apoptosis in the skeletal muscle cell represents an adaptive mechanism providing a comfortable niche for the parasite.

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Alcohol based fixatives provide excellent tissue morphology, protein immunoreactivity and RNA integrity in paraffin embedded tissue specimens

Rositsa Milcheva[a,c], Pavol Janega[a], Peter Celec[b], Russy Russev[c], Pavel Babál[a],∗

[a]Department of Pathology, Faculty of Medicine, Comenius University in Bratislava, Sasinkova 4, 81372 Bratislava, Slovakia
[b]Department of Molecular Biomedicine, Faculty of Medicine, Comenius University in Bratislava, Sasinkova 4, 81372 Bratislava, Slovakia
[c]IEMPAM, Bulgarian Academy of Sciences, "Acad. G. Bonchev" Str. 25, 1113 Sofia, Bulgaria

Abstract:

Fixation techniques preserving morphological fidelity, protein antigenicity and integrity of nucleic acids can have a high impact on both basic and applied biomedical sciences and diagnostic pathology. Different types of mouse tissues were fixed with neutral buffered formalin, ethanol supplemented with acetic acid and modified methacarn (methanol-Carnoy) fixative. The alcohol-fixed samples were processed in an Autotechnicon tissue processor or in an incubator. The preservation of tissue morphology was assessed in all specimens and the immunoreactivity was evaluated with antibodies specific for proteins with nuclear, membrane or cytoplasmic localization. RNA was extracted from all groups of fixed hind limb skeletal muscle specimens and was assessed versus unfixed tissue for preservation of its quantity and quality by amplification of gene-specific fragments of different lengths. Both alcohol-based fixatives preserved the tissue architecture and the specificity of immunoreactivity in excellent quality; the trimming approach did not result in detectable differences. Oligonucleotide fragments of length between 108 and 577 base pairs were amplified from all groups of alcohol-fixed skeletal muscle specimens in amounts comparative to the unfixed muscle tissue. We conclude that both alcohol-based fixatives are an excellent tool for storage of tissue samples designed for immunohistochemical and mRNA expression studies when the access to fresh samples is limited.

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The occupation of intestinal epithelium by *Trichinella spiralis* in BALB/C mice is not associated with local manifestation of apoptosis related factors

Rositsa Milcheva & Svetlozara Petkova & Zuzana Hurniková & Pavol Janega & Pavel Babál

Abstract: *Trichinella spiralis* actively passes through the epithelial cells of the intestinal mucosa but morphologically, these cells do not manifest apparent damage. The possible activation of apoptotic mechanisms in the small intestine mucosa after infection with larvae and adults of *Trichinella spiralis* was explored by immunohistochemistry. Sporadic individual cells of normal intestinal epithelium showed activation of caspase-3, increased expression AIF, or Bax. The larval stage of intestinal trichinellosis was characterized by distortion of cells on the villus tips that were strongly reactive to caspase-3, Bax, and survivin antibodies. There was a transient loss of the survivin expression on the brush border of the epithelial cells at 15-h post infection, which reappeared on the fifth day. Bcl-2 changed its normal apical distribution and relocalized to the basal part of the epithelial cells. No significant changes of expression of the selected apoptosis-related proteins were observed in the intestinal epithelial cells immediately surrounding the worms. The presence of *Trichinella* affects intestinal epithelial cells, but unlike in muscle cells, invading them does not initiate apoptotic factors activation.

Доклади на Българската академия на науките
Comptes rendus de l'Acad´emie bulgare des Sciences
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SERUM SIALIC ACID LEVELS IN *TRICHINELLA SPIRALIS* INFECTED RATS

Dimitar Ivanov, Rositsa Milcheva, Svetlozara Petkova, Margarita Gabrashanska

Abstract: Sialic acids a large family of neuraminic acid derivatives, are acidic monosaccharides, present as terminal component of oligosaccharide chains of many glycoproteins and glycolipids. They play an important role in many physiological and pathological processes like cell-cell communications, cell-substrate interaction, adhesion, maintenance of serum glycoproteins in circulations, regulation of the immune response, and protein targeting. Studies have shown that the serum sialic acid evaluation can be a valuable indicator for diagnostics and prognosis of many inflammatory diseases. In the present study we investigated the content of free, lipid and protein bound sialic acid in normal serum and in serum of rats with *Trichinella spiralis* infection in different phases of the disease. The experiment covered 8 weeks post infection. The data analysis disclosed that the serum sialic acid concentrations in the *Trichinella* infected rats were significantly higher (at week 8) than those found in the healthy controls. A strong positive correlation was observed between the serum levels of different forms of sialic acid and the development of the disease.
Key words: Sialic acid, *Trichinella spiralis*, acute phase proteins, inflammation

Доклади на Българската академия на науките
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Tome 67, No 3, 2014

NUCLEAR DISTRIBUTION OF PROTEINS IN SKELETAL MUSCLE FIBRE INVADED BY *TRICHINELLA SPIRALIS*

Rositsa Milcheva, Svetlozara Petkova, Dimitar Ivanov, Ivan Iliev, Pavol Janega, Pavel Babal

Abstract: The nurse cell – *Trichinella spiralis* complex has been a subject of research interest for many decades because of the tendency of the striated myofibre to respond to the needs of the invasive parasite without any effort to defend itself. This biological system is an excellent model to explore the broad and still unidentified adaptive capacities of the striated muscle tissue. In this work we demonstrate up-regulation of the two nuclear proteins poly (ADP-ribose) polymerase-1 (PARP-1) and proliferating cell nuclear antigen (PCNA) within the nuclei of the occupied portion of skeletal muscle fibre in the time course of its transformation towards a Nurse cell after invasion by *T. spiralis*. We also show that the process of transformation is associated with nuclear distribution of apoptosis inducing factor (AIF), Bcl-2 associated protein X (Bax), and caspase-3 that normally reside in other cellular compartments, and proteins of *Trichinella* origin. Apparently, *Trichinella* has the ability to regulate the intracellular systems, which is still not fully understood. The presented results open some new views concerning plasticity of skeletal muscle cells.
Key words: nucleus, skeletal muscle, *Trichinella*

Доклади на Българската академия на науките
Comptes rendus de l'Acad´emie bulgare des Sciences
Tome 67, No 1, 2014

INFLUENCE OF FUMONISIN B1 AND DEOXYNIVALENOL ON THE IMMUNE SYSTEM OF CHICKENS AFTER APPLICATION IN QUANTITIES, NATURALLY PRESENTED IN FODDERS

Katerina Todorova, Petar Dimitrov, Reneta Toshkova, Simona Lazarova, Elena Gardeva, Liliya Yossifova, Boyka Andonova-Lilova, Rositsa Milcheva, Russy Russev

Abstract: Fumonisin B1(FB1) and deoxynivalenol (DON) are mycotoxins the consummation of which could lead to severe intoxications, alterations in the structure and function of different organs, immunosuppression or cancer. The aim of this research was focused on the influence of FB1and DON on the immune system of chickens in concentrations that normally exist in nature. FB1 and DON were applied either separately or in combination in the diet of chickens for a period of two weeks. Assays on the viability and functional activity of lymphocytes and macrophages were performed *in vitro*. Morphostructural changes were defined by routine histopathological and ultrastructural examinations of thymus, spleen and *bursa Fabricii*. Our results showed that the number of viable blood lymphocytes from the FB1/DON consuming group was significantly decreased, as well as their proliferative activity and mitogenic response. The splenic lymphocytes showed lowered proliferation, but preserved mitogenic response. The macrophage functions – spreading and phagocytosis were also decreased significantly. The histological and ultrastructural findings revealed alterations in the lymphoid organs mainly distinguished in FB1 and FB1/DON groups. We concluded that the applied FB1 and DON concentrations, and particularly their combination, can affect the health and the immune status of poultry.
Key words: deoxynivalenol, fumonisin B1, poultry

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Increased sialylation as a phenomenon in accommodation of the parasitic nematode *Trichinella spiralis* (Owen, 1835) in skeletal muscle fibres

Rositsa Milcheva[1,2], Dimitar Ivanov[1], Ivan Iliev[1], Russy Russev[1], Svetlozara Petkova[1], Pavel Babál[2]

[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria; [2]Department of Pathology, Faculty of Medicine, Comenius University in Bratislava, Bratislava, Slovakia

Abstract: The biology of sialic acids has been an object of interest in many models of acquired and inherited skeletal muscle pathology. The present study focuses on the sialylation changes in mouse skeletal muscle after invasion by the parasitic nematode *Trichinella spiralis* (Owen, 1835). Asynchronous infection with *T. spiralis* was induced in mice that were sacrificed at different time points of the muscle phase of the disease. The amounts of free sialic acid, sialylated glycoproteins and total sialyltransferase activity were quantified. Histochemistry with lectins specific for sialic acid was performed in order to localise distribution of sialylated glycoconjugates and to clarify the type of linkage of the sialic acid residues on the carbohydrate chains. Elevated intracellular accumulation of α-2,3- and α-2,6-sialylated glycoconjugates was found only within the affected sarcoplasm of muscle fibres invaded by the parasite. The levels of free and protein-bound sialic acid were increased and the total sialyltransferase activity was also elevated in the skeletal muscle tissue of animals with trichinellosis. We suggest that the biological significance of this phenomenon might be associated with securing integrity of the newly formed nurse cell within the surrounding healthy skeletal muscle tissue. The increased sialylation might inhibit the affected muscle cell contractility through decreased membrane ion gating, helping the parasite accommodation process.

Keywords: glycosylation, sialic acid, nurse cell

Доклади на Българската академия на науките
Comptes rendus de l'Acad´emie bulgare des Sciences
Tome 68, No 5, 2015

FUMONISIN B1 CYTOTOXICITY AND SUBCELLULAR LOCALIZATION IN DUCK EMBRYO CELL LINE DEC 99

Katerina Todorova, Ivan Ivanov, Ani Georgieva, Simona Lazarova, Rosica Milcheva, Petar Dimitrov, Rumen Dimitrov, Russy Russev

Abstract: A comparative study on the cytotoxic effect of fumonisin B1 (FB1) was carried out on BALB/c 3T3 and DEC 99 cell lines. The newly tested cell line DEC 99 appeared as more sensitive than BALB/c 3T3 line according to the performed Neutral red uptake assay. Light microscopic investigations of DEC 99 cultures treated with FB1 showed altered monolayer with free of cells spaces and abundance of dead cells. The immunocytochemical techniques (immunofluorescent and immunogold labelling) proved the influx of the toxin through the cell membranes. The toxin was visualized in the cytoplasm and in the nucleus of the treated cells.
Key words: fumonisin B1, DEC 99 cell line, immunocytochemistry

Доклади на Българската академия на науките
Comptes rendus de l'Acad_emie bulgare des Sciences
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INHIBITORY EFFECT OF SOME NUCLEOTIDES, AND NUCLEOTIDE SUGAR DERIVATIVES ON THE MICROSOMAL SIALYLTRANSFERASE ACTIVITY OF MCF-7 CELLS

Dimitar Ivanov, Radostina Alexandrova, Rositsa Milcheva, Katerina Todorova

Abstract: Sialylation of glycoproteins and glycolipids plays an important role in many cell surface processes like cell-cell communication, cell surface interaction, adhesion, and regulation of the immune response. Therefore, the inhibitors of sialyltransferases for regulation of sialylation might be of medicinal interest, especially in the therapy of cancer diseases. In the present study we investigated the inhibition of cytidine 50-monophosphate N-acetylneuraminic acids total sialyltransferase activity in MCF-7 microsome fraction using diferent nucleotide inhibitors and newly synthesized derivatives of the neuraminic acid. The cytidine nucleotides showed the highest inhibitory efect on the compounds tested in this study. The power of inhibition for all nucleotides increased with the number of phosphate groups. We found that 4 mM AMP did not inhibit the enzyme in MCF-7 cells, whereas 2 mM ATP inhibited the enzyme activity by 50.9%.
Key words: MCF-7 cells, sialyltransferase, nucleotide inhibitors

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Accumulation of α-2,6-sialyoglycoproteins in the muscle sarcoplasm due to *Trichinella sp.* invasion

Rositsa Milcheva*, Pavol Janega, Peter Celec, Svetlozara Petkova, Zuzana Hurniková, Barbora Izrael-Vlková, Katerina Todorova, Pavel Babál

Abstract: The sialylation of the glycoproteins in skeletal muscle tissue is not well investigated, even though the essential role of the sialic acids for the proper muscular function has been proven by many researchers. The invasion of the parasitic nematode *Trichinella spiralis* in the muscles with subsequent formation of Nurse cell-parasite complex initiates increased accumulation of sialylated glycoproteins within the affected area of the muscle fiber. The aim of this study is to describe some details of the α-2,6-sialylation in invaded muscle cells. Asynchronous invasion with infectious *T. spiralis* larvae was experimentally induced in mice. The areas of the occupied sarcoplasm were reactive towards α-2,6-sialic acid specific *Sambucus nigra* agglutinin during the whole process of transformation to a Nurse cell.The cytoplasm of the developing Nurse cell reacted with *Helix pomatia* agglutinin, *Arachis hypogea* agglutinin and *Vicia villosa* lectin-B4 after neuraminidase pretreatment. Up-regulation of the enzyme ST6GalNAc1 and down-regulation of the enzyme ST6GalNAc3 were detected throughout the course of this study. The results from our study assumed accumulation of sialyl-Tn-Ag 6`-sialyl lactosamine, SiA-α-2,6-Gal-β-1,3-GalNAc-α- Ser/Thr and Gal-β-1,3-GalNAc(SiA-α-2,6-)-α-1-Ser/Thr oligosaccharide structures into the occupied sarcoplasm. Further investigations in this domain will develop the understanding about the amazing adaptive capabilities of skeletal muscle tissue.

Keywords: Nurse cell; sialic acids; skeletal muscle; *Trichinella*

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ABSENCE OF ST3GAL2 AND ST3GAL4 SIALYLTRANSFERASE EXPRESSIONS IN THE NURSE CELL OF *TRICHINELLA SPIRALIS*

R. S. MILCHEVA[1], P. JANEGA[2], S. L. PETKOVA[1], K. S. TODOROVA[1], D. G. IVANOV[1] & P. BABÁL[2]

[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum – Bulgarian Academy of Sciences, Sofia, Bulgaria, [2]Department of Pathology, Faculty of Medicine, Comenius University in Bratislava, Bratislava, Slovakia

Summary: This study was aimed to describe some glycosylation changes in the Nurse cell of *Trichinella spiralis* in mouse skeletal muscle. Tissue specimens were subjected to lectin histochemistry with *Maackia amurensis* lectin (MAL), Peanut agglutinin (PNA) and neuraminidase desialylation in order to verify and analyse the structure of α-2,3-sialylated glycoproteins, discovered within the affected sarcoplasm. The expressions of two sialyltransferases were examined by immunohistochemistry. It was found out that the occupied portion of skeletal muscle cell responded with synthesis of presumable sialyl-Tantigen and α-2,3-sialyllactosamine structure, that remained accumulated during the time course of Nurse cell development. The enzymes β-galactoside-α-2,3-sialyltransferases 2 and 4, which could be responsible for the sialylation of each of these structures, were however not present in the invaded muscle portions, although their expressions in the healthy surrounding tissue remained persistent. Our results contribute to the progressive understanding about the amazing abilities of *Trichinella spiralis* to manipulate the genetic programme of its host.
Key words: Nurse cell, sialic acids, sialyltransferases, *Trichinella spiralis*

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CYTOTOXICITY OF THE *FUSARIUM* MYCOTOXIN DEOXYNIVALENOL ON MAMMALIAN AND AVIAN CELL LINES

K. TODOROVA[1], A. GEORGIEVA[1], R. MILCHEVA[1], D. IVANOV[1] & I. KALKANOV[2]

[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum – Bulgarian Academy of Sciences, Sofia, Bulgaria; [2]Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

Summary: Trichothecenes are mycotoxins that occur in grains and can lead to acute and chronic poisoning in animals and humans. Deoxynivalenol (DON) is a type B trichothecene affecting protein synthesis, immune system, leading to brain, blood and kidney disorders. The aim of this work was to evaluate *in vitro* the cytotoxicity and the pathological effects of DON in short-term experiments on cells from non-tumour and tumour permanent cell lines and to compare their sensitivity. Cell cultivation of BALB/c 3T3, DEC 99, MDA-MB-231, MCF-7 and Hela cells was performed. Quantitative and qualitative methods evaluating cytotoxicity on the base of statistical and morphological analyses for determining the impact on the viability and proliferative activity were used: Neutral Red Uptake (NRU) cytotoxicity test, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test and fluorescence microscopy. The cytotoxic effect of DON was assessed after an exposure period of 24 h. DON treatment induced significant alterations in the growth and morphology of the cells, involving early and late apoptosis and necrosis signs. Statistically significant decrease of the viability of all cell lines was established at concentrations of DON starting from 1.9 μg/mL to 3.7 μg/mL, the mean IC50 concentrations were calculated. According to the IC50 values the hierarchical order of cell lines' sensitivity was determined.

Key words: cytotoxicity, deoxynivalenol, permanent cell lines

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Expression of sialyltransferases from the *St3gal*, *St6gal* and *St6galnac* families in mouse skeletal muscle and mouse C2C12 myotubes

Rositsa S. Milcheva*, Any K. Georgieva, Katerina S. Todorova, Svetlozara L. Petkova

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

ABSTRACT: In skeletal muscles, the sialic acids have a great significance for their functional maintenance and proper structural organization. Our work described the expressions of *St3gal*, *St6gal* and *St6galnac* sialyltransferases specific for glycoproteins in mouse skeletal muscles and murine C2C12 myotubes. Lectin histochemistry, cytochemistry and lectin blot were used to demonstrate the membrane localization and the electrophoretic profi les of α-2,3- and α-2,6-sialylated glycoproteins. The expression levels of sialyltransferases were analysed by real time RT-PCR and western blot. The enzymes *St6gal2* and *St6galnac1* were not expressed in skeletal muscle tissue and C2C12 myotubes. In both experimental groups, mRNAs of the *St3gal* family prevailed over the mRNA expressions of the *St6gal* and *St6galnac* families. The profiles of sialyltransferase expressions showed differences between the two experimental groups, illustrated by the absence of expressions of the mRNA for the *St3gal6* and *St6galnac3* genes in the C2C12 cell samples and by the different shares of the enzymes *St3gal3* and *St3gal4* in both experimental groups. The different patterns of enzyme expressions in both experimental groups corresponded with differences between their α-2,3- and α-2,6-sialylated glycoprotein profiles. These results could be a useful addendum to the knowledge concerning the glycosylation of the skeletal muscle tissue.
KEY WORDS: C2C12 myotubes, sialylation, sialyltransferases, skeletal muscles

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Sonochemically engineered nano-enabled zinc oxide/amylase coatings prevent the occurrence of catheter-associated urinary tract infections

Aleksandra Ivanova[a], Kristina Ivanova[a], Ilana Perelshtein[b], Aharon Gedanken[b], Katerina Todorova[c], Rositsa Milcheva[c], Petar Dimitrov[c], Teodora Popova[d], Tzanko Tzanov[a],*

[a]*Group of Molecular and Industrial Biotechnology, Department of Chemical Engineering, Universitat Polit`ecnica de Catalunya, Rambla Sant Nebridi 22, 08222, Terrassa, Spain*
[b]*The Department of Chemistry and Institute for Advanced Materials and Nanotechnology, Bar-Ilan University, Ramat-Gan 52900, Israel*
[c]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Geo Milev, 1113 Sofia, Bulgaria*
[d]*Faculty of Veterinary Medicine, University of Forestry, 10 Sveti Kliment Ohridski Ave, 1756 Sofia, Bulgaria*

ABSTRACT: Catheter-associated urinary tract infections (CAUTIs), caused by biofilms, are the most frequent health-care associated infections. Novel antibiofilm coatings are needed to increase the urinary catheters' life-span, decrease the prevalence of CAUTIs and reduce the development of antimicrobial resistance. Herein, antibacte-rial zinc oxide nanoparticles (ZnO NPs) were decorated with a biofilm matrix-degrading enzyme amylase (AM) and simultaneously deposited onto silicone urinary catheters in a one-step sonochemical process. The obtained nano-enabled coatings inhibited the biofilm formation of *Escherichia coli* and *Staphylococcus aureus* by 80% and 60%, respectively, for up to 7 days *in vitro* in a model of catheterized bladder with recirculation of artificial urine due to the complementary mode of antibacterial and antibiofilm action provided by the NPs and the enzyme. Over this period, the coatings did not induce toxicity to mammalian cell lines. *In vivo*, the nano-engineered ZnO@AM coated catheters demonstrated lower incidence of bacteriuria and prevent the early onset of CAUTIs in a rabbit model, compared to the animals treated with pristine silicone devices. The nano-functionalization of catheters with hybrid ZnO@AM coatings appears as a promising strategy for prevention and control of CAUTIs in the clinic.

Keywords: Sonochemistry Amylase Zinc oxide nanoparticles Biofilm inhibition Bacterial infections prevention

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The synthesis of UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE), α-dystroglycan, and β-galactoside α-2,3-sialyltransferase 6 (ST3Gal6) by skeletal muscle cell as a response to infection with *Trichinella spiralis*

R. MILCHEVA[1],*, K. TODOROVA[2], A. GEORGIEVA[3], S. PETKOVA[4]

Institute of experimental morphology, pathology and anthropology with museum – Bulgarian Academy of Sciences. Acad. G. Bonchev Str., block 25, Sofi a 1113, Bulgaria, 1,*ORCID 0000-0003-1103-9026, E-mail: [1]**rosicamilcheva@abv.bg*, [2]*katerinagencheva@yahoo.com*, [3]*georgieva_any@abv.bg*, [4]*svetlozarapetkova@abv.bg*

Summary: The Nurse cell of the parasitic nematode *Trichinella spiralis* is a unique structure established after genetic, morphological and functional modification of a small portion of invaded skeletal muscle fiber. Even if the newly developed cytoplasm of the Nurse cell is no longer contractile, this structure remains well integrated within the surrounding healthy tissue. Our previous reports suggested that this process is accompanied by an increased local biosynthesis of sialylated glycoproteins. In this work we examined the expressions of three proteins, functionally associated with the process of sialylation. The enzyme UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE) is a key initiator of the sialic acid biosynthetic pathway. The α-dystroglycan was the only identified sialylated glycoprotein in skeletal muscles by now, bearing sialyl-α-2,3-Gal-β-1,4-GlcNAc-β-1,2-Man-α-1-O-Ser/Thr glycan. The third protein of interest for this study was the enzyme β-galactoside α-2,3-sialyltransferase 6 (ST3Gal6), which transfers sialic acid preferably onto Gal-β-1,4-GlcNAc as an acceptor, and thus it was considered as a suitable candidate for the sialylation of the α-dystroglycan. The expressions of the three proteins were analyzed by real time-PCR and immunohistochemistry on modified methacarn fixed paraffin tissue sections of mouse skeletal muscle samples collected at days 0, 14 and 35 post infection. According to our findings, the up-regulation of GNE was a characteristic of the early and the late stage of the Nurse cell development. Additional features of this process were the elevated expressions of α-dystroglycan and the enzyme ST3Gal6. We provided strong evidence that an increased local synthesis of sialic acids is a trait of the Nurse cell of *T. spiralis*, and at least in part due to an overexpression of α-dystroglycan. In addition, circumstantially we suggest that the enzyme ST3Gal6 is engaged in the process of sialylation of the major oligosaccharide component of α-dystroglycan.

Keywords: α-dystroglycan; GNE; Nurse cell; Sialic acid; Skeletal muscle; *Trichinella spiralis*

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Trichinella spiralis (Owen, 1835) Induces Increased Dystrophin Expression in Invaded Cross-striated Muscle

Rositsa Milcheva[1], Iveta Mečiarová[2], Katerina Todorova[1], Valeria Dilcheva[1], Svetlozara Petkova[1], Pavel Babál[3]

Abstract:
Purpose: Dystrophin and the dystrophin glycoprotein complex serve as a cytoskeletal integrator, critical for muscle membrane stability. The aim of the present study was to clarify the expression of dystrophin protein and mRNA in the skeletal muscle tissue during the muscle phase of trichinellosis in mice.
Methods: Muscle tissue was collected from mice experimentally infected with *Trichinella spiralis* at days 0, 14 and 40 after infection. The expression of dystrophin in the muscle tissue was investigated by immunohistochemistry with antibodies against three different domains of the protein, and the expression levels of *Dys* mRNA by real-time PCR.
Results: The presence of dystrophin protein was increased in the de-differentiating cytoplasm at the early stage of muscle infection and was persisting also in the mature Nurse cell harbouring the parasite. It was accompanied by significantly elevated expression of *Dys* mRNA at days 14 and 40 after infection.
Conclusion: Our findings indicate that dystrophin plays a role in regeneration of the muscle and in the Nurse cell formation and stability for security of the parasite survival.
Keywords: Dystrophin, Nurse cell, Skeletal muscle, *Trichinella spiralis*

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Novel Triple Stimuli Responsive Interpenetrating Poly(Carboxybetaine Methacrylate)/Poly(Sulfobetaine Methacrylate) Network

Konstans Ruseva[1],*, Katerina Todorova[2] , Tanya Zhivkova[2], Rositsa Milcheva[2], Dimitar Ivanov[2], Petar Dimitrov[2], Radostina Alexandrova[2] and Elena Vassileva[1],*

[1]Laboratory on Structure and Properties of Polymers, Department of Pharmaceutical and Applied Organic Chemistry, Faculty of Chemistry and Pharmacy, Sofia University "St. Kl. Ohridski", 1 J. Bourchier Blvd., 1164 Sofia, Bulgaria
[2]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 25, 1113 Sofia, Bulgaria
***** Correspondence: ohtkr@chem.uni-sofia.bg (K.R.); ohtev@chem.uni-sofia.bg (E.V.);
Tel.: +359-281-614-95 (K.R.)

Abstract: The study reports the synthesis and characterization of novel triple stimuli responsive interpenetrating polymer network (IPN) based on two polyzwitterionic networks, namely of poly(carboxybetaine methacrylate) and poly(sulfobetaine methacrylate). The zwitterionic IPN hydrogel demonstrates the ability to expand or shrink in response to changes in three "biological" external stimuli such as temperature, pH, and salt concentration. The IPN hydrogel shows good mechanical stability. In addition, other important features such as non-cytotoxicity and antibiofouling activity against three widespread bacteria as *P. Aeruginosa*, *A. Baumanii*, and *K. Pneumoniae* are demonstrated. The in vivo behavior of the novel zwitterionic IPN hydrogel suggests that this smart material has very good potential as a biomaterial.

Keywords: interpenetrating polymer network; poly(sulfobetaine methacrylate); poly(carboxybetaine methacrylate); triple stimuli responsiveness; biomaterial