Абстракти на статиите на английски език
на
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участник в конкурс за заемане на академична длъжност „доцент” в професионално направление 6.4. Ветеринарна медицина, научна специалност 04.03.06 ”Патология на животните”, обявен в ДВ, брой 107 от 16. 12. 2021г.

1. Dimitrov, P., Simeonov, K., **Todorova, K**., Ivanova, Z., Toshkova, R., Shikova, E., Russev, R.. Pathological features of experimental bovine leukaemia viral (BLV) infection in rats and rabbits. The Bulletin of the Veterinary Institute in Pulawy, 56, 2, National Veterinary Research Institute, Poland, 2012, 115-120. JCR-IF (Web of Science):0.357 Q3 (Scopus).

Abstract
Rabbits and rats were inoculated with material derived from FLK cells producing permanently bovine leukaemia virus (BLV). The viral presence in the inoculum was proved by transmission electron microscopy, immunofluorescence, immunogold labelling demonstrating viral Tax protein, and PCR analysis. About 30 % of the infected animals sustained BLV seropositivity during the experiment, and demonstrated symptoms of lympholeukaemia – clinical manifestation of an immunosuppressive condition, increased number of lymphocytes and lymphoblasts, and preneoplastic lymphoid cell accumulations in the liver, lungs, kidneys, and lymph nodes. BLV DNA, detected by PCR in diseased animals, indicates the role of BLV as an aetiological factor of lympholeukaemia, developed in these animals after BLV infection. The alterations in rats were more pronounced than those in rabbits. The results prove that these two species of laboratory animals, especially rats, are suitable models for the in vivo studies of leukaemogenesis caused by BLV/HTLV infections.

2. **Todorova, K**., Dimitrov, P., Toshkova, R., Lazarova, S., Gardeva, E., Yossifova, L., Andonova-Lilova, B., Milcheva, R., Russev, R.. Influence of fumonisin B1 and deoxynivalenol on the immune system of chickens after application in quantities, naturally presented in fodders. Comptes Rendus de L'Academie Bulgare des Sciences, 67, 1, 2014, 139-144. SJR (Scopus):0.21, JCR-IF (Web of Science):0.29 Q3 (Scopus).

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.

3. Arnaudova-Matey, A., Yankovska, T., Kirilova, T., **Todorova, K**,. Mehmedov, T., Ivanova, S., Dilov P, Angelov, G.. Utilisation of iron methionate in broiler chickens compared to iron sulphate. Bulgarian Journal of Agricultural Sciences, 19, 4, Agricultural Academy, 2013, 854-859. SJR:0.262, ISI IF:0.214 Q3 (Web of Science).

Abstract

35-day tests with broiler chickens treated with Bulgarian iron methionate administered through the food compared to the iron sulphate (heptahydrate) in doses of 60 ppm and 300 ppm were conducted. The test involved 55 broiler chickens aged 10 days, divided into 5 groups of 11 chickens. The tests started on May 21st, 2012 and continued 35 days. The basic mixed feed was prepared by using a recipe for growing broiler chickens and an average content of 85.6 ± 2.4 mg Fe/kg. The appetite, health status (clinical one) and individual weight of the chickens were controlled. On the 15th day samples of the liver from three euthanized chickens of each group were taken for histological and chemical studies. On the 35th day four more chickens of each group were subjected to the same studies. The liver samples intended for chemical analysis were frozen at-18o C and after 22 days were thawed out and tested for iron content by optical emission spectrophotometer ICP-OES 715-S. Samples of the cloacal content were taken from the chickens euthanized on the 15th and 35th day. They were also frozen and then thawed out, dried and analysed for iron content by using atomic absorption spectrophotometer equipped with graphic cuvette, model Spectra AA 800. The statistical results were processed by three different methods - parametric (Anova one-way), non-parametric (Mann-Whitney U-test) method and by using the tables of Student-Fisher. During the test period no clinical symptoms and signs of disease or mortality were found in all treated chickens; there were no pathomorphological changes in the liver of the chickens. In general, the utilisation was more favourable for the iron methionate compared to the iron sulphate. It was better expressed in the low concentration (60 ppm) - a steady growth, trend for better deposition in the liver and significantly smaller amount (up to two times) of iron in the cloacal content (beneficial for the environment). The iron deposited in the liver of the treated chickens was from 40 to 60% more than that in the control ones.

4. **Todorova, K**., Ivanov, I., Georgieva, A., Lazarova, S., Milcheva, R., Dimitrov, P., Dimitrov, R., Russev, R.. Fumonisin B1 cytotoxicity and subcellular localization in duck embryo cell line DEC 99. Comptes rendus de l'Academie bulgare des Sciences, 68, 5, „Проф. Марин Дринов", 2015, 617-622. SJR (Scopus):0.21, JCR-IF (Web of Science):0.233 Q3 (Scopus).

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.

5. Bratslavska, O., Kozireva, S., Baryshev, M., Russev, R., Alexandrov, M., Uzameckis, D., **Todorova, K.,** Dimitrov, P., Murovska, M.. Parvovirus B19 infection increases proliferative activity of non-permissive cells. Comptes rendus de l'Academie bulgare des Sciences, 68, 1, 2015, 49-58. SJR (Scopus):0.21, JCR-IF (Web of Science):0.284 Q3 (Web of Science).

Abstract

Parvovirus B19 (B19) is associated with a wide spectrum of diseases in humans, including autoimmune disorders and is possibly involved in the pathogenesis of some carcinomas. The appearance of B19 infection in non-permissive cells is poorly explored. Our study demonstrates that B19 virus is able to infect epithelial-like HOS TE85 cells. Although the virus particles were not found, the virus genomic DNA was detected in HOS cells during 3–11 passages. Moreover, the expression of B19 VP1 and NS1 mRNAs and the presence VP1/VP2 proteins were revealed in infected cells. The manifestation of B19

infection was dependent on virus dose. At the high dose (5000–8000 virus genomes/cell) massive apoptosis developed on the 6th day after infection and the cells were lost. Morphological and electron microscopy studies showed that the cytopathic effect was associated with apoptotic alteration in HOS cells. At the lower virus dose (100–1000 virus genomes/cell) the B19 infection in HOS cells was accompanied by a 1.5–1.8 times elevated IL-6 level and by 1.5–1.9 times increased proliferative activity of the infected cells. An intact VP1 unique region and a VP1u with a point mutation in the sPLA2 catalytic site were expressed in E. coli. The intact VP1u protein increased the cells proliferative activity by up to 2–3.5 times in a dose-dependent manner while mutated sPLA2 deficient VP1u had no effect on the cell proliferation. We suggest that the stimulation of the cells' proliferation is associated with phospholipase A2activity of B19 capside protein VP1.

6. Sultanova, A., Cistjakovs, M., Cunskis, E., **Todorova, K**., Russev, R., Murovska, M.. Thyrocytes as the target cells for HHV-6 infection in patients with autoimmune thyroiditis. Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences., 70, 4, Latvian Academy of Sciences, 2016, 160- 166. SJR (Scopus):0.14 Q4 (Scopus).

Abstract

Human herpesvirus-6 (HHV-6) is a ubiquitous betaherpesvirus with immunomodulating properties that have been suggested to play an important role in the development of several autoimmune disorders. Although the primary targets for HHV-6 replication, both in vitro and in vivo, are CD4+ and CD8+ T lymphocytes, some studies have reported the presence of HHV-6 sequences in different solid organs, including in the thyroid gland, showing possible involvement of this herpesvirus in development of autoimmune thyroid disease. The aim of this study was to determine loads of HHV-6 in thyroid gland tissue in comparison to those in peripheral blood of patients with autoimmune thyroiditis. Seven patients [women mean age 45 (28–65)] with histologically confirmed autoimmune thyroiditis were enrolled in this study. Fluorescence-activated cell sorting was used to distinguish and sort lymphocyte populations from peripheral blood mononuclear cells of patients. HHV-6 load was determined by real-time PCR for peripheral blood and thyroid gland tissue samples. Additionally, all results from molecular analyses were compared with histological results obtained by light microscopy. Viral load was detected only in one (46 viral copies/ 1×106 cells) blood sample; others were under the detection limit of the used kit. However, in all HHV-6 positive tissue samples viral load was detected in the range of 132–1620 viral copies/106 cells. Substantial HHV-6 load in lymphocyte subpopulations was detected in two of seven patients. HHV-6 load was detected in NK and CD95+ cells of two patients. The obtained results show that thyroid gland cells (tyrocytes) act as target cells for HHV-6.

7. Ivanov D., Alexandrova R., Milcheva R., **Todorova K**.. Inhibitory effect of some nucleotides and nucleotide sugar derivatives on the microsomal salyltransferase activity of MCF-7 cells. Comptes rendus de l'Academie bulgare des Sciences, 70, 8, 2017, 1107-1114. SJR:0.207, ISI IF:0.253 Q4 (Web of Science).

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 different nucleotide inhibitors and newly synthesized derivatives of the neuraminic acid. The cytidine nucleotides showed the highest inhibitory effect 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%.

8. Kalkanov, I., Dinev, I., **Todorova, K**., Alexandrov, M., Ananiev, Y., Galabova, M.. Ultrastructural and Immunohistochemical Investigations in Calves with Coronavirus Pneumoenteritis Syndrome. Kafkas Universitesi Veteriner Fakultesi Dergisi, 24, 6, Faculty of Veterinary Medicine, Kafkas University, 2018, 791-797. SJR (Scopus):0.231, JCR-IF (Web of Science):0.411 Q3 (Scopus).

Abstract

The aim of present studies was the structural and morphogenetic investigation of spontaneous pneumoenteritis syndrome in newborn and growing calves with regard to confirmation of some structural features of disease morphogenesis. The research was done with 370 calves from 6 cattle farms in 4 regions of the country, at the age of 24 h - 25 days. For rapid antigenic and viral detection of pathogens, Multiscreen Ag ELISA, Bovine respiratory, Pulmotest respiratory tetra ELISA kit for antigenic diagnosis of BoHV-1, BVDV, BRSV, and BPI-3 sandwich test for tissue lysates (BIOX Diagnostics, Belgium) and Rainbow calf scour 5 BIO K 306 Detection of Bovine Rotavirus, Coronavirus, Escherichia coli F5, Cryptosporidium parvum and Clostridium perfringens in bovine stool (BIOX Diagnostics, Belgium) were used. In 5% of cases, laboratory antigenic tests of lung tissue lysates from pneumonic calves detected co-infections with BoHV-1, BVDV, BRSV and BPI-3. The utilised antigenic, ultrastructural and virological diagnostic methods allowed concluding that they could be successfully used in the diagnostics of pulmonary and gastrointestinal viral infections in juvenile calves. Electron microscopy and immunohistochemical methods of lung and intestinal tissue are also important and applicable for diagnostics and in differential diagnostic recognition of the condition from other common diseases as IBR, BVD, BRSV, Mannheimia haemolytica, Cryptosporidium parvum, BRV and E. coli K99 (F5).

9. Ruseva, K., Ivanova, K., **Todorova, K**., Gabrashanska, M., Hinojosa-Caballero, D., Tzanov, Tz., Vassileva, E.. Poly(sulfobetaine methacrylate)/poly(ethylene glycole) hydrogels for chronic wounds management. European Polymer Journal, Elsevier Ltd., 2019, 391-401. SJR (Scopus):0.967, JCR-IF (Web of Science):3.621 Q1 (Web of Science).

Abstract

Polyzwitterions (PZI) recently emerged as biomaterials with excellent bio- and haemo-compatibility, demonstrating lower protein adsorption on their surfaces even compared to the golden standard in the field – poly (ethylene glycol) (PEG). Although PZI combine many beneficial for chronic wound treatment properties as nonfouling ability and high capacity to absorb wound exudate, their potential for such demanding application is still unrevealed. In this work, polysulfobetaine (PSB) networks were synthesized using PEG-based crosslinking agent, thus combining in one material two polymers with inherent antifouling properties. The obtained PSB hydrogels demonstrate linear temperature dependence of their swelling capacity in water between 20 and 70 °C. Moreover, they all exhibit strong antipolyelelctrolyte behavior, increasing their swelling ratio between 10 and 22 times depending on their crosslinking degree as the NaCl concentration increased. The study also demonstrates the PSB high ability to bind water - ∼40% bound water was determined for almost all PSB hydrogels, which is considered as the main reason for their ultra-low non-specific protein binding ability. Moreover, PZI networks effectively absorb and retain the major enzymes causing chronicity of the wounds as 30–40% myeloperoxidase (MPO) was loaded into the PSB hydrogels depending on their crosslinking degree. At the same time, PZI hydrogels do not inhibit neither MPO nor the collagenase activity, thus ensuring a decrease in their excessive amount in the chronic wounds but at the same not hampering the enzyme activity necessary for the proper wound healing. All PSB hydrogels demonstrated antibiofilm activity against S. aureus, a common bacterial representative in chronic wounds. The non-cytotoxicity and biocompatibility of the hydrogels were proved in vitro and in vivo. Thus, the study demonstrated the PSB hydrogels' advantages as dressing materials for chronic wound healing, namely: (i) high ability to absorb wound exudate; (ii) high ability to bind water; (iii) good control on the enzymes concentration in the chronic wounds through absorption (iv) without inhibiting their

activity; (v) antibiofilm activity against common for the chronic wounds bacteria; (vi) non-cytotoxicity and (vii) in vivo proved very good tolerance by the surrounding tissues.

10. Panayotova-Pencheva, M., **Todorova, K**., Dakova, V.. Pathomorphological studies on wild boars infected with Metastrongylus spp., Ascarops strongylina and Macracanthorhynchus hirudinaceus. Journal of Veterinary Research, 63, De Gruyter Open Ltd., 2019, 191-195. SJR (Scopus):0.291, JCR-IF (Web of Science):0.829 Q2 (Scopus).

Abstract
Introduction: Pathomorphological changes in the lungs, stomach, and small intestines of wild boars infected with Metastrongylus spp., Ascarops strongylina, and Macracanthorhynchus hirudinaceus were investigated. Material and Methods: Dissection of 11 wild boars was performed, and parasitised organs were histologically investigated by common techniques. Results: Macroscopic lesions in the lungs infected with Metastrongyus spp. were seen within the apical parts of the large lobes, irregular in form, pale greyish in colour, and compact in consistency. The main pathohistological findings were: the presence of parasite forms, and lymphocytes and neutrophils in the lumen of bronchi and bronchioles, desquamation of the bronchial and bronchiolar epithelium, emphysema, thickening of alveolar septa, hyperaemia, alveolitis, infiltration of the interstitial tissue with giant cell, monocytes and eosinophils, and peribronchial and disseminated lymphoid hyperplasia. The principal observations accompanying infection with A. strongylina were inflammation and focal mucosal damage in the stomach, the latter clearly demarcated from the surrounding tissues. Severe injuries in the place of attachment of M. hirudinaceus to the wall of the small intestine were seen. Intestinal villi, underlying mucosa, and submucosa were destroyed, and an intense inflammatory reaction was present. Conclusion: The histopathological lesions showed wide diversity, varying from mild to severe; but none of them were lethal.

11. Dimitrov, P., **Todorova, K**., Petrichev, M., Russev, R.. Bovine leukemia virus – pathogenicity in animals and potential impacts in humans. The Cyprus Journal of Sciences, 10, American College, Cyprus, 2012, 101-109.

Abstract
Data on the spreading of Enzootic Bovine Leucosis (EBL) and the pathogenicity of Bovine Leukemia Virus (BLV) for different animal species and humans have been presented. Cattle are the natural host of the virus, buffaloes and capybaras are strongly sensitive, sheep and goats are susceptible as well. The infection in other species including human cells in vitro is not an exception. Certain authors attribute the increased percentage of leukemic disorders in men from regions with a high EBL seroprevalence and high rate of consumption of dairy and cattle products. BLV sequences have been detected in materials from breast cancer.

12. Dimitrov, P., **Todorova, K**., Milcheva, R., Gabev, E., Rusev, R.. BLV infected rats and rabbits as a model of human lymphocytic 10/n leukaemia. Proceedings of the IV workshop of experimental models and methods in biomedical research, 2013, 24-33.

Abstract
Human T leukaemia is known to be quite identical with enzootic bovine leukosis due to common genome organization of their aethilogical agents - Human T leukaemia virus (HTLV) and Bovine leukaemia virus (BLV). Because of the very long period of the latency and the pathogenesis of the illness in cows, we tried to infect rabbits and rats with BLV. The inoculated material was derived from FLK cells permanently producing BLV. The viral presence in the inoculum used was proved by PCR, transmission electron microscopy and light and electron immunocytochemistry.. About 1/3 of the infected animals sustained BLV seropositivity during the experiment and developed symptoms of lympholeukaemia – immunosuppressive clinical condition, marked leukocytosys (predominantly lymphocytes and lymphoblasts) and lymphoid cell accumulations in most viscera. BLV DNA detected in diseased animals by PCR indicated the role of BLV as an aetiological factor of lympholeukaemia. The

pathological changes in rats were more pronounced than those in rabbits proved by the statistical analyzes of results from haematological studies and survival. The results proved these two species of laboratory animals and especially the rats to be a suitable model to study the leukaemogenesis due to BLV/HTLV infections.

13. **Todorova, K**., Dimitrov, P., Toshkova, R., Lazarova, S., Gardeva, E., Yossifova, L., Andonova-Lilova, B., Milcheva, R., Russev, R.. Morphological and immunological assessment of the individual and combined effects of two mycotoxins-fumonisin b1 and deoxynivalenol in vivo. Proceedings of the eighth workshop biological activity of metals, synthetic compounds and natural products, 2013, 93-100.

Abstract

Mycotoxins are health problem as they are natural contaminants of corn, wheat, oats, barley, rice, ect. and their by-products used in food and feed industry. Two of them-fumonisin B1 (FB1) and deoxynivalenol (DON) are the predominant representatives detected in samples of grains from different regions of Bulgaria. They lead to acute and chronic intoxications, and different pathological alterations in humans and animals. Chickens are potential target, because their feed is based on wheat and corn. More investigations are needed to evaluate the impact of FB1 and DON toxicity on the profitability of the poultry industry. The objective of our study was to investigate the morphological and immunological effects of fumonisin B1 and deoxynivalenol, and their combination in chickens, used in concentrations found in grains from Bulgaria. Materials and methods: FB1- 10 mg/kg and DON-1,15 mg/kg forage were applied, either separately or in combination, in the diet of 30 days old Lohmann brown female chickens for a period of two weeks. Histological and ultrastructural examinations were performed. Thiazolyl blue tetrazolium bromide test (MTT), trypan blue assays, tests for proliferation and mitogenic response of blood and spleen lymphocytes and spreading and phagocytosis of macrophages were carried out. The results showed reduced viability, proliferative activity and mitogenic response of the blood lymphocytes. The spleen lymphocytes proliferation was diminished too. The functions-spreading and phagocytosis of peritoneal macrophages were decreased significantly 93 after the treatment. The histological and ultrastructural findings revealed alterations in the lymphoid organs, esophagus and duodenum that were highly expressed in the FB1 and DON consuming group. We concluded that the applied FB1 and DON concentrations and particularly their combination can compromise the health of chickens.

14. Арнаудова Мейти, А., Тодоров, Т., **Тодорова, К.**, Димитрова, Д., Мехмедов, Т., Шиндарска, З., Иванова, С., Ангелов, Г., Дилов, П.. Поносимост и субхронична токсичност на железен метионат при пилета бройлери сравнено с железен сулфат. Животновъдни науки, LI, 3, 2014, 31-40.

Abstract

Not available

15. Пеев, И., Тодоров, Т., **Тодорова, К**.. Изследване за субхронична токсичност на птаквилозид, изолиран от орлова папрат (Pteridium aquilinum (L.) Kuhn) при морски свинчета., Tradition and Modernity in Veterinary Medicine, 2013, 91-100.

Abstract

The study presents hematological, biochemical, histological and urinalysis changes of guinea pigs treated for 35 days with norsesquiterpen glycoside ptaquiloside derived from Bracken fern (Pteridium aquilinum L. Kuhn). Hematological analysis revealed statistically signifi cant abnormalities of leukocytes, lymphocytes, erythrocytes, platelets, packed cell volume and mean levels of hemoglobin in erythrocytes. The tests on the biochemistry profi le determined statistically signifi cant changes in the levels of gamma glutamyl transferase, total protein and albumin. In urinalysis proteinuria was the most common fi nding. Histopathology study did not revealed signifi cant changes.

16. **Todorova, К**., Georgieva, A., Dimitrov, P., Ivanov, I., Russev, R.. Cytotoxicity and immunolocalization of the mycotoxin fumonisin B1 in permanent cell lines. Trakia Journal of Sciences, 2, 13, 2015, 74-80.

Abstract

Fumonisins are widespread mycotoxins, produced by several species of Fusarium moulds that contaminate mainly crops, and food and feed based on cereals. These metabolites represent a significant health treat for humans and animals because of their toxic and carcinogenic effects. The present study aims to ascertain the cytotoxic effects of FB1 on permanent lines and its subcellular localization. In the present study, the cytotoxic effect of fumonisin B1 (FB1) on DEC99 (duck embryo cells) and BALB/c 3T3 (mouse embryo fibroblasts) cell lines was assessed by Neutral Red Uptake (NRU) test and standard Pappenheim and fluorescent AO/PI double staining. Intracellular distribution of FB1 in DEC99 cells was examined by immunofluorescence and immunoelectron microscopy methods. The results from the NRU test indicated that DEC99 cells are more sensitive to the toxic effect of FB1 than BALB/c 3T3 cells. AO/PI double staining revealed morphological features of apoptosis of cells treated with 300 μg FB1/ml. The immunofluorescent and immunogold labeling of FB1 revealed the localization of the micotoxin in the cytoplasm and the nucleus of the cells. The presented results clearly indicate that the cell line DEC99 is a useful model system for studies on the cytotoxic effects of fumonisins.

17. **Todorova, K.**, Dimitrov, P., Milcheva, R., Roga, S., Russev, R.. Comparative study of several cases of human breast cancer and mammary cancer in domestic dogs and cats. Acta Morphol. Anthropol., 23, 2016, 66-70.

Abstract

A useful model for studying tumor systems, which is close to the human analogue, is very necessary for development of modern approaches and methods in cancer researches. As breast cancer is the second leading cause of cancer deaths in women, spontaneous mammary tumors in domestic animals are feasible solution for valid tumor systems model. In this study we present histological diagnosis and grading of human, canine and feline mammary tumors and evaluate their histological and biological behavior. Histological diagnosis of animal tissue samples found five cases of ductal carcinoma (n=5, 62.5%), one lobular carcinoma, one squamous cell carcinoma and one case of metaplastic carcinoma with osteosarcomatous differentiation, graded from I to III: I (n=1, 12.5%), II (n=4, 50%) and III (n=3, 37.5%). Four of the human cases were diagnosed as invasive ductal (n=2, 40%) and lobular (n=2, 40%) carcinoma, one case - metaplastic carcinoma, all scored as grade III.

18. Иванова, С., Димитрова, Д., **Тодорова, К**., Мехмедов, Т., Калинова, Г.. Сравнително проучване на субхронична токсичност на цинков метионат и цинков сулфат при пилета бройлери. Ветеринарна сбирка, 1-2, 2016, 42-50.

Abstract

The purpose of these studies is to evaluate toxicological risks of administering in the feed ration of zinc methionate (Zn-Met) at broiler chickens for 35 days (subchronic toxicity) as compared with zinc sulphate heptahydrate (ZnSO4.7H2O). To conduct the study was used zinc methionate containing 14.6% zinc, 16,6% H2O and 68.8% methionine, in comparison with zinc sulfate heptahydrate, (ZnSO4.7H2O), containing 22.7% zinc. Were used 56 broiler chickens aged 15 days. The basic feed contained an average of 41,54 ± 1,84 ppm Zn, as well as all the necessary vitamins and minerals, but without the an additional zinc. The chickens their divisions by 8 pieces per group and received zinc methionate and zinc sulphate in the following concentrations: 60 ppm, 300 ppm, 600 ppm. The highest weight at the end of the experiment was observed in chickens of I group (Zn-Met 60 ppm - 2,013 kg ± 0,057), compared to all other groups. It was foundalbeit not significant difference in the number of leukocytes on the 35th day, between I and IV group (Zn-Met 60 ppm and Zn SO4 60 ppm) and II and Group V (Zn-Met 300 ppm and Zn SO4 300 ppm) in favor of ZnSO4 ($p < 0,01$). On 35-th day highest hemoglobin (100,125 g / l) was observed in the group receiving an average dosage Zn-Met (300 ppm), and the lowest (92,0 g / l) in the group receiving the most high dosage Zn-Met (600 ppm). It has been

found statistically significant difference in the quantity of albumin (P < 0,05) between I group – ZnMet 60 ppm (16,4g / l) and the control (13,9 g / l). For the remaining studied of us biochemical parameters (glucose, urea, creatinine, alkaline phosphatase, ASAT, ALAT) was not observed statistically significant difference, and levels were within the normal range. Pathologic research don't showed evidence of the organs damage.

19. Nanev, V., Vladov, I., Dimitrov, P., **Todorova, K**., Tsocheva Gaytandzhieva, N., Ilieva, R., Gergulova, R., Gabrashanska, M.. Biohemical and histological studies in rat models with experimental implants based on modified beta – TCP. Proceedings of the eight workshop experimental models and methods in biomedical research, 2017, 42-48.

Abstract
Calcium-phosphate (Ca-P) cements are widely used as bone substitutes in orthopedic, reconstructive and maxillofacial surgery because they have good biocompatibility and extensive bone conductivity. Many bivalent trace metallic ions have demonstrated their beneficial effects in bones tissues engineering applications. A bone-related enzyme alkaline phosphatase (ALP) together with bone – related minerals (Ca, P, Mg and Zn) act actively in bone formation. The aim of this study was to asses some bone turnover parameters (alkaline phosphatase, Ca, P, Mg and Zn) and histological response in rat models with experimental subcutaneous beta - TCP implants modified with doped trace elements (Zn and Mg). The newly synthesized three types cements - β-tricalcium phosphate doped with Zn/Mg were studied in a rat experimental model during 12 week. Slight deviations were observed in the studied bone turnover markers. There was an absence of inflammation and necrosis, suggesting that there were no toxic effects in the surrounding tissues and no disorders observed during degradation of materials. Results obtained showed that TCP with dual dopants of Mg and Zn has the potential to be used in orthopedics and dentistry.

20. Nanev, V., Vladov, I., Dimitrov, P., **Todorova, K**., Tsocheva Gaytandzhieva, N., Ruseva, K., Nikolova, D., Simeonov, M., Vassileva, E., Gabrashanska, M.. Biochemical studies in rats with polymeric hydrogel implants. Proceedings of the twelfth workshop with International Electronic Participation on biological activity of metals, synthetic compounds and natural products, 2017, 61-67.

Abstract
Hydrogels are polymeric materials distinguished by high water content and diverse physical and chemical properties. Polymer hydrogels are widely used as scaffolds and 61 materials for biomedical application due to the close mechanical behavior that they possess as compared to the body tissues. The aim of our study was to investigate the biocompatibility of polyzwitterionc hydrogel systems as bone implants. To this purpose, three groups of polymeric networks were studied. The 1st type was poly (sulfobetaine metacrilate) network, obtained by cross-linking with 4 wt% N, N'-methylene-bis-acrylamide neat (PSB4) and hybride, obtained after in situ formation of calcium phosphates (PSB4-CaP). The 2nd type was poly (sulfobetaine metacrilate) network, obtained by cross-linking with polyethylene glycol diacrylate (PEGDA) with different concentration of the crosslinking agent PEGDA. The 3rd group was poly (carboxybetaine methacrylate) network, obtained by cross-linking with PEGDA with different concentrations (6p CBMA and 7p CBMA). The 4th group are control- without implants. The materials were implanted in the soft tissues of male albino Wistar rats (subcutaneously and intramuscularly). There was a control group of animals with surgically made skin pockets but without hydrogels placement in them. The rats were euthanized 3 months after the implantations. Blood samples were collected to study a serum alkaline phosphatase activity and serum Ca and P contents. Ca and P contents were determined biochemically in the soft tissues surrounding the implants. The results showed that the studied biochemical parameters were in normal physiological values with non-significant deviations. There were no signs of inflammation or harmful effects in the soft animal tissues in the places of implantations. The conducted in vivo studies indicated that the investigated polymer hydrogels were welltolerated, non-toxic and biocompatible. Key words: Polymer hydrogels,

polycarboxybetaines (PCB), polysulfobetaines (PSB), carboxybetaine methacrylate (CBMA), biocompatibility, bone implants.

21. Milcheva, R., **Todorova, K**., Petkova, S., Vladov, I., Dilcheva, V., Georgieva, A., Ivanov, D., Iliev, I., Kirazov, L.. The muscle phase of trichinellosis in mice is associated with increased ST6GalNAc1 sialyltransferase activity in sceletal muscle fibers. Acta Morphologica et Anthropologica, 25, 3-4, 2018, 76-79.

Abstract
We previously showed that the de-differentiation of the occupied portion of muscle fibers toward Nurse cell after invasion by Trichinella spiralis is associated with increased intracellular accumulation of α-2,6-sialylated glycoproteins and novel gene activation of ST6GalNAc1. With this work we demonstrate ST6GalNAc1 expression in mouse skeletal muscles invaded by T. spiralis. Muscle samples were collected at certain time points after invasion. Immunochistochemistry was performed using rabbit polyclonal antibody against ST6GalNAc1 sialyltransferase. We found short up-regulation of the enzyme ST6GalNac1 that faded within the transformation of the occupied area into a Nurse cell. The enzyme ST6GalNAc1 is not synthesized in healthy mouse muscle tissue and is rarely expressed in normal tissues. It is responsible for the formation of the cancer-associated sialyl-Tn antigen in variety of carcinomas, blocking regular carbohydrate chain elongation. The functional role of this enzyme for the Nurse cell formation of T. spiralis in muscles has to be elucidated.

22. **Todorova, K**., Georgieva, A., Dikovska, A., Toshkova, R., Milcheva, R., Nikolov, B., Kozlov, V., Murovska, M., Russev, R.. Surface interactions and cellular uptake of metal nanoparticles in primary lymphocyte cultures Brief report. The Cyprus Journal of Sciences, 16, American College, Cyprus, 2018, 51-58.

Abstract
Gold and silver nanoparticles of 20, 40 and 100 nm in diameter were introduced into the culture medium of short-term lymphocyte cultures for studying the possibility to use them as carrier for therapeutic and immunomodulatory agents. Lymphocyte cultures from human volunteers and laboratory rats of Wistar strain were used. The ultrastructural analysis revealed that the nanoparticles congregated mostly in the extracellular space around the cell membrane without changes in the cell morphology being observed. Some methodological details associated with the use of suspension cultures are described. The results are discussed against the background of similar studies still few in the literature.

23. **Todorova, K**., Milcheva, R., Nikolov, B., Sultanova, A., Cistjakovs, M., Petkova, S., Russev, R.. Histochemical evaluation of increased sialylation in skeletal muscle fibers invaded by Trichinella spiralis. Proceedings of the ninth workshop on experimental models and methods in biomedical research, 2018.

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.

24. **Todorova, K**., Sultanova, A., Cistjakovs, M., Cunskis, E., Milcheva, R., Murovska, M.. Evidence of productive HHV-6 infection in autoimmune thyroiditis patients' thyroid gland tissue samples. The Cyprus Journal of Sciences, 16, The e-Journal of American College, 2018, 59-69.

Abstract

The present work describes several cases of confirmed autoimmune thyroiditis (AIT) in two females and one male, patients with clinical diagnosis of Struma nodosa after long-term medical treatment following a total thyroidectomy. In the thyroid gland tissues of all three patients significant HHV-6 load (500> copies/106 cells) was accounted. (By means of transmission electron microscopy were observed HHV associated viral-like particles and characteristic feature of herpes virus infections - multivesicular bodies (MVBs)).

25. Kolyovska, V., **Todorova, K**., Milcheva, R., Sultanova, A., Cistjakovs, M., Petrova, Z., Spasov, R., Murovska, M.. Can human herpes viral infection be a factor in triggering autoimmune thyroiditis?. Proceedings of the ninth workshop on experimental models and methods in biomedical research, 2019.

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.

26. Milcheva, R., Janega, P., Celec, P., Petkova, S., Hurniková, Z., Izrael-Vlková, B., **Todorova, K**., Babál, P.. Accumulation of α-2,6- sialoglycoproteins in the muscle sarcoplasm due to Trichinella sp. invasion. Open Life Sciences, 14, De Gruyter, 2019, 470-481. SJR (Scopus):0.266, JCR-IF (Web of Science):0.504 Q3 (Scopus).

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-αSer/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.

27. **Todorova, K**., Nanev, V., Vladov, I., Dimitrov, P., Vassileva, E., Dyulgerova Taneva, E., Vassileva, R., Gabrashanska, M.. Newly synthesized polymer hydrogels and hydroxyapatite nanoparticles (nhap) for biomedical application: histological and biochemical studies in rats. Acta Morphologica et Anthropologica, 26, 1-2, 2019, 44-51. SJR (Web of Science).

Abstract

The development of biocompatible zwitterionicpolymers and polymer-reinforced calcium phosphate pastes and cements in combination with specific drugs, has been considered as a promising strategy in bone tissue engineering and dental medicine. The main purpose of this work was to evaluate the relationship between physicochemical and mechanical properties of newly synthesized polymer hydrogels and hydroxyapatite nanoparticles (nHAP) and their biocompatibility in vivo. Standard hematological, biochemical and histological laboratory tests with Wistar rats and statistical analysis of the data obtained were performed. The results from the histological, hematological and biochemical analyses revealed that all tested materials are characterized by good biocompatibility and biodegradation. No hard inflammatory effects were noticed, only slight foreign body reaction responses were observed. The histological findings made by us confirmed the acceptance of the implanted materials and the good tolerance to their componential compounds.

28. **Todorova, K**., Peev, I., Kanchev, K., Ivanov, D., Milcheva, R., Todorov, T., Popova, T.. A fatal mixed bacterial infection in guinea pigs - clinical symptoms, microbiology and pathomorphology - Case report. Tradition and Modernity in Veterinary Medicine, 4, 1, 2019, 27-33.

Abstract

The occurrence of acute and chronic spontaneous or food born bacterial infections is a serious problem in the research practices that can compromise the normal course of experiments. Although the compliance with the necessary rules for means of transport, housing and feeding of laboratory animals, it is possible to import ubiquitous pathogens via food or animal bedding. Especially dangerous are some pathogenic spore-forming soil microorganisms that can contaminate concentrated and rough forages, fruit and vegetables both in harvesting and improper storage. This article presents a case of an acute infection in guinea pigs bred as laboratory animals, graduated lethally. Several weeks after their purchase the animals begin to show signs of depression, food and water refusal. Symptoms characteristic for toxicoinfection – cachexia, fever with drop of temperature below normal, spiking of hair, dehydration, watery to bloody profuse diarrhea, difficult breathing, bluish mucous membranes and subsequent respiratory paralysis were noticed for a period of 2-3 days. The disease was fatal to all animals within several days after the onset of the clinical signs. Materials from dead animals were taken for microbiological, parasitological and pathohistological examinations and clarification of the cause of death. The aim of this study was to clarify the cause of the acute lethal infection in guinea pigs reared in laboratory conditions.

29. Georgieva, A., **Todorova, K**., Iliev, I., Dilcheva, V., Vladov, I., Petkova, S., Toshkova, R., Velkova, L., Dolashki, A., Dolashka, P.. Hemocyanins from Helix and Rapana snails exhibit in vitro antitumor effects in human colorectal adenocarcinoma. Biomedicines, 8, 7, 2020, 194, SJR (Scopus):1.508, JCR-IF (Web of Science):4.717 Q1.

Abstract

Hemocyanins are oxygen-transporting glycoproteins in the hemolymph of arthropods and mollusks that attract scientific interest with their diverse biological activities and potential applications in pharmacy and medicine. The aim of the present study was to assess the in vitro antitumor activity of hemocyanins isolated from marine snail Rapana venosa (RvH) and garden snails Helix lucorum (HlH) and Helix aspersa (HaH), as well the mucus of H. aspersa snails, in the HT-29 human colorectal carcinoma cell line. The effects of the hemocyanins on the cell viability and proliferation were analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the alterations in the tumor cell morphology were examined by fluorescent and transmission electron microscopy. The

results of the MTT assay showed that the mucus and α-subunit of hemocyanin from the snail H. aspersa had the most significant antiproliferative activity of the tested samples. Cytomorphological analysis revealed that the observed antitumor effects were associated with induction of apoptosis in the tumor cells. The presented data indicate that hemocyanins and mucus from H. aspersa have an antineoplastic activity and potential for development of novel therapeutics for treatment of colorectal carcinoma.

30. Iliev, I., Ivanov, I., **Todorova, K**., Dimitrova, M.. Effects of a cotinus coggygria ethyl acetate extract on two human normal cell lines. Acta Morphologica et Anthropologica, 27, 3-4, 2020, 25-29 SJR – индексиран в WoS или Scopus (Web of Science).

Abstract

The effect of ethyl acetate extract from Cotinus coggygria (sumac) leaves on the viability of two normal human cell lines (BJ and MCF-10A) is tested. Both cell lines are known to express fibroblast activation protein α (FAP) – a serine protease involved in tumorigenesis and tumor progression. The extract is shown to contain one or more components that inhibit FAP. Using the Neutral Red Uptake Test, it is found that in the line of activated human fibroblasts (BJ), the treatment leads to inhibition of cell proliferation. Conversely, the extract has no adverse effect on MCF-10A cells (mammary gland epithelium cells) at low concentrations even increasing the cells proliferation by 13 %. It is concluded that the tested extract contains a potent inhibitor of FAP, which may be useful as an anti-cancer agent, but should be used with caution due to its dual effect on normal epithelial cells.

31. **Todorova, K**.. In the small world of the molds from the genus Fusarium. Proceedings of the 15th workshop on biological activity of metals, synthetic compounds and natural products, 2020, 5-10.

Abstract

Molds from the genus Fusarium are fungal species, multi-cellular mycelium organisms, adapted to almost every corner of our planet including human and animal food and feed products. Molds can cause two groups of diseases: mycoses - by colonizing the host organism mycotoxicoses-acute and chronic food –borne diseases and poisonings. The most common in nature of the representatives of the genus Fusarium are: F. graminearum, F. verticillioides (moniliforme), F. poae, F. equiseti, F. culmorum, F. sporotrichoides, F. proliferatum, F. oxysporum, F. solani and others, which are widespread in all climatic zones. Predisposing factors for their development of these fungi are high temperatures and humidity, problems in the agronomic measures and crop storage, insects and others. Fusarium molds produce three main groups of mycotoxins - fumonisins, trichothecenes and zearalenone, which are of a great ecomonic and health risk value, because of the various effect and diseases and are in the focus of research priorities of the World Health Organization (WHO) and European Food Safety Authority (EFSA).

32. Дакова, В., **Тодорова, К**., Димитров, П., Панайотова-Пенчева, М.. Паразити и противопаразитно третиране на лабораторни зайци, отглеждани в клетка. Proceedings of the 11th workshop on experimental models and methods in biomedical research, 2020, 22-27.

Abstract

The aim of the present study was to identify possible infestation of laboratory rabbits and the effectiveness of their subsequent administration treatment. Helminths of two nematode genera were found - Passalurus, invasion extent (EI) 10.5% and Nematodirus with EI 5.3%, and protozoa of the genus Eimeria (EI 57.9%), represented by the species E.magna, E. Media E.perforans. Of the Eimers, species E was found in most animals. Three of the rabbits infested with eymeria were markedly clinical signs of the disease. The infested animals were treated individually according to the established parasitological status with Fenbendazole in a dose 25mg / kg, Sulfachlorpyrazi n30% at a dose of 1g per liter of water for 10 days and multivitamin preparations. The parasitological status of the rabbits

was examined again on day 10 and 40 days after treatment, as when establishing parasites were again treated with the appropriate for the respective etiological agent antiparasitic agent. The results of what was done study showing the following: Treatment of laboratory rabbits with signs of eimeriosis with Sulfachlorpyrazine and multivitamin preparations leads to their clinical recovery but does not stop oocyst carrier and secretion. Administration of fenbendazole results in cessation of excretion of nematode eggs of the genera Passalurus and Nematodirus, but 40 days later treatment again observed emission of pasalurus eggs, which indicates that a single treatment with fenbendazole is not sufficient for the final cure of rabbits from pasalurosis.

33. Mladenov, G., Popova, T., **Todorova, K**.. Experimental staphylococcal infection in budgerigar (Melopsittacus undulatus) – diagnostics and therapy. Tradition and Modernity in Veterinary Medicine, 5, 1(8), 2020, 8-19.

Abstract

Staphylococcal infection in the budgerigar is one of the most common diseases in this species of animal. Studies have been carried out using various methods of experimentally infecting budgerigars with Staphylococcus aureus. The course of the disease, the clinical manifestation of staphylococcosis, as well as the pathological-anatomical changes are followed. Studies have been conducted on the effect of antibiotic therapy, depending on the stage of the infection

34. Ruseva, K., Ivanova, K, **Todorova, K**., Vladov, I., Nanev, V., Tzanov, T., Hinojosa-Caballero, D., Argirova, M., Vassileva, E.. Antibiofilm poly(carboxybetaine methacrylate) hydrogels for chronic wounds dressings. European Polymer Journal, 132, 2020, SJR (Scopus):0.864, JCR-IF (Web of Science):3.862 Q1.

Abstract

The current study demonstrates the benefits of poly(carboxybetaine methacrylate) hydrogels in chronic wound healing. These hydrogels demonstrate high absorbing capacity upon swelling in salt solutions thus revealing great potential as dressings for highly exuding chronic wounds. Moreover, upon swelling they expand, increasing their volume by 25%, which makes them patient friendly ensuring also the proper wound healing. Poly(carboxybetaine methacrylate) hydrogels were also shown to absorb collagenase and myeloperoxidase, two enzymes that are specific for chronic wounds, reducing in this way their amount by 30–45% in the wound bed without entirely inhibiting their activity, as the latter is necessary for the wound healing process. The hydrogels were also shown to be non-cytotoxic as well as to prevent the biofilm formation of S. aureus. The in vivo implantation in rats showed no immune response to moderate immune reaction for both studied PCB hydrogels. Thus, the properties of the PCB networks revealed in the study demonstrate their potential as chronic wounds dressing materials.

35. Sultanova, A., Cistjakovs, M., Sokolovska, l., **Todorova, K**., Cunskis, E., Murovska, M.. HHV-6 infection and chemokine RANTES signalling pathway disturbance in patients with autoimmune thyroiditis. Viruses, 12(6), MDPI Multidisciplinary Digital Publishing Institute, 2020, 689. SJR (Scopus):1.633, JCR-IF (Web of Science):3.816 Q1.

Abstract

The aim of this study was to investigate the role of human herpesvirus-6 (HHV-6) in autoimmune thyroiditis (AIT) development. We examined the possible involvement of HHV-6 gene expression encoding immunomodulating proteins U12 and U51 in AIT development and their role in the modulation of chemokine signaling. One hundred patients with autoimmune thyroiditis following thyroidectomy were enrolled in this study. Nested polymerase chain reaction (nPCR) was used to detect the HHV-6 sequence in DNA samples. Reverse transcription PCR (RT-PCR) with three different HHV-6 gene targets (U79/80, U51 and U12) was to detect active infection markers. HHV-6 load was identified using a commercial real-time PCR kit. Immunohistochemistry was performed to investigate the expression of the HHV-6 antigen and RANTES (Regulated upon Activation, Normal T Cell Expressed

and Secreted) in thyroid gland tissue. Different commercial immunosorbent assay kits were used for the detection of RANTES, IFNγ, IL-6, and TNFα levels in the AIT patient group and controls. We detected 98% presence of the HHV-6 genomic sequence in AIT patients' thyroid gland tissues. Markers of active HHV-6 infection (HHV-6 U79/80, U12 and/or U51 mRNA) were predominant in AIT patients' thyroid tissue samples in comparison with the control group (56% vs. 6%). Evidence from immunofluorescence microscopy showed that HHV-6 can persist in thyrocytes and can interact with RANTES. Visual confirmation of the intense immunofluorescence signal of RANTES detected in thyroid tissues could indicate high expression of this chemokine in the thyroid gland. On the other hand, immunosorbent assays showed very low RANTES levels in AIT patients' peripheral plasma. These results indicate that RANTES level in AIT patients could be influenced by HHV-6 activation, which in turn may aid AIT development.

36. Georgieva, A., **Todorova, K**., Iliev, I., Dilcheva, V., Vladov, I., Petkova, S., Toshkova, R., Velkova, L., Atanasov, V., Dolashki, A., Dolashka, P.. In vitro antitumor activity of hemocyanins isolated from Helix aspersa and Helix lucorum in human bladder carcinoma cells. Compt. rend. Acad. bulg. Sci., 74, 9, 2021, 194, SJR (Scopus):0.244, JCR-IF (Web of Science):0.378 Q2 (Scopus).

Abstract

The present study aims to assess the in vitro antineoplastic potential of total hemocyanins isolated from Helix aspersa and Helix lucorum (HaH-total; HlH-total), their structural subunits (βc-HaH; α-HaH; βc-HlH; α-HlH) and Helix aspersa mucus in human urinary bladder carcinoma cell line 5637. The effects of the hemocyanins on the cell viability and proliferative activity was determined by MTT test. The morphological changes induced by hemocyanins in the tumour cells were analyzed by fluorescent microscopy after staining with acridine orange/ethidium bromide and DAPI. The results of the MTT test showed a significant antiproliferative effect of all tested hemocyanin samples. The antitumour effects of subunits α-HaH and βc-HlH were most clearly pronounced. Microscopic analysis of the hemocyanin-treated bladder carcinoma cells revealed typical morphological features of apoptosis. The results of our study indicate that in addition to the known immunogenic effects, the molluscan hemocyanins also have a direct antitumour activity against urinary bladder cancer.

37. Georgieva, A., Toshkova, R., **Todorova, K**., Tsoneva, R.. Antineoplastic effects of erufosine on Graffi myeloid tumour in hamsters. Bulgarian Journal of Veterinary Medicine, 24, 3, 2021, 442-449. SJR (Scopus):0.211 Q3 (Scopus).

Abstract

Cancer has become one of the most significant health challenges for both human and veterinary medicine. The present study examined the antineoplastic and antimetastatic activity of the novel membrane-targeting anticancer agent erufosine. The antitumour effects of erufosine on Graffi virusinduced experimental myeloid tumour in hamsters was assessed by histopathological methods and evaluation of some biometric parameters of tumour growth. Two schemes of experimental antitumour therapy were applied – one that started simultaneously with the tumour transplantation and a second one that started after the appearance of palpable tumours. The results demonstrated protective antitumour effect of erufosine, expressed by decrease of transplantability, tumour growth inhibition, suppression of metastatic activity and extension of mean survival time. The effectivity of the experimental therapy was more pronounced when it was started simultaneously with the transplantation of the tumour cells. Presented results suggest that erufosine is a promising drug candidate for treatment of haematological malignances.

38. Iliev, I., Ivanov, I., **Todorova, K**., Tasheva, D., Dimitrova, M.. Cotinus coggygria Non-Volatile Fraction Affects the Survival of Human Cultured Cells. Acta Morphologica et Anthropologica, 28, 1- 2, 2021, 13-18. SJR (Web of Science).

Abstract

Ethyl acetate extract from Cotinus coggygria (smoke tree) leaves contains non-volatile components, some of which are potent inhibitors of prolyl oligopeptidase (POP) and fibroblast activation protein α (FAP). Those enzymes are known to participate in tumorigenesis and tumor growth. Effects of the above extract on several human cultured cells, originating from the most common and aggressive cancers were examined using the Neutral Red Uptake Test. The IC50 values were determined and selectivity indices (SI) versus non-tumorigenic cell lines MCF-10A and BJ were calculated. According to the results, C. coggygria extract has a highly selective effect on HeLa cells and can be considered as a potential therapeutic agent in cervical carcinoma. Additionally, it is shown that the simultaneous suppression of POP and FAP has a pronounced impact on the cell proliferation of both tumor and normal human cells at concentrations > 12 μg/ml, which proves the enzymes' role in the control of cell proliferation.

39. Ivanov, D., **Todorova, K**.. Multiple forms of serum sialyltransferase in normal rats and rats bearing Zajdelam hepatoma. Compt. rend. Acad. bulg. Sci., 73, 9, 2021, 1247-1253, SJR (Scopus):0.244, JCRIF (Web of Science):0.378 Q2 (Scopus).

Abstract

Comparative studies on the sialyltransferase activity in normal serum as opposed to serum of rats bearing Zajdela hepatoma (ascitic and solid forms) have been conducted. About 3-fold elevation of sialyltransferase activity in the serum of rats bearing Zajdela hepatoma ascitic form was established. The multiple forms of sialyltransferase in all three serum types have also been studied using preparative isoelectric focusing. The presence of 8 forms of sialyltransferase was detected within the pH range 3.54–8.95 for ascitic form and pH 3.54–9.66 for solid form. Forms with pI 3.54, 4.64, 5.35, 8.02, 8.48 and 8.95 could be considered specific to the ascitic form, while forms with pI 3.54, 4.36 and 8.02 are specific to the solid form.

40. **Todorova, K**., Angelov, A.. Morphological Characteristics of Rabbit Cornea in Norm and Wound Healing Cytoarchitecture. Acta Morphologica et Anthropologica, 28, 1-2, 2021, 19-27. SJR (Web of Science).

Abstract

Cornea is an avascular structure with an important role in vision, which could be impaired by different conditions. The most suitable animal model in ophthalmology research, comparable to human, is the rabbit model. Data on the morphology of rabbit cornea are capable to predict or to be used for comparison of the fundamental processes in corneal wound healing. In this study the morphological aspects of corneal postoperative wound healing processes were assessed in comparison with the normal histology of corneal layers in rabbits. Our findings demonstrated that the corneal wound healing was between the phases of proliferation and maturation-tissue regeneration restored the integrity for three months but lamellar organization and remodeling still were not completed. Strong postoperative keratitis was observed in one case and structure similar to the human pre-Descemet's layer was noticed.

41. **Todorova, K**.. The molds of the genus Aspergillus and their toxinshealth hazards in food. Proceedings of the Eleventh Workshop on Experimental models and methods in biomedical research, 2020, 2021, 46-51.

Abstract

Micheli, an Italian priest and naturalist, first described and named in 1729 the fungi Aspergillus (aspergillum from the Latin term aspergere, "to scatter"- a device used for sprinkling holy water), because of their shape. These microscopic fungi are widespread saprophytes or parasites, especially in warmer regions. However, our most frequent contacts with Aspergillus remain plants and products of plant origin. In immunocompromised patients the fungus is causative agent of the disease aspergillosis, affecting the brain and participates in the formation of mycetomas in the pulmonary upper airways, alveoli, the maxillary sinuses or sabotages bone marrow transplants. Also, in the period

of 1960-1970, were identified highly toxic metabolites of Aspergillus molds– aflatoxins. They were grouped in four main groups- aflatoxins B1, B2, G1 and G2 and along with more than 17 derivatives of their compounds are pro causative agents of mycotoxicoses and cancer.

42. Sapundjiev, E., Chervenkov, M., Popov, G., **Todorova, K**.. Adrenal glands histological structure in Brown Bear (Ursus arctos, Linnaeus, 1758). Acta Morphologica et Anthropologica, 28, 1-2, 2021, 32-37, SJR (Web of Science).

Abstract
The adrenal glands exhibit species specific differences in the outer layer of the glandular parenchyma cortex where in ruminants, some laboratory animals and human the cells form glomeruli, but in carnivores, horse and pig they are arranged in arches. The purpose of this study was to examine histologically adrenal gland of a deceased adult male Brown bear during summer time and to compare its morphology with those of other domesticated animals and human. In our study we found endocrine cell clusters in the capsule of the gland which was described only in horse adrenal gland. We also established that in outer cortical zone of the adrenal glands parenchyma the cells form arches which resembled the shape and the height of the dog's glands. The remaining inner cortical zones and the medulla were situated similarly to those of the bovine, horse, pig, dog and human adrenal glands and did not show structural peculiarities.

43. Toshkova-Yotova, T., Georgieva, A., **Todorova, K**., Pilarski, P., Toshkova, R.. Antitumor properties of vegetable oil extract from green microalga Coelastrella sp. BGV. Journal of Microbiology, Biotechnology and Food Sciences, 11, 2, 2021, SJR (Scopus):0.16 Q4 (Scopus).

Abstract
Microalgae are of great importance for the production of original natural substances of interest for food, health or biotechnological applications. Mass cultures of green microalgae are used for production of carotenoids such β-carotene, astaxanthin, canthaxanthin, lutein etc. The aim of this study was to obtain an oil extract from a Bulgarian green microalga strain Coelastrella sp. BGV and to assess its anticancer and apoptogenic activity in vitro against human tumor cells HeLa by means of MTT and fluorescence microscopy analyses. The results showed that the oil extract obtained by a direct extraction using a common vegetable oil reduces proliferation and induces apoptosis in HeLa cells. In contrast to widely used organic solvents for the production of carotenoids, the applied bio-solvent (sunflower oil) can be a valuable alternative approach for the needs of the food industry. The resulting oil extract showed promising antitumor and apoptosis-inducing activity against HeLa cells in vitro and potential for future use in practice.

44. Rafael, S., Ivanova, K., Stefanov, I., Puiggali, J., del Valle, L., **Todorova, K**., Dimitrov, P., Hinojosa-Caballero, D., Tzanov, Tz.. Nanoparticle-driven self-assembling injectable hydrogels provide a multi-factorial approach for chronic wound treatment. Acta Biomaterialia, Elsevier BV, 134, 2021, 131-143, SJR (Scopus):1,94, Q1 (Scopus).

Abstract
Chronic wounds represent a major health burden and drain on medical system. Efficient wound repair is only possible if the dressing materials target simultaneously multiple factors involved in wound chronicity, such as deleterious proteolytic and oxidative enzymes and high bacterial load. Here we develop multifunctional hydrogels for chronic wound management through self-assembling of thiolated hyaluronic acid (HA-SH) and bioactive silver-lignin nanoparticles (Ag@Lig NPs). Dynamic and reversible interactions between the polymer and Ag@Lig NPs yield hybrid nanocomposite hydrogels with shear-thinning and selfhealing properties, coupled to zero-order kinetics release of antimicrobial silver in response to infectionrelated hyalurodinase. The hydrogels inhibit the major enzymes myeloperoxidase and matrix metalloproteinases responsible for wound chronicity in a patient's wound exudate. Furthermore, the lignin-capped AgNPs provide the hydrogel with antioxidant properties and strong antibacterial activity against Staphylococcus aureus and Pseudomonas

aeruginosa. The nanocomposite hydrogels are not toxic to human keratinocytes after 7 days of direct contact. Complete tissue remodeling and restoration of skin integrity is demonstrated in vivo in a diabetic mouse model. Hematological analysis reveals lack of wound inflammation due to bacterial infection or toxicity, confirming the potential of HA-SH/Ag@Lig NPs hydrogels for chronic wound management.

45. Yankova, I., Ivanova, E., **Todorova, K**., Georgieva, A., Dilcheva, V., Vladov, I., Petkova, S., Toshkova, R., Velkova, L., Dolashka, P., Iliev, I.. Assessment of the toxicity and antiproliferative activity of hemocyanins from Helix lucorum, Helix aspersa and Rapana venosa. Bulgarian Chemical Communications, 53, Special Issue A, 2021, 15-21. SJR (Scopus):0.179 Q4 (Scopus).

Abstract

Hemocyanins (Hcs) are respiratory, oxygen-carrying metalloproteins that are freely dissolved in the hemolymph of many molluscs and arthropods. The interest in hemocyanins has grown significantly since it was found that they can be successfully used in immunotherapy of neoplastic diseases as non-specific or active stimulators of the immune system. The present study aims to assess the cytotoxicity, in vivo toxicity and antiproliferative activity of hemocyanins isolated from marine snail Rapana venosa (RvH), garden snails Helix lucorum (HlH) and Helix aspersa (HaH). For in vitro safety testing, 3T3 Neutral Red Uptake (NRU) test was used. The experiments for antiproliferative activity of the hemocyanins were performed by MTT assay on a panel of cell lines - a model of breast cancer. The in vivo toxicological assessment was performed by regular clinical examinations of hemocyanin-treated laboratory mice and histopathological analysis of hematoxylin/eosin stained preparations of parenchymal organs. The evaluation of the in vitro cytotoxicity showed that the tested hemocyanins does not induce toxic effects in nontumorigenic epithelial cell lines. In contrast, significant reduction of the viability of human breast carcinoma cell lines was found after treatment with high concentrations of hemocyanins. The in vivo experiments showed no signs of organ and systemic toxicity in the hemocyanin-treated animals. The presented data indicate that Hcs show a potential for development of novel anticancer therapeutics due to their beneficial properties, biosafety and lack of toxicity or side effects.

46. Milcheva, R., Petkova, S., **Todorova, K**., Ivanov, D.. Absence of ST3GAL2 and ST3GAL4 sialyltransferase expressions in the Nurse cell of Trichinella Spiralis. Bulgarian Journal of Veterinary Medicine, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, 2020, SJR (Scopus):0.164 Q3 (Scopus).

Abstract

I. Background. In skeletal muscles the sialic acids have a great significance for their functional maintenance and proper structural organization. Our work for the first time described the expressions of ST3Gal, ST6Gal and ST6GalNAc sialyltransferases specific for glycoproteins in mouse skeletal muscles and murine C2C12 myotube cell cultures. II. Methods and Results. Lectin histochemistry, cytochemistry and lectin blot were used to demonstrate the membrane localization and the electrophoretic profiles of α-2,3- and α-2,6-sialylated glycoproteins. The expression levels of sialyltransferases were analyzed 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 STR expressions showed differences between the two experimental groups, illustrated by the absence of expressions of the mRNA for the ST3Gal6 and ST3GalNAc3 enzymes 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. III. Conclusions. These results could be a useful addendum to the knowledge concerning the glycosylation of the skeletal muscle tissue. In addition, this report would be helpful and informative for any

researches in future where the C2C12 myotube cell cultures will take a place as an experimental model.

47. Milcheva, R., Petkova, S., **Todorova, K.,** Ivanov, D.. Absence of ST3GAL2 and ST3GAL4 sialyltransferase expressions in the Nurse cell of Trichinella Spiralis. Bulgarian Journal of Veterinary Medicine, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, 2020, SJR (Scopus):0.164 Q3 (Scopus).

Abstract
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.

48. **Todorova, K.,** Georgieva, A., Milcheva, R., Ivanov, D., Kalkanov, I.. Cytotoxicity of the Fusarium mycotoxin deoxynivalenol on mammalian and avian cell lines. Bulgarian Journal of Veterinary Medicine, Faculty of Veterinary Medicine, Trakia University, Stara Zagora,online first, 2020, SJR (Scopus):0.164 Q3 (Scopus).

Abstract
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.

49. **Тодорова, К**.. Експериментални in vivo модели в офталмологията Proceedings of the XII workshop experimental models and methods in biomedical research, 2021.

Abstract
About 80% of the information our brain receives comes from the visual system and any of its dysfunctions can significantly reduce the quality of life. The purpose of this review is to present some experimental animal models for various diseases of the cornea, retina and choroid used in preclinical research in ophthalmology and surgery. The presence of specialized animal models is a necessity that allows for study of the molecular mechanisms of pathological processes and for testing of new

therapeutic interventions in ophthalmology. Appropriate animal models facilitated the identification of genes involved in ocular pathology. The choice of an appropriate model depends mainly on the nature of the study - the type of disease, its mechanisms or the therapeutic process to be applied. All this mandatory use of experimental animal models in medical research to develop new approaches in the treatment of eye diseases in humans, which goes with its advantages and difficulties.

50. **Тодорова, К**.. Експериментални примери за използване на ТЕМ в биомедицинските изследвания. Proceedings of the XII workshop experimental models and methods in biomedical research, 2021.

Abstract

Electron microscopy is a technique for examining biological and non-biological samples. It can be combined with various other methods. The main types of electron microscopes are transmission (TEM) and scanning (SEM). TEM is used to study thin samples through which electrons can pass, generating projection image. Scanning electron microscopy gives an image based on the radiation of secondary electrons from the sample surface. It will be presented in this review the use of TEM in biomedical research. Transmission microscopy has high image resolution and is widely used in biomedical research at the level of cells, organelles and macromolecular complexes, viruses, bacteria, study of nanoparticles in biological samples, etc. The high resolutionimage capability in TEM is the result of the use of high frequency electron flux (electrons that have very short wavelengths) as a source of illuminating radiation. The cathode is used for the resource of electrons, and the anode accelerates them in directional beam focused by electrostatic or electromagnetic lenses. This is how the beam is formed passes through sufficiently thin objects about 0.1 microns and the image depends on whether the material is permeable to electrons or diverts them from the beam. Thanks to TEM in IEMPAM - BAS determines the biological phase and physiological role of cells, types of cell death, phases of division, intercellular connections and contacts, excretionor the intake of extracellular products and substances; extracellularly or intracellularly localization, viruses, bacteria, yeasts and parasites; nanocomplexes with different therapeutic values and many others. These observations are performed both in vitro in cell cultures and in vivo in human and animal tissues.

51. **Todorova, K**., Ivanov, I., Iliev, I., Dimitrova, M.. Biological activity of orally given ethyl acetate extract from Cotinus coggygria in albino mice with solid and ascites forms of Ehrlich's tumor, Acta Morphologica et Anthropologica, 28, 3-4, 2021, 3-9, SJR (Web of Science).

Abstract

Biological activity of ethyl acetate extract from the leaves of Cotinus coggygria Scop. (smoke tree) was studied in an in vivo model of mice both healthy and developing solid or ascites form of Ehrlich's mammary gland carcinoma. Thus, the toxicity of the extract, applied per os, as well as its possible antitumor activity were evaluated. Clinical and pathomorphological studies were carried out. According to the results, no signs of overall or organ-specific toxicity were found. The extract did not prevent the development of Ehrlich's tumor but reduced the solid tumor grade by enhancing the cells differentiation. Additionally, the herb was shown to possess a mild tissue-protective activity expressed by less pronounced pathological changes in the internal organs. Another beneficial effect of the extract application was the prolonged life expectancy of treated mice.

52. **Todorova, K**., Dimitrov, P.. Development and outcome of Feline injection - site sarcoma in an adult cat – case report. Tradition and Modernity in Veterinary Medicine, приета за печат с писмо от издателя, 2021.

Abstract

Feline injection – site sarcomas (FISS) are aggressively behaving fibrosarcomas with a rate of metastasis ranging from 10 to 25%. The main treatment of soft tissue sarcomas is surgical excision with clean margins and subsequent radiotherapy and systemic chemotherapy. We present a case of an adult male cat with recurrent fibrosarcoma, which anamnesis and aggressive tumor development

pointed to FISS. A fine needle biopsy first suggested and then histopathology confirmed that diagnose. Two months after the surgery a new tumor mass appeared on the site of the excision. The rapidly growing tumor penetrated the abdomen wall, occupying almost entire abdomen, affecting the internal organs and the right lumbar region muscles, observed by a conventional X-ray examination. A developing central necrosis provoked fistulation and formation of a large wet ulcerative wound in the state of a constant inflammation and tissue disintegration. The local treatment failure and the progressing poor general condition leaded to carry out a humane euthanasia of the animal.

53. Ivanova, A., Ivanova, K., Perelshtein, I, Gedanken, A., **Todorova, K**., Milcheva, R., Dimitrov, P., Popova, T., Tzanov, T.. Sonochemically engineered nano-enabled zinc oxide/amylase coatings prevent the occurrence of catheter-associated urinary tract infections. Materials Science and Engineering: C, Available online 26 October 2021, 112518, Journal Pre-proof 2021, SJR (Scopus):1.23, JCR-IF (Web of Science):7.3 Q1.

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, antibacterial 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.