Bingol University

Oles Honchar Dnipro National University

Palladin Institute of Biochemistry of NASU

Dnipro State Agrarian and Economic University

Workshop BU-DNU 2021

"ACTUAL ASPECTS OF MOLECULAR BIOLOGY, GENETICS, BIOCHEMISTRY AND VETERENARY MEDICINE"

April 29, Thursday 12:00

Program

1. <u>12:05</u> – Welcome: Prof. Dr. Ibrahim Çapak, Rector of Bingol University

2. <u>12:10</u> – Welcome: Prof. Dr. Sergey Okovytyy, Vice-Rector of Oles Honchar Dnipro National University

3. <u>12:15</u> – Welcome: Prof. Dr. Anatolii Kobets, Rector of Dnipro State Agrarian and Economic University

----------10 min presentation plus 5 min Q/A/D for each talk----------

Section-A: Veterinary medicine

1. <u>12:20</u> – Viktor Evert, Dnipro State Agrarian-Economic University, Ukraine

“Endemical situation with leptospirosis of farm animals in Ukraine”

2. <u>12:35</u> – Dmitro Masiuk and Iryna Volovyk, Dnipro State Agrarian-Economic University, Ukraine

“Current diagnostic techniques of animal diseases, safety animal feeding and human consumers”

3. <u>12:50</u> – Irina Subotsina and Igor Gromov, Vitebsk State Academy of Veterinary Medicine,

“Circulation of sars-cov-2 among various animal species, clinical and pathoanatomical manifestation of covid-19 in animals”, Republic of Belarus

4. 13:05 – Yasin Özturk, Bingol University, Turkey

“Evaluation of farmer`s knowledge and application of veterinary antibiotics usage”

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Section-B: Molecular and genetic mechanisms of the diseases.

1. <u>13:25</u> – Victor Nedzvetsky, Bingol University, Turkey, Oles Honchar Dnipro National University, Ukraine

“Signaling functions of muramyl peptides”

2. 13:40 – Musa TARTIK, Bingol University, Turkey

“Microbial Cell Factories: an engineered yeast for high-production of flavonoids“

3. 13:55 – Artem Tykhomyrov, Palladin Institute of Biochemistry of NASU, Ukraine

“Reparative autophagy and TIGAR up-regulation as resistance factors against plasmin-induced apoptosis/anoikis in lung adenocarcinoma A549 cells”

4. 14:10 – Galyna Ushakova, Oles Honchar Dnipro National University, Ukraine

“Modification of serum proteins after experimental brain hemorrage in rat under type 2 diabetes mellitus”

5. 14:25 – Ramazan Gundogdu, Bingol University, Turkey

“Personalised Cancer Treatment: Synthetic lethal interactions in DNA repair pathways”

6. 14:40 – Olena Dovban. Oles Honchar Dnipro National University, Ukraine

“Astrocytes under modified restraint water-immersion stress and myocardial damage.”

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Section-C: Environmental Biochemistry and Agriculture

1. 14:55 – Viktor Gasso, Oles Honchar Dnipro National University, Ukraine

“Biomarkers and biomonitoring of aquatic environment pollution”

2. 15:10 – Veysel Turan, Bingol University, Turkey

“The permanent problems of the global environment pollution”

3. 15:25 – Nusret Özbay, Bingol University, Turkey

“Modern automatic system of the tomatoes production”

4. 15:40 – Yelena Sukharenko, Kerch State Maritime-Technology University, Moscow State Academy of Veterinary Medicine and Biotechnology by K.I. Skryabin, Russia, Bingöl University, Bingöl, Turkey.

“The study of specific molecular markers in the fish exposed to realistic concentration of mercury”

5. 15:55 – Yuriy Murdasov, Oles Honchar Dnipro National University, Ukraine

“The ions levels and oxidative stress in kidney of rats with type 2 diabetes and intracerebral hemorrhage, and impact of metformin and vitamin D”

6. 16:10 – Alla Shevtsova, Oles Honchar Dnipro National University, Ukraine

“Modification of serum proteins after experimental brain hemorrhage in rats under type 2 diabetes mellitus”

---------- **Round Table** ----------

Chiefs: Veysel Turan, Nevzat Esim, Galyna Ushakova and Victor Gasso

Collaboration and exchange possibility between universities of Turkey, Ukraine and Belarus

ABSTRACTS

ANTIOXIDANT PROTECTION UNDER THE INFLUENCE OF THE MAGNETIC FIELD IN THE SIMULATION OF EROSIVE-ULCER LISTER OF THE STOMACH IN RATS

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It is known that disruption of the pro- and antioxidant systems (AOS) of the body leads to the development of "oxidative stress" - a pathogenetic factor in many diseases. The growing number of free radical pathologies, which include gastroesophageal reflux disease and peptic ulcer disease, makes it important to search for factors that can affect the system of oxidative homeostasis.

Purpose: to evaluate the effect of magnetic field (MF) on lipoperoxidation processes and antioxidant defense system in tissues of rats with experimental pathology of the gastroduodenal zone.

The study was performed on white outbred male rats weighing 220-250 g (n = 24). Animals were divided into five groups: I - control group (n = 6) consisted of rats, which were injected intragastrically through a tube with saline (1 ml / 100 g). Intragastric group II rats were injected with saline and the abdominal area was treated with MF with the following characteristics: modulation frequency - 75–85 Hz, radial component - 5–10 mTl, tangential component - 0.5–15 mTl, exposure - 15 min. Group III (n = 6) included animals with erosive-ulcerative damage (EUD) of the stomach. EUD simulation was performed by intragastric administration of medical bile (1 ml / 100 g) for seven days. Rats of group IV (n = 6) simultaneously with the simulation of EUD received applications of the magnetic field. At the end of the experiment, euthanasia was performed under ketamine anesthesia at a dose of 1 mg / 100 g by decapitation.

Object of research: blood, tissues of the stomach, liver, brain (B) of rats. In blood, homogenates of tissues of the stomach, liver, brain, LPO activity was determined by the content of TBA-active products (TBAAP) in the reaction with

thiobarbituric acid. In the experimental tissues, the state of the antioxidant system was investigated by the level of reduced glutathione (RG), which was determined by the Ellman reaction, and by the activity of enzymes of anti-peroxide protection. The activity of catalase (Kat) was evaluated by the reaction with ammonium molybdate, glutathione reductase (GR) - by the oxidation rate of HADPH, glutathione peroxidase (GPO) - by the method based on the reaction of the interaction of Ellman's reagent with SH-groups, superoxide dismutase (SOD) – by inhibiting the reduction of nitrosine tetrazolium.

Statistical data processing was performed using the software package Statistica 6.0 (StatSoft, USA). The significance of the difference was assessed using Student's t-test.

Studies of the effect of MF on rats of group II did not reveal significant changes in the indicators of the AOS and the floor in the blood, liver tissue, B and stomach. It should be noted that in experimental rats without pathology, the application of MF led to a slight activation of lipoperoxidation processes in the B tissue, as evidenced by the tendency to increase the level of TBAAP. Modeling of gastric EUD in group III rats was accompanied by intensification of free radical processes in the blood, which led to an increase in plasma TBAAP by 94% ($P<0,05$), in erythrocytes - by 62% ($P<0,05$) relative to the corresponding control indices. In the liver tissue of animals of group III, gastric EUD modeling led to the activation of LPS processes with an increase in the amount of TBAAP by 93% ($P<0,05$), while inhibiting the enzymes of antiradical and antiperoxide protection. Thus, there was an inactivation of SOD by 38% ($P<0,05$), inhibition of enzymes of the glutathione system GPO - by 35% ($P<0,05$), GR - by 30% ($P<0,05$) with depletion of the level of RG by 57% ($P<0,05$) in comparison with the corresponding control indices. These changes occurred with the activation of the antiradical enzyme SOD by 29% ($P<0,05$) on the background of inhibition of Kat by 63% ($P<0,05$) compared with similar control groups. The protective system of glutathione in the blood of rats of group III responded to the formation of erosive-ulcerative process in the stomach of experimental animals by activating GPO by 44% ($P<0,05$) while reducing the pool of RG by 35% ($P<0,05$) according to similar control data. In B tissue, an imbalance in the work of the enzymatic antioxidant link led to increased lipoperoxidation, an increase in the amount of TBAAP in 2,4 times ($P<0,05$), activation of SOD - by 76% ($P<0,05$) with simultaneous inhibition of Cat - by 35% ($P<0,05$), GPO - by 49% ($P<0,05$), GR - by 28% ($P<0,05$), depletion of the pool of RG in 1, 9 times ($P<0,05$) according to the control indicators. Modeling of gastric EUD in group III animals was accompanied by accumulation of LPO products in gastric tissue, as evidenced by an increase in the level of TBAAP by 56% ($P<0,05$) relative to control values. in the blood of animals of group IV there was an inhibition of LPO processes, as evidenced by a decrease in plasma TBAAP by 46% ($P<0,005$) and erythrocyte TBAAP - by 45% ($P<0,005$) relative to the indicators of group III rats. There were tendencies

to SOD activation, as well as significant inhibition of GPO and GR by 42% (P<0,005) and 46% (P<0,005), respectively, and replenishment of the RG pool by 34% (P<0,005) relative to similar indices in animals III groups. Under the action of MF in rats of group IV, the activity of Kat increased according to the index of animals of group II by 80% (P<0,005).

In the liver tissue of rats of group IV, in contrast to the effect on blood, the effect of MF caused the activation of lipoperoxidation processes, which is confirmed by an increase in the amount of TBAAP by 9% (P<0,05) compared with group III. Activation of SOD by 56% (P<0,005) was accompanied by inhibition of Kat by 9% (P<0,005) and increase in GPO activity by 60% (P<0,005) relative to the corresponding indices of group III. The decrease in GR activity by 8% (P<0,005) did not prevent an increase in the level of RG by 14% (P<0,005) compared with similar indicators of group III rats. In rats of group IV in the tissue of B under the action of MF there was an increase in the number of TBAAP in 2 times (P<0,005) and an increase in the pool of RG by 71% (P <0,005) relative to these animals of group III, respectively. At the same time, the activity of B antioxidant enzymes in animals of group IV had no significant changes compared to the indices of rats of group III, but relative to the data of animals of the control group SOD activity was increased by 43% (P<0,05), inhibition of GPO and GR was 59% (P<0,005) and 22% (P<0,005), respectively. The effect of MF led to an increase in TBAAP by 34% in the gastric tissue of rats of group IV (P<0,005) compared with the corresponding indicator of animals of group III.

Thus, the effect of MF on experimental animals depended on the initial functional and metabolic state of the organism and its systems, on organs and tissues, their metabolism. Applications of the magnetic field to animals with simulated gastroduodenal pathology resulted in inhibition of LPO processes in the blood of experimental rats by activating free radical reactions in liver, brain and stomach tissues against the background of partial inactivation of glutathione eeenzymes enzymes due to increased reduced glutathione.

CIRCULATION OF SARS-COV-2 AMONG VARIOUS ANIMAL SPECIES, CLINICAL AND PATHOANATOMICAL MANIFESTATION OF COVID-19 IN ANIMALS

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Between December 2019 and April 2021, scientists and medical professionals around the world have learned quite a lot about the coronavirus infection called COVID-19, caused by the recently discovered (identified) SARS-CoV-2 virus. This infectious disease for the second year does not allow humanity to return to a normal active life and communication in all spheres. In many countries, strict quarantine measures are still in place, lockdowns have been introduced in a number of countries, countries are closed to the entry of foreign citizens and the exit of their own citizens, and trade, political and social relations between countries are hindered. To date, 3 million people have already fallen victim to this disease.

One feature of SARS-CoV-2 is of concern to scientists and epidemiologists (medical and veterinary) around the world – this is the lack of strict species specificity. Initially, it was proved that COVID-19 is a zoonosis-a disease transmitted to humans from an animal. Potential sources of the new virus have been identified, one of the main ones today is considered to be a bat, although there are disputes about the additional participation in the process of mutation and transmission of the pathogen to humans of pangolin and snake.In the first days and months of the pandemic, the disease was registered and studied only in humans, but today this situation has changed dramatically. Based on the official data of the OiE, FAO, WHO, CDC, AVMA and a number of other international organizations, officially today this virus has been isolated from the body of a fairly large number of animals, and the virus not only circulates in the body of animals, but also as in humans, it can cause the development of diseases in individual animal species and even death. Diseases is

registered and the clinical picture is partially described in representatives of the cat family (domestic cat, lion, leopard, tiger, puma), in fur-bearing animals (European mink, ferret). Chinese researchers conducted an experiment and proved the transmission of SARS-CoV-2 from individual to individual within the domestic cat population. Italian scientists conducted an extensive study of cats and dogs in the most affected areas of COVID-19 in Italy and identified a fairly high percentage of animals with antibodies to SARS-CoV-2, which indicates the susceptibility of these animal species to the new virus. To date, in a number of European countries and in the United States, outbreaks of this disease have been registered and are being recorded among the mink population and about 20 million animals of this species have already been destroyed. In the United States, the circulation of a new coronavirus in the wild (free-living) population was detected mink. There are data on the possibility of infection of laboratory animals (white mice), hamster and guinea pig, raccoon dog, badger, pigs (in experimental infection). Scientists still doubt that animals can transmit the virus to humans, but they are confident that the reverse process is possible.

We conducted our own screening studies among the livestock of animals (domestic (cats, dogs, ferrets, guinea pigs, parakeet, chinchilla), zoo (ferrets, mongoose, rhinoceros, monkeys), agricultural (pig, cattle, horses, donkey, poultry), wild (red deer), fur (European mink, fox)) In the Republic of Belarus, it was possible to detect the circulation of the virus and the development of the disease with characteristic clinical symptoms in the following animal species:

* mink (clinical symptoms and death were observed);

*domestic cats (clinical symptoms and deaths were observed, there is evidence of pathological births, deformities, stillbirths and non-viability of kittens);

* dogs (clinical symptoms have been shown, there is evidence of pathological births, deformities, stillbirths and non-viability of puppies);

* ferrets (clinical symptoms and case were observed);

When studying the duration of the incubation period, the features of clinical and pathoanatomical manifestations, and histological changes, the following was found.

1. The main clinical symptoms in infected animals are: refusal to feed, depression, shortness of breath, shortness of breath, cough, cyanosis of the mucous membranes, tachycardia, less often - diarrhea and fever. The incubation period averaged from 6 to 10 days.

2. The main pathoanatomical changes in infected animals are: hurricane (membranogenic) pulmonary edema ("carmine lungs") with areas of alveolar emphysema and small-focal pneumonia with predominant localization in the anterior and middle lobes, pronounced blood clotting in the arteries and veins of medium caliber, acute expansion of the atria and right ventricle ("pulmonary heart"), pulmonary vein systems. Pronounced postmortem blood clotting in the heart cavities, large arteries and veins, fatty dystrophy with pronounced liver

edema, acute venous hyperemia of the kidneys, fatty dystrophy of the cortical substance, pronounced edema of the medulla, hypotrophy (congenital, postnatal).

3. At the histological level, the following changes were noted: pronounced proliferation of interlobular and interalveolar connective tissue, lymphoid-macrophage peribronchitis and perivasculitis, formation of nodular lymphoid tissue, extensive areas of alveolar emphysema, atrophy or absence of alveolar epithelium; liver – total small – drop fatty dystrophy, pronounced edema (dilation of Disse spaces); kidneys – venous hyperemia, serous edema of the glomeruli and interstitial connective tissue; heart – serous edema of the myocardium; spleen-pronounced lymphoid hyperplasia of the white pulp.

The data obtained confirm the need for further study of the circulation of the new coronavirus both in the population of domestic and farm animals, and in the population of wild animals, to identify the features of the clinical and pathoanatomical manifestations of the disease, and to develop effective treatment and prevention tools. To resolve these issues, we consider it necessary to conduct joint work and mutual exchange of information between representatives of human and veterinary medicine.

THE IONS LEVELS AND OXIDATIVE STRESS IN KIDNEY OF RATS WITH TYPE 2 DIABETES AND INTRACEREBRAL HEMORRHAGE, AND IMPACT OF METFORMIN AND VITAMIN D

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Aim: It is known that one of the most common and severe complication of type 2 diabetes is diabetic nephropathy, which is characterized by proteinuria, glucosuria, polyuria etc. and may lead to chronic kidney disease. At the same time, several investigations suggest, that intra-cerebral hemorrhage may be a reason of renal function declining. Ion and oxidation balances are vitally important in maintaining the renal function, but also suffer in mentioned pathologies. Metformin is a well-known drug for the blood glucose reduction and vitamin D – as an antioxidant. The aim of this work is to investigate whether vitamin D and metformin may prevent the pathological changes in rat's kidney with type 2 diabetes and intra-cerebral hemorrhage.

Methods: The studies were performed on adult male Wistar rats in accordance with the ethical standards of work with laboratory animals. All rats were divided into 5 groups (n = 6): 1 - control group; 2 - rats with experimental streptozotocin-induced diabetes mellitus (DM2); 3 – rats with DM2 and intracerebral hemorrhage (ICH); 4 – rats with DM2 and ICH under metformin administration; 5 - rats with DM2 and ICH under vitamin D administration. The level of oxidative stress was determined in cytosolic extracts obtained from the kidneys of the studied rats, according to the following parameters: catalase activity and MDA concentration. Catalase activity was estimated by the H_2O_2 decreasing in the incubation medium according to Korolyuk; MDA concentration - in the reaction of interaction with thiobarbituric acid according to Andreeva. Changes in ions concentrations were determined by the content of potassium and chlorine using the appropriate standard test kits LLC SPE "Philisit-Diagnostics" (Ukraine). Statistical data processing was performed using

one-way analysis of variance ANOVA, data were considered probable under conditions of $P < 0,05$.

Results: According to the results of the study, an increase in catalase activity can be observed, while the concentrations of MDA were unchanged, what may be explained as an early stage of oxidative damage transformation of cells compartments. The increase in catalase activity in rats of the 2nd group occurred by 4,7%, in the 3rd group - by 3,3%. The administration of vitamin D and metformin significantly decreased the catalase activity to control levels.

The changes of the content of potassium and chlorine ions were also significant for experimental groups compared to the control animals. The decrease in potassium and chlorine concentrations in the kidneys of rats of the 2nd group occurred by 33,9% and 9,5%, respectively, compared with the control group of healthy rats, in the kidney extracts of rats from the 3rd group - by 49,7% and 8,9%, respectively. The administration of vitamin D and metformin increased the ions concentration in rats' kidneys homogenates to control levels.

Conclusion: Development of DM2 and ICH may affect renal function, and the occurrence of ICH under condition of DM2 may worsen the kidney's biochemical parameters. High glucose levels in DM2 lead to elevated oxidative damaging of tissues and the activation of renin-angiotensin-aldosterone system (RAAS); on the other hand, ICH causes a systemic inflammation in organism, hypertension and activates RAAS too. Oxidative stress, hypertension and "doubly" activated RAAS are the reasons of renal impairment and ion imbalance, what may end as a chronic kidney disease. The administration of vitamin D and metformin significantly decreases the level of oxidative stress and turn the ions concentrations to normal values.

REPARATIVE AUTOPHAGY AND TIGAR UP-REGULATION AS RESISTANCE FACTORS AGAINST PLASMIN-INDUCED APOPTOSIS/ANOIKIS IN LUNG ADENOCARCINOMA A549 CELLS

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BACKGROUND. Activation of plasminogen system plays a key role in cancer progression and metastasis. In cancer, cell-associated plasmin proteolysis contributes to the loss of cell adhesion and dissociation from the primary site, tissue remodeling, invasion, and metastasis. Pericellular plasmin generation induces apoptosis/anoikis in normal adherent cells. In contrast, cancer cells are notoriously resistant to anoikis, enabling metastasis and new tumor growth beyond their original environment. Autophagy is thought to be a major contributor to anoikis resistance of cancer cells, which provide their survival during a travel through the vascular system to disseminate distant organs. Mechanisms of cancer cell acquiring anoikis resistance may represent a relevant pharmacological target for reducing tumor malignancy by lowering metastatic potential of tumors.

AIM. To evaluate capacity of lung adenocarcinoma cells to activate plasminogen, to assess effects of pericellular plasmin on cell survival, and to clarify if plasmin is able to induce autophagy in cancer cells.

MATERIALS AND METHODS. Adenocarcinoma A549 cell line is one of the most commonly used human non-small cell lung cancer cell lines for basic research and drug discovery. These cells have relatively high proteolytically active phenotype by overexpressing proteins of plasminogen activation system, including surface-bound uPA/uPAR cascade. Human non-small lung adenocarcinoma cells A549 were incubated with the native Glu-plasminogen (0.1 - 1.0 μM) for 24 h. Pericellular plasmin activity was measured spectrophotometrically by a cleavage of the specific chromogenic substrate S-2251. Cell survival of adenocarcinoma cells A549 and normal fibroblasts 3T3, both affected to plasmin, was assessed by MTT-test. Fibronectin degradation products, levels of autophagy markers (beclin-1 and LC3) and

glycolysis/penthosophosphate pathway regulator TIGAR were evaluated by western blot in cell lysates. Cellular localization of plasminogen and LC3 protein was visualized by immunocytochemistry with the use of confocal laser scanning microscope LSM510 (Zeiss, Jena, Germany).

RESULTS AND DISCUSSION. It was shown that plasminogen is localized on the surface of plasma membrane, where is rapidly converted into plasmin in a concentration-dependent manner by endogenous activators (presumably, urokinase, or uPA). Plasmin cleaved one of the most important cell adhesive substrate, fibronectin, thus disrupting cellular adhesive contacts, which resulted in cell detachment. However, A549 cells preserved their viability after plasminogen treatment for 24 h, while 0.75 - 1.0 μM of plasminogen appeared to be cytotoxic for non-transformed fibroblast cells. Plasminogen 0.1, 0.5, and 1.0 μM induced 7.08-, 5.18-, and 3.78-fold elevation of TIGAR expression ($P<0.05$), respectively. Enhanced TIGAR expression indicates switch of glucose oxidation in pentosephosphate shunt rather than in glycolysis to produce more NADPH in order to protect against oxidative stress, prevent apoptosis through DNA repair. Incubation of adenocarcinoma cells with plasminogen in concentrations of 0.1 and 0.5 μM caused 1.74- and 2.19-fold elevation of early autophagy marker beclin-1 expression vs. untreated cells ($P<0.05$), respectively. Plasminogen treatment (0.1 - 0.5 μM) resulted in increased expression of LC3-I and stimulated rapid conversion of late autophagy marker LC3-I and its rapid conversion into LC3-II, a lipoconjugate associated with an autophagosome membrane. Up-regulation of beclin-1 levels and enhanced LC3-I/II conversion in plasminogen-exposed A549 cells means autophagy induction and progression. In addition, immunocytochemistry data showed increased LC3 puncta and autophagosome formation after incubation with plasminogen that supports autophagy activation. In the context of cancer progression and metastasis, upregulation of autophagy is a necessary step for supporting metabolism of cancer cells by partial autodigestion of cellular structures and macromolecules that allows traveling cells survival until they recover their adhesion in distant organs and form metastasis.

CONCLUSIONS. We highlighted here that plasmin-induced activation of autophagy in adenocarcinoma cells may provide them resistance to apoptosis/anoikis after detachment from the primary site. Thus, mechanisms of plasmin-induced autophagy in cancer cells are needed to be explored, while blocking autophagic flux may be a promising strategy to counteract tumor invasion and metastasis.

THE POSSIBLE MECHANISMS OF HEPATOTOXICITY OF STREPTOZOTOCIN AS AN INDUCTOR OF TYPE 2 DIABETES MELLITUS

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Nowadays, diabetes mellitus is a well-studied pathology, there are many drugs and modern treatment regimens for this disease. However, not all aspects of the pathogenesis of this disease are sufficiently studied and evidence of this is the morbidity rate, high mortality, and complications. Considering the data on the multifactorial pathogenesis of type 2 diabetes mellitus (T2DM), it is undoubtedly relevant to study various experimental models of insulin deficiency, which would at the pathogenetic level correspond to the development of this disease and could be the basis for searching new promising antidiabetic drugs, ways to correct various complications caused by T2DM. It is very important to study the toxic effects of streptozotocin (STZ) on the liver. When creating an experimental model for the reproduction of the hyperglycemic state, streptozotocin is preferred as the most stable and less toxic agent (Lenzen, 2008).

The aim of the study was to determine the possible mechanisms of streptozotocin hepatotoxicity as a T2DM inducer.

The experiment was performed on adult male Wistar rats weighing 230-250 g in accordance with standards of management, requirements, and rules of laboratory animals' treatment: the rules of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986); Physician Ethics and Human Rights: Regulations on the Use of Animals in Biomedical Experiments (2003) and Regulations on the Use of Animals in Biomedical Experiments.

An experimental model of T2DM was a pathological process that develops in animals while using a diabetogenic substance streptozotocin (Lenzen, 2008). The formation of pathology was induced by intraperitoneal streptozotocin at a dose of 65 mg/kg as a 5% solution in citrate buffer, pH 4,5 (Galenova, 2010) and nicotinamide 230 mg/kg. T2DM was confirmed by measuring blood glucose with an Optium Omega glucometer (Abbot Diabetes Care Inc., USA) on the third day after reproduction of the pathology and 6-hour

food deprivation under conditions of free access to water. In further studies, only animals with high levels of fasting glucose were used (8-14 mmol/l).

Markers of hepatotoxicity are the activity of hepatic alanine aminotransferase (ALT) and alkaline phosphatase (ALP) (Danan&Teschke, 2015), as well as the activity of aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and gamma-glutamyltransferase (GGT), and lipid concentration were measured in the liver.

It was established that under the conditions of streptozotocin-induced T2DM there were no significant changes in the activity of the studied enzymes compared with the group of intact animals. The changes observed occurred within the reference values.

However, it is necessary to note certain changes in markers of lipid metabolism hat occurred in the liver under conditions of this pathology. Thus, in the group of animals with induced T2DM, the concentration of triacylglycerols (TAG) was increased by 1,5 times compared with the group of intact animals. At the same time, the concentration of total cholesterol was within the values of intact animals. Therefore, these results provide additional evidence for the formation of T2DM, which is associated with changes in lipid metabolism of adipose tissue. Under the conditions of decreasing insulin concentration in the blood, activation of triacylglycerol lipase is observed, as a result of which TAG are released to the bloodstream. Then, TAG are delivered to the liver, where they are normally used for the synthesis of ketone bodies. The accumulation of TAG in the liver indicates a violation of their utilization mechanisms, which can lead to steatosis in conditions of T2DM.

Thus, the results suggest that the streptozotocin-induced model of T2DM is the closest to the human pathology and the least toxic to the liver, and therefore can be used in further animal studies for in-depth study of the T2DM mechanisms.

MODIFICATION OF SERUM PROTEINS AFTER EXPERIMENTAL BRAIN HEMORRHAGE IN RATS UNDER TYPE 2 DIABETES MELLITUS

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Introduction. Worldwide, cerebrovascular diseases and type 2 diabetes mellitus (T2DM) are common disorders that are among the top ten leading causes of death. Both types of pathology are tightly associated: it was shown that diabetes increases the risk of stroke, and stroke, in turn, is a risk factor for diabetes. Intracerebral hemorrhage (ICH) is the most dramatic subtype of stroke that is associated with higher mortality, particularly in T2DM population. Few studies have focused on the impact of T2DM on ICH and discussed the blood-brain barrier disruption, brain edema, and hematoma formation. More recently, investigating the role of oxidative damage of proteins in T2DM-ICH animal models has gained attention.

Aim of the study. To study the oxidative modification of serum proteins in rats with experimental T2DM after ICH and to establish the possibility of using these indicators as non-invasive markers of brain hematoma.

Materials and methods. All experimental procedures were performed in accordance with national and institutional guidelines for protecting animal welfare as well as the protocol approved by the Biomedical Ethics Committee. 24 Adult male Wistar rats weighted 269.1±12.1 were randomly divided into 3 groups (8 rats in each): 1 – intact rats, 2 – rats with T2DM, and 3 – rats with T2DM+ICH. T2DM was induced by intraperitoneal (i.p.) injection of streptozotocin65 mg/kg (STZ, Adooq Bioscience, USA) and nicotinamide 230 mg/kg (NAD, Sigma-Aldrich, USA). Blood glucose levels were measured with the blood glucose meter Bionime Rightest GM300 (Bionime Corporation, Switzerland) after the collection of blood samples from the tail vein, and glycated hemoglobin HbA1c was measured by Bio-La-Test® Glycated Hemoglobin. ICH was induced on the 55th day by the microinjection of sterile saline containing 0.2 IU bacterial collagenase (Type IV-S, Sigma-Aldrich, 1.0μL of 0.2U/μL) into the striatum after a burr hole. Samples of serum were obtained

at the end of the experiment by the intracardiac punction followed by centrifugation at 1500 g for 10 min. Advanced Oxidation Protein Products (AOPP) were measured by the method of Witko-Sarsat, proteins carbonyls (PC) – by the reaction of dinitrophenylhydrazine (2,4-DNPH) with oxidized radicals of amino acids, advanced glycated end products (AGEs) – by the quantitative fluorescence, and ischemia modified albumin (IMA) – by albumin cobalt-binding test. Data were tested with the ANOVA; nonparametric Mann-Whitney U test was used for the assessment of differences between the selected groups.

Results. According to our results, the mean level of AOPP in rats did not significantly change in T2DM group, while AGEs, IMA, and neutral and basic PC were increased in these rats. The more significant changes were observed in T2DM+ICH group: all studied indices were increased (excluding IMA that was significantly decreased in comparison with groups 1 and 2). All these changes occurred on the background of unchanged content of albumin and other blood proteins and higher levels of HbA1c. It should be noted that there were no association between AGEs and HbA1c. At that time, changes of AGEs in groups 2 and 3 correlated with changes of protein carbonyls, particularly with their basic forms PC_{430}, where Pearson correlation coefficient was 0.68 ($p<0,05$) and 0.66 ($p<0,05$) in groups 2 and 3, respectively.

Conclusion. Determination of oxidatively modified proteins in the blood can be useful in preclinical diagnostic of ICH and monitoring of its complications in patients with T2DM.

HUMAN EAR SULFUR COMPONENTSSTUDY AND DIAGNOSTIC VALUE

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ВИВЧЕННЯ ТА ДІАГНОСТИЧНА ЦІННІСТЬ КОМПОНЕНТІВ ВУШНОЇ СІРКИ З ОРГАНІЗМУ ЛЮДИНИ

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Останнім часом за розвитку пандемії набувають все більшої актуальності неінвазивні скрінінгові методи дослідження показників організму людини. Одним з таких доступних субстратів для вивчення є вушна сірка. Доведено, що вушна сірка несе у собі важливу інформацію щодо здоров'я та стану організму людини.

У дорослої людини майже 2000 сірчаних залоз в кожному зовнішньому слуховому проході, які виділяють 12–20 мг вушної сіркина місяць. Основні компоненти вушної сірки – ланостерол, сквалені холестерин. Крім того увушній сірці зустрічаються відшаровані клітини епідермісу, гіалуронова кислота, білки (імуноглобуліни), мінеральні солі, часточки пилу. Склад сірки дещо відрізняється у чоловіків та жінок, наприклад, чоловіча сірка має менш кислу реакцію, ніж жіноча (у середньому pH від 4,0 до 6,0). Він також залежить від національності людини. Так у представників азіатських народностей сірка містить більше білків та по консистенції більш суха, а у представників європеоїдної та негроїдної рас вона багата жирами и більш м'яка за структурою.

Цей секрет виробляється потовими, сальними та сірковими залозами вушного каналу і містить у своєму складі ряд біохімічних компонентів, зокрема насичені та ненасичені жирні кислоти, спирти, холестерин, білкові молекули, мінеральні солі, тощо.

Детальний біохімічний склад сірки змінюється у залежності від харчування, етнічної приналежності, віку та стану навколишнього середовища. Серед білків у вушній сірці містяться лізоцим, імуноглобуліни, лактоферин, пептиди та інші білкові молекули.

Діагностична цінність визначення компонентів вушної сірки має великі перспективи.

Вже у нинішній час саме за сіркою можна діагностувати суттєві порушення обміну речовин – лейциноз та алкаптонурію - до їхнього виявлення за аналізами крові та сечі. Також є розробки японських вчених у галузі онкодіагностики за допомогою вушної сірки.

Наші пілотні дослідження спрямовані на визначення вмісту імуноглобулінів у складі вушної сірки у нормі та при патологічних змінах різного характеру із застосуванням імуноферментних методів – сендвіч аналізу ELISA. У контрольній групі практично здорових людей (7 осіб молодого віку жіночої статі) виявлено слідові кількості імуноглобулінів А та G. Такі показники підтверджуються літературними даними. У подальших пошуках планується визначення вмісту цих білків за різних патологічних станів організму людини та встановлення спрямованості дії отриманих антитіл.Також потрібно проаналізувати загальний білковий склад сіркових виділень з вуха у різних вікових та статевих груп населення міста Дніпра та України в цілому.

ASTROCYTES UNDER MODIFIED RESTRAINT WATER-IMMERSION STRESS AND MYOCARDIAL DAMAGE

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A lot of physical and mental disorders are caused by the effects of stress. The brain is the central organ involved in the processes of perception and adaptation to psychological and physical stressors. Restraint water immersion stress (RWIS) – the model of stress that includes both psychological and physical stimulation. Long-term effects of stress were found to induce myocardial ischemia. Myocardial damage is a serious health problem that causes substantial morbidity and mortality. Primary changes in the response of the nervous system to stress are widely studied in neurons. However, the functional state of neurons directly depends on astrocytes.

The aim of the study was to compare the sensitivity of astrocytes to modified stress conditions and myocardial damage in different areas of the brain and to assess the effects of 2-oxoglutarate on the brain after such conditions.

30 Wistar rats were used for investigation divided into 5 groups (n=6): 1 – control for RWIS; 2 – control for myocardial damage; 3– rats subjected to modified RWIS for 3 days with changing temperature and permanent light stressors; 4 – animals with 14 days period of physiological recovery after subjection to modified RWIS for 3 days; 5 – animals with pituitrin-isadrine-induced myocardial damage (Belenichev et al., 2012). All procedures were conducted according to the guidelines established by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. After the experiment, all animals were decapitated under mild anesthesia. The different areas (hippocampus, thalamus, cerebellum, visual cortex) isolated from the rat`s brains were used for differential ultracentrifugation, and fractions with cytoskeletal proteins were obtained. The level of filamentous GFAP was determined due to competitive ELISA using monospecific polyclonal antibodies (Santa Cruz Biotechnology Inc., USA). Statistics were provided using the Student's test and the one-way analysis of variance (ANOVA) followed by Tukey's test for multiply comparisons. Values with $P < 0.05$ were considered reliable.

Under the modified RWIS effect, there was a significant upregulation of the level of filamentous GFAP by 23% and 18% in the visual cortex and thalamus respectively compared to the control group. However, the concentration of filamentous GFAP was decreased by 31% in the hippocampus

under immobilization stress. No changes of GFAP concentration in the cerebellum were observed under 3-daysstress condition. The physiological recovery after stress for 14 days showed restored levels of GFAP in the studied brain areas.

The results obtained indicate significant increases in the level of the filamentous GFAP under the pituitrin-isadrine-induced myocardial damage in all investigated areas of the brain. In the cerebellum by 18%, in the cerebral cortex –13%, in the thalamus –12 %, and in the hippocampus by 19%.

Our results show that 3-days modified restraint water-immobilization stress and the pituitrin-isadrine-induced myocardial damage are clearly responsible for the increased contents of fGFAP in the rat’s brain depending on the area.

EVALUATION OF COMMERCIAL METHODS TO SEPARATE NUCLEIC ACIDS FROM INTESTINAL TISSUES OF PIGS FOR DIAGNOSIS OF PORCINE EPIDEMIC DIARRHEA

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Nucleic acid extraction is the process of separating different forms of DNA and RNA from other biological macromolecules using a specific sequence of biochemical and biophysical methods (Busa et al., 2016). The first protocol for the isolation and purification of nucleic acids was developed by Johannes Friedrich Miescher in 1869 (Greenly et al., 2015). The process of nucleic acid extraction is a fundamentally important step in modern molecular genetic studies, such as polymerase chain reaction (PCR), sequencing, restriction analysis, molecular hybridization, etc (Ali et al., 2017; Akshara, 2018). For testing, all these methods require a solution of nucleic acids with a high degree of purification and a minimal level of degradation of their molecules (Hardy et al., 2017).

Intracellular nucleic acids are divided into genomic (chromosomal) and plasmid DNA as well as separate types of RNA. In general, despite some differences in the composition of RNA and DNA (the presence of uracil or thymine) and the unique three-dimensional conformation of these linear biopolymers, the basic physicochemical properties of nucleic acids are similar. Current methods of nucleic acid extraction have been successfully used, with only minor modifications, both for DNA and RNA isolation. An important difference between DNA and RNA extraction methods is that RNA has a higher lability and sensitivity to a wide range of RNA, which increases the risk of degradation of the ribonucleic acid molecule (Chacon-Cortes, 2014).

The quality of nucleic acid extraction is one of the important steps for molecular genetic studies, in particular polymerase chain reaction (PCR). It directly affects the kinetics, which depend on the concentration of the extracted nucleic acids, their degree of destruction and purification of nucleic acid

solution from PCR enzyme inhibitors (Chacon-Cortes et al., 2014; Cui et al., 2015; El-Maklizi et al., 2015). PCR enzyme inhibitors are quite important in the extraction of genetic material from samples, which are rich in low molecular weight substances that have inhibitory properties against enzymes, as well as enzymes that increase the destruction of nucleic acids – DNA and RNA (Busa et al., 2016). The most common material is faecal or intestinal specimens, which in most cases are used as an object of research for the indication and identification of the etiology of gastrointestinal infections by PCR (Choi et al., 2014; Carvajal et al., 2015).

For today, there is a wide range of nucleic acid extraction methods, among which the most effective and consequently the most common are solid phase methods. These include modern variants of methods of extracting nucleic acids on magnetic balls, silicate membrane, silicon sorbent, etc. (Fong et al., 2015; Busin et al., 2016).

One of the most common diseases in pigs in Western European countries and since 2014 in Ukraine is porcine epidemic diarrhea (PED) which leads to significant economic losses (Cochrane et al., 2015; Gerber et al., 2016; Yonghyan et al., 2017; Wanitchang et al., 2019). PED is caused by the RNA genomic coronavirus and is accompanied by diarrhea in pigs of different sex-age groups and causes high mortality (up to 100%)

The aim of our study was the evaluating commercial methods for extracting nucleic acids from pig intestinal tissues for the diagnosis of PED. The study was based on samples of small intestine tissues and faeces from 3–5 day old pigs which died from PED. Nucleic acid extraction was performed using commercial kits with different nucleic acid separation strategies based on: silicon-sorbent; silicate membrane fixed in a microcentrifuge column and magnetic balls. The studies were conducted in two stages. The first was a comparison of the results of the amplification of the obtained nucleic acid extracts from the homogenate of the intestines of piglets by using the above-mentioned commercial kits for the extraction of nucleic acids. For this purpose, samples of homogenate were used which in weight corresponded to the guideline for the application of the test kits.

The second step was directed to determining the efficiency of extraction of DNA and RNA from homogenate samples with a weight of 10, 50, 100 and 200 mg. Determination of the optimal methodological strategy of nucleic acid

extraction for the diagnosis of porcine epidemic diarrhea by PCR has been investigated. The results of the PCR studies of RNA of the PED virus and a unique pig DNA fragment indicate that the extraction of nucleic acids by commercial kits has different levels of efficiency and depends on different factors. According to the research, it was found that the most important of them are the adsorption capacity of the solid-phase sorbent, its configuration and nature, which binds RNA and DNA molecules, the type of sample from which extraction takes place, its volume, or the tissue mass used for extraction. Based on the obtained results, it has been estimated that the most effective PED virus RNA extraction is by “ArtBioTech”, “Bio Extract Column”, and “Viral DNA/RNA.

MURAMYL PEPTIDE INHIBITS MIGRATION AND UPREGULATE CELLULAR REACTIVITY OF U373MG CELL

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Background and aim. The Muramyl peptides (MPs) are the main component of bacterial walls into peptidoglycan's structure. Peptidoglycan is confirmed as one of important natural source of bioactive compounds which are potent to stimulate both innate immunity and exhibit anti-cancer activity in various cell types. The enzymatic cleaving of bacterial peptidoglycan generates the biology active fragments containing N-acetylmuramyl peptides named muropeptides. Muramyl pentapeptide is bigest muropeptide which contains longes aminoacid chain. Anti-tumor effects of different muropeptides were reported with respect to various tumor types including sarcoma, leukemia, melanoma and lung cancer. All of aforementioned activities are mediated by specific stimulation of MPP-specific cell surface and intracellular reseptors. Furthermore, MPP-induced cell response lead to metabolic energy expenditure via binding TLR and the NOD(1-2) which are members of the pattern recognition receptors for bacterial peptidoglycan. NODs sense distinct monomeric peptidoglycan (PGN) fragments and constantly interact with the actin cytoskeleton, which facilitates their rapid relocalization upon stimulation. Other sensor of MPP is Hexokinase which control metabolic energy production. Besides NOD stimulation initiates the transcripitional regulation through NF-kB in various cell types. Thus, MPP could be a new molecular tool to regulate cellular response and energy expenditure. Every MPP-dependent signalling is related to stimulation various pathways and energy consumption. It is hypotezed that MPP stimulation of cancer cells could deplete metabolic energy through upregulation of several mechanisms of cellular response.

As a rule, tumoricidal activity of muropeptides accompanied by the modulation cytokines and chemokines production. Elevated cytokines production can initiate either cell surviving or cell death in depend on the power and the duration of stimuli. The inhibition of the glial cell viability by stimulation with proinflammatory factors is well known phenomenon. Glial cells exhibit reactivity via NF-kB upregulation. NF-kB is one universal adaptor of cellular response by cytokines production. Other widespread regulator of cell reactivity is PARP which provides the regulation of cell function via ADP-

ribosilation to control various vital functions. Furthermore, PARP can serve as a coactivator of NF-kB and modulate its transcriptional activity. Unique muropeptide stimulus can induce functional cooperation of PARP and NF-kB in course of cell response. In addition, cellular response which accompanied by extensive activation of both NF-kB and PARP can initiate cell death via dysregulation in cytokines production and extended metabolic energy expenses.

Glioma is a prevailing brain cancer type of astrocyte-derived tumors. Glioblastoma is most aggressive grade of the gliomas among brain tumors. Glioblastoma cells have a high rate of migration and extremely potent to invasion . Therefore, the search for agents with anti-migration activity is actual to limit glioma aggressiveness. Taking into acount that muropeptide exposure could initiate in glioma cells abnormal cellular response, it hypotesed as anti-inasive tool to suppress aggressive glioma phenotype. study were to elucidate the role of PARP1 and NF-kB in anticancer effect of muramyl pentapeptide from Lactobacillus delbrueckii strain in glioma U373MG cells.

Methods. MPP (MurNAc-l-Ala-d-Glu-l-Lysd-Ala-d-Ala) was isolated and purified from Lactobacillus delbrueckii subsp. Cell viability was determined with MTT assay. Metaboilc energy production was estimated by NADPH measuring. PARP1 and NF-kB expressions were detected by western blot. Cytotoxic effect of MPP was characterized in vitro with cell viability and migration scratch-assay.

Results. The metabolic energy deficit in glioblastoma cells initiated by the exposure to 25 μg/ml - 200 μg/ml MPP was determined as dose dependent decrease in NADPH content. Cytotoxic effect of MPP was characterized in vitro with cell viability and migration scratch-assay. The results of cell viability measuring in control and treated with MPP glioblastoma U373MG cells showed dosedependent cytotoxic effect. The results on the impact of MPP onto U373MG cells migration test in a course of scratch-assay have shown dose-dependent effect in a range concentration from 25 to 200 μg/ml.

The PARP1 expression in control and exposed to MPP glioblastoma U373MG cells was upregulated except the lowest dose exposure. The results of NF-kB expression in control and exposed to MPP glioblastoma U373MG cells have showed an increase in almost all treated cell groups.

Conclusion. Muramyl pentapeptide exposure induces disturbances in NADH content, inhibits migrative capability and upregulate PARP1 and NF-kB expression in glioblastoma U373MG cells. Obtained results evidence that muramyl pentapeptide could initiate a lack of migration via metabolic energy expenditure.

EFFECT ON IMIDACLOPRID LOW DOSES ON OXIDATIVE STRESS AND NEURAL CELL DISRUPTION IN *EISENIA FETIDA*

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Imidacloprid is a widely used pesticide that belongs to the class of neonicotinoids. There is rising evidence that neonicotinoids exert cytotoxic effects in non-target organisms including vertebrate species such as mammals. Nevertheless, dose limiting toxicity and molecular mechanisms of neonicotinoids deleterious effects are still poor understood. In accord to imidacloprid fate in the environment, the most part of used pesticide is absorbed in a soil. Therefore, earthworms, which are prevailing soil organism, could be considered as a target of neonicotinoids toxicity. The earthworm's simple nervous system is a prospective model for neurotoxicological studies.

There are many evidence that neonicotinoids exert the multiple toxic effects in various organisms (Chevillot et al., 2017; De Lima e Silva et al., 2020). These deleterious effects are accompanied by metabolic disorders, DNA damage, reactive oxygen species (ROS) production and suppression of antioxidant systems in earthworms (Dani et al., 2019; Zhang et al., 2014; 2021). However, limited information is available on the molecular mechanism neonicotinoid toxicity for earthworms.

Recent study has focused on the neural tissue cells disturbances. The similar to insect abnormalities of nicotinic acetylcholine receptors were detected in various species (Rawi et al., 2019). Therefore, neonicotinoids are potent to provoke plural disturbances, which associated with its direct effect on nicotinic acetylcholine receptors. Recent studies have shown the impairment of earthworm metabolomics caused with imidacloprid (Zhang et al., 2021, Dani et al., 2019). Considering that oxidative stress and metabolic disruptions are the main initiators of programmed cell death (Redza-Dutordoir & Averill-Bates, 2016), a decline in worm populations viability may be relate to the cell death switching in neonicotinoid-polluted areas.

Earthworms *Eisenia fetida* were placed in wooden boxes with natural soil substrate to acclimatize for 20 days (18 ± 2 °C). The exposure to imidacloprid was carried out using the filter paper contact test (OECD 1984). The earthwormi were treated with doses 0.1, 0.2 and 0.4 μg/cm^2 that was carried out by

application accordingly 1.4 μg, 2.8 μg and 5.6 μg imidacloprid doses applied to 14 cm^2 filter paper placed into Petri dish.

Each earthworm was individually treated with one of aforementioned doses under conditions of 20 ± 1 °C in the darkness and a relative humidity of 80–85% for 24 hours. After 24 h treatment, the earthworms were removed into artificial soil in a 500-mL beaker at 20 ± 1 °C for 24 h in the darkness for rehabilitation. The exposure–rehabilitation procedure was repeated for 14 days. Totally, every earthworm was exposed to respective imidacloprid dose for 7×24 hours.

Earthworms were scarified according to the rules adopted by the Bioethics Committee of Oles Honchar Dnipro National University. The earthworm tissue was washed with ice-cold phosphate buffer saline (PBS). Then the PBS was removed with filter paper and the tissue samples were subjected to protein extraction. We use standard homogenization and centrifugation methods of protein analysis.

The reactive oxygen species (ROS) level was measured by using fluorometric method based on DCFHDA reaction. The ROS measuring was performed with a SpectraMax spectrofluorometer with 485 nm wavelength excitation and 530 nm emission.

Western blotting was performed with using SDS-PAGE electrophoresis in 5-20% gradient of acrylamide. After standard procedures, the membranes were incubated overnight at +4 ° C with the primary rabbit monoclonal anti-NSE (1:3000) and mouse monoclonal anti-β-actin (1:5000). The results of western blot were developed with ECL kit based on chemiluminescence method with the use of the automatic X-ray machine. Densitometric analysis of immunostained polypeptide zones was performed with the use of TotalLab TL120 software. The intensity value obtained with scanning every individual band was normalized to the intensity in respect with correspondent actin band.

Oxidative stress is confirmed as the common driver of both structural and functional disruption in different cell types of diverse groups of organisms. Since pesticides are known as xenobiotics, which powerful to induce the redox imbalance in various organism types, we have been measured the main indices of oxidative stress ROS and lipid peroxidation (LPO) levels in earthworms. In order to investigate the realistic effects of mercury low doses the measuring of ROS level was carried out in the whole tissue worm extracts. A dose-dependent increase in ROS content was observed in the groups exposed to imidacloprid with 1.4 – 5.6 μg doses compared to untreated control. Since LPO level is a function of ROS generation and reflects membrane disruption, we measured this index to estimate oxidative damaging of cellular membranes. Statistically significant increase in LPO level has observed in the groups treated with 2.8 and 5.6 μg doses compared with untreated control.

In order to investigate the possible neurotoxic effect of imidacloprid on the earthworm, neurone-specific enolase (NSE) expression was detected with western blot. The progressive decline in NSE expression was observed in groups

exposed 2.8 and 5.6 μg doses. Besides, there was not detected statistic significant changes in group treated with dose 1.4 μg in compare with control.

Taking together the obtained results, observed toxic effects of imidacloprid is associated with oxidative stress generation and decline in the neurospecific protein NSE content in earthworm exposed to low doses of this neonicotinoid. Furthermore, imidacloprid cytotoxicity is accompanied by dose-dependent increase of ROS and LPO levels.

Thus, low doses of imidacloprid induce oxidative stress in earthworms, which is associated with the increase in NSE expression. Imidacloprid-caused cytotoxicity is accompanied by neuronal damage and neurotoxicity could be recognized as a particular scenario of the general imidacloprid cytotoxicity.

RELATION OF NEUROTOXICITY AND GENOTOXICITY INDUCED BY INORGANIC MERCURY IN FISH BRAIN: DNA REPAIR MACHINERY

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Mercury is known as one of the most toxic heavy metal environmental pollutants for aquatic biota, terrestrial animals, and human beings. Global mercury environmental pollution generates a risk factor for natural ecosystems due to high mercury bioaccumulation rate in living organisms. The neurotoxicity of mercury is well known (Pletz et al., 2016; Cariccio et al., 2019).

Mercury compounds are mainly presented in water bodies and fish accumulate it. There is a direct interaction of inorganic mercury with the blood-brain barrier (BBB); it brings the trouble in the BBB integrity (Zheng et al., 2003). The delayed effect of low doses inorganic mercury in the fish was also lately confirmed (Pereira et al., 2015).

One of the unspecific consequences of many ecotoxicants is launching the redox imbalance. Reactive oxygen species (ROS) products can induce injuries of DNA. Mercury substances have genotoxic effects (Chang et al., 2017). Unfortunately, the mercury influence on DNA repairing machinery is not well known.

The single-strand breaks (SSB) are the often-happened DNA lesions. Environmental pollutants can cause a growth of DNA SSB (Meland et al., 2019). The base excision repair (BER) is a stable enzymatic system renovating the SSB with the apurinic/apyrimidinic endonuclease 1 (APE1) as a key BER enzyme. Dysregulation of APE1 leads to deviations in cell viability. The DNA repair machinery is implicated in a cellular response to genotoxic damage, and the problems with DNA reparation could cause genomic instability and cell viability decrease. The anti-apoptotic protein Bcl-2 is a mitochondria-associated. It is vulnerable to intracellular signalling that regulates cell viability and apoptosis.

Rainbow trouts (*Oncorhynchus mykiss*) (n=28) were divided into four equal groups and exposed to mercury chloride low doses of 9, 18, and 36 $\mu g \times L^{-1}$

for 60 days. After the treatment, the fish were sacrificed according to the rules of Oles Honchar Dnipro University Bioethics Committee. The ROS level was measured by the fluorometric method using DCFHDA. The analysis of APE1 and Bcl-2 was carried out with western blotting. Comparisons were considered statistically significant as $P < 0.05$.

We found the decline in APE1 expression in fish exposed to 18 and 36 $\mu g \times L^{-1}$ mercury chloride. The finding of anti-apoptotic protein Bcl-2 decrease demonstrated that inorganic mercury exposure is powerful to inhibit the apoptosis suppressor. Data obtained show that inorganic mercury cytotoxicity accompanies the dose-dependent rise of ROS and the drop in the APE1 and Bcl-2 levels in treated fish brain tissue.

The APE1 activity is predominantly related to DNA repair pathways. Furthermore, the APE1 is a key player in the BER pathway and delivers the vital genome stability in different cells. Obviously, found ROS production in the fish brain is able making DNA breaking. As observed in our study, the failure in APE1 level echoes the inhibition of BER, which is an evolutionary conservative pathway restoring the SSB preventing the transcription progressions (Iftode et al., 1999).

The defects in DNA repairing can directly initiate a decline in cell viability and a lack of neuronal functioning. Thus, the detection of DNA repair enzymes in the brain cells exposed to toxic agents is a prospective way to evaluate both neurotoxic and genotoxic effects of environmental contaminants including mercury. Total coordination of cellular response initiated by DNA lesions launches the DNA damage response and the BER pathway is the largest part of the total DNA damage response machinery.

APE1 could be considered as one of the important targets for mercury genotoxicity. The suppression of APE1 in neural tissue cells can inhibit DNA repair response, upsurge genomic instability and activate. Thus, similar irreversible abnormalities intrude brain cell viability and functions. Moreover, mercury-induced redox imbalance can initiate apoptosis straight through the initiation of mitochondria-dependent controllers.

As observed in our study, the decrease in anti-apoptotic protein Bcl-2 demonstrates that mercury exposure initiates apoptotic deviations. Therefore, mercury is forceful to induce programmed cell death in neural cells through two found effects: genome instability and mitochondria-associated apoptosis.

Thus, exposure of fish to low doses of inorganic mercury makes oxidative stress, impedes the DNA repair, and stimulates mitochondria-dependent apoptosis in neural tissue. The APE1 expression can be a soon-to-be instrument to disclose the harmful subsequence of the environmental pollution.

IMIDACLOPRID AFFECTS INTERCELLULAR ADHESION AND LAUNCHES PROINFLAMMATORY ALTERATIONS IN INTESTINAL CELLS

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Imidacloprid is a comparatively new neonicotinoid insecticide developed in 1990s, which is a first commercially introduced by Bayer Crop Science (Tomlin, 1997). The neonicotinoids are specific to synaptic target due to high affine agonisticness of the nicotinic acetylcholine receptors (nAChR) that responsible for the control of ligand-gated ion channels and neurotransmission (Tomizawa & Yamamoto, 1993). The neonicotinoids are highly selective toxic for insects contrary to mammals (Liu & Casida, 1993). Nevertheless, some reports on its toxicity to mammals have been published (Schulz-Jander et al., 2002; Schulz-Jander & Casida 2002).

The permanent increase in neonicotinoid insecticides use in agriculture throughout the world could resulted in the growing of their accumulation in agricultural products (Wu et al., 2020). Therefore, the digestive system could be a first target of the neonicotinoid toxicity. Intestines fulfils digesting food and intake nutritive substances. However, another important function of the intestine system is a barrier one. It is kept with specialized adhesion of intestine epithelial cells. These epithelial cells form a tight cell monolayer that exaggerated by infectious agents and toxic compounds consumed with food and water. Thus, unique barrier mechanisms of intestine epithelial cells are a first target of swallowed neonicotinoids.

Subchronic exposure to imidacloprid can disrupt the gut barrier function and could be potentially toxic to mammals (Yang et al., 2020) and affect cell viability in the human Caucasian colon adenocarcinoma (Caco-2) epithelial cell model (Shi et al., 2021). The intestine barrier can be disordered by breaking the intercellular junctions. Therefore, chronic exposure to neonicotinoid low doses should be assessed as a risk factor for non-target organisms.

Reactive oxygen species (ROS) production is a common unspecific disruption caused by different toxic compounds. The inflammation, in its turn, is an important innate defence against infectious and toxic agents. Oxidative stress is influential to inspire the proinflammatory changes in most cell types. The intestine cells develop biological barriers, such as a gut-blood barrier, which are exceedingly vulnerable to damaging agents and produced the inflammatory

cytokines (Banks et al., 2015). Among the cytokine family of tumour necrosis factors (TNF) the TNF-α and inducible oxide nitrogen synthase (iNOS) successfully indicate the inflammatory imbalance in different cells (Shen et al., 2021).

The barrier function of intestinal system is mediated by specialized epithelial cells of gut. These polarized cells form the tight contacts to each other through the characteristic to epithelial cells tight and adherens junctions. The tight and adherens junctions are formed with specialized proteins occludin and E-cadherin respectively (Hartsock & Nelson 2008). The adhesive contacts of epitheliocytes provide the strength bindings between contiguous cells and maintain intestinal barrier function. The intestine barrier function could be disrupted under the influence of different toxicants. The effect of nicotinoids on the intestinal barrier function remains undefined. Taking into account that intestine epithelial cells is potent to absorb neonicotinoids, we hypothesized that imidacloprid could affect a vital barrier function of these cells probably through the disruption of intercellular adhesion and proinflammatory changes. Therefore, the integrative detection of intercellular adhesion and proinflammatory cytokines production could be a prospective tool to elucidate the cytotoxic effect of the contaminants as well as the molecular mechanisms of neonicotinoids toxicity in respect to barrier function of intestinal system. In order to understand the mechanisms of cytotoxic effect of neonicotinoids we have studied the expression of proinflammatory cytokine, iNOS as inflammatory initiator, specific for epithelial cells adhesive and tight junctions proteins in Caco-2 cell culture model under imidacloprid chronic exposure.

The Caco-2 cell culture was incubated at 37 °C and 5% carbon dioxide up to 90% confluence. Eighteen passages with using 0.25% trypsin-EDTA (0.25% trypsin, 0.02% EDTA) were performed. Then, the cells (7×10^5 cells per /well) were placed in 6 cm Petri and cultured to 100% confluence. The Caco-2 cells at completely confluence were exposed to imidacloprid doses 0.10, 0.25 and 0.75 $\mu g\times mL^{-1}$ for 4 days.

We use the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay to estimate the imidacloprid cytotoxicity on intestine epithelial cells. All the procedures were carried out accordingly to the manufacturer's recommendation. The Caco-2 cells were exposed to 0.10, 0.25 and 0.75 $\mu g\times mL^{-1}$ doses of imidacloprid and then incubated at 37° C for 96 h. Then the cells were washed by phosphate saline buffer (PBS) and were exposed to the 20 μL MTT and 180 μL PBS. The incubation with MTT reagent was carried out for 4 h at 37 °C in a humidified atmosphere with 5% CO_2. After incubation, MTT solution was removed and in each well was completed with 180 μL DMSO for 10 min. The absorbance level was scanned at 570 nm length wave in the presence of 20 μL Sorensen's buffer.

The ROS production was determined with DCFHDA with a SpectraMax spectrofluorometer at 485 nm wavelength excitation and 530 nm wavelength

emission. The control and exposed to imidacloprid Caco-2 cells were centrifuged and lysed in RIPA buffer containing proteinase and phosphatase inhibitor cocktail. The proteins were separated with polyacrilamide gel electrophoresis with using 5–20% gradient of acrylamide and then proteins were transferred from gel onto polyvinylidene fluoride membrane with applying the electric field (10 $V \times cm^{-1}$). After necessary procedures with primary antibodies, washing membrane was incubated with according secondary anti-rabbit or anti-mouse IgG antibodies conjugated with horseradish peroxidase. Immunostaining was made with luminol-hydrogen peroxide solution by the enhanced chemiluminescence method with the use of X-ray films (Konica Minolta, Japan).

Densitometric analysis of the immunostained polypeptide zones was performed with the use of TotalLab TL120 software (USA). The intensity value obtained with scanning every individual band was normalized to the intensity in respect with correspondent GAPDH band. Every track on the scanned picture was corrected to background level, which corresponds to nonreactive area on the X-ray film.

The completely confluence Caco-2 cells were exposed to 0.10, 0.25 and 0.75 μg/mL imidacloprid for 96 h. The decrease in cell viability was observed in the Caco-2 cells treated with 0.25 and 0.75 $\mu g \times mL^{-1}$ imidacloprid. These doses reduced cell viability by 81% and 72% respectively.

The dose-dependent imidacloprid effect was detected for the ROS level up-regulation in the Caco-2 cells exposed to 0.25 and 0.75 $\mu g \times mL^{-1}$ doses. Occludin and E-cadherin are biomarkers of tight junctions and adherent junctions proteins responsible for the intestine barrier function. Obtained data revealed that both occludin and E-cadherin were downregulated in exposed to 0.25 and 0.75 $\mu g \times mL^{-1}$ imidacloprid Caco-2 cells.

We have found the dose-dependent increase in TNF-α expression under imidacloprid exposure of 0.25 and 0.75 $\mu g \times mL^{-1}$ doses. We found that imidacloprid low doses induce intestine epithelial Caco-2 cell reactivity that is accompanied by meaningful TNF-α and iNOS upregulations.

Thus, low imidacloprid doses promote TNF-α and iNOS upregulation in human intestine epithelial cells that could be considered as proinflammatory biomarkers. The imidacloprid exposure downregulates occludin and E-cadherin that are tight and adherens junctions proteins. Thus, the imidacloprid can disturb the intestine barrier function through the growth of the proinflammatory cytokine production and reduction of the enterocyte adhesive properties.

BIOMARKERS IN BIOMONITORING OF AQUATIC ENVIRONMENTAL POLLUTION WITH A USE OF SNAKE BRAIN GFAP

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The term "biological marker" was firstly introduced by K. A. Porter in 1950s. At present, many valid and informative biomarkers are used for early diagnostics in medicine. In 2000, De Lafontaine defined the term biomarker as a “biochemical and/or physiological change(s) in organisms exposed to any pollutant. It represents initial responses to environmental perturbation and contamination“. To use biomarker concept De Kock and Kramer in 1994 developed the concept of active biomonitoring.

Biomonitoring is defined as the system of observations and assessments of the state and ongoing changes in ecosystems, components of biodiversity and landscape, including the types of natural habitats, populations and species. Biomarkers have successfully been used in environmental monitoring and assessment around the world to detect exposure to and effects of chemicals. Many different biomarkers have been used or are under development, and they all have different strengths and weaknesses.

The biomarkers were given scores for five properties that were considered important for environmental monitoring. Those were:

- specificity for chemicals,
- ecological relevance,
- ability to provide early warning,
- feasibility of the analysis, etc.

About 30 years ago the number of used in environmental monitoring biomarkers was limited by about two tens of indices, e.g. Acetylcholinesterase in a brain, metallothioneins, superoxide dismutase, T- and B- immune system functional activity, DNA adducts, etc. All of them are currently used as well. However, new developments propose molecular parameters as valuable biomarkers of environmental stress.

For the earliest identification of harmful impact, the biochemical and molecular markers are mostly effective as long as imbalance in molecular pathways reflects the abnormalities in a biosystem at the earliest stages.

The present study was designed to evaluate the responsiveness of modulation of glial fibrillary acidic protein (GFAP) content and its

fragmentation in the snake brain as a biomarker of local industrial pollution of aquatic ecosystems. Despite GFAP being a well known cytoskeleton marker of astrocytes' reactivity in the brain of vertebrates, its expression in the snake brain remains insufficiently described.

Snakes are usually at the third or fourth level of trophic pyramid and, therefore, accumulate different pollutants. Dice snake (*Natrix tessellata*) is widespread and numerous in fresh, brackish and salt waters of the Black Sea basin in Turkey and Ukraine. Thus, this species could be used for environmental assessment in case of biomarkers suitability.

Mature individuals of the dice snake were caught in three locations in the Dnieper River (Ukraine) in 2014–2016: in polluted habitats adjoining Prydniprovska Thermal Power Station and Zaporizhia industrial metallurgical and merchant-coke enterprises, and in the relatively clean Majorova Balka site.

The snakes were studied according to the rules adopted by the Bioethic Committee of Oles Honchar Dnipro National University. The snake brain was isolated, washed with PBS, cleaned on the ice, homogenized in the 10-fold volume of 50 mm Tris buffer with 2 mM EDTA, 1 mM EGTA and standard additives. Obtained homogenate was centrifuged in the refrigerating centrifuge for 45 min at 60,000 g. The supernatant (S1) of first after centrifugation contains soluble fraction of cytosolic proteins. To extract the cytoskeleton proteins of brain nervous cells, the pellet of first centrifugation was resuspended in 4-fold volume of the Tris buffer with 4 M urea. The suspended matter was incubated for 30 min at 4°C and centrifuged for 45 min at 60,000 g (Baydas et al., 2003). The supernatant (S2) contains insoluble cytoskeleton fraction of brain cell proteins (Nedvetsky, Nerush, 1999).

The proteins of both fractions were separated with polyacrylamide gel electrophoresis in gradient of acrylamide (5–20 %) with 0.1% SDS-PAAG. The estimation of GFAP content and polypeptide fragments composition of glial intermediate filaments were carried out with immunoblotting analysis as it was described earlier (Nedzvetsky et al., 2011). The results of immunoblotting were scanned and evaluated densitometrically.

We found the significant ROS increase in snake brain from both polluted sites of Prydniprovska TPS and Zaporizhia city by 1.6 and 1.7 times respectively.

To estimate unfavorable effect of industrial pollution we carried out comparative analysis of the GFAP expression and its fragmentation in animal brain. Significant higher GFAP content was shown in brain tissue of the snakes from polluted sites. The elevated content of filamentous cytoskeleton (insoluble) fraction (c-GFAP) found in the snake brain from the contaminated sites in comparison with the control animals. The c-GFAP level in the snake brain from polluted biotopes of the Power Station and Zaporizhia city were found as 1.7 and 2.2 times augmentation respectively.

Our data have demonstrated significant alterations in cytosolic soluble GFAP (s-GFAP) in snakes subjected to ecotoxicants. The s-GFAP in dice snakes from the vicinity of the Power Station escalates by 3.8 times in contrast with a control population. In much the same way, the snakes from the polluted sites in Zaporizhia are characterized by 5.6 times raised s-GFAP content. Substantial growth of the s-GFAP form attests the increased production of intermediate filament proteins and release of cleaved subunits from the cell cytoskeleton structures. The abnormality in the ratio of the GFAP fractions (s-GFAP/c-GFAP) was also found in the brain of snakes from both polluted sites.

Thus, industrial pollution of aquatic ecosystems launches astrogliosis in the brain of dice snakes. The astroglial reactive response is accompanied by an active reorganization of the glial cytoskeleton. GFAP upregulation in the course of astrogliosis is accompanied by increase in both soluble cytosolic and insoluble filamentous GFAP fractions. Therefore, their indices in the dice snake brain could be recognized as valid biomarkers of industrial pollution of water bodies.