ORIGINAL ARTICLE

CONTENTS

Features of metabolism and peculiarities of organs' structural reorganization by sodium nitrite with underlying tobacco intoxication

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ABSTRACT

Aim: To determine the characteristics of metabolism in rats of different ages exposed by sodium nitrite with underlying tobacco intoxication.

Materials and Methods: The experiments were conducted on male white rats kept on a standard vivarium diet. The rats were divided into three age groups: sexually immature with a body weight of 60–80 g (3 months old), sexually mature with a body weight of 180–200 g (12 months old), and elderly with a body weight of 300–350 g (18 months old).

The animals received a single intragastric dose of sodium nitrite via a probe in the form of an aqueous solution at a dose of 45 mg/kg body weight, which is 1/4 of the LD50. The study was conducted 24 and 72 hours after exposure to this toxicant.

Results: It has been established, that during the whole experiment, after the affection of the rats, the hyperproduction of active forms of oxygen is present. Simultaneous exposure of rats to sodium nitrite and tobacco intoxication leads to increased generation of active oxygen species by neutrophil granulocytes in the blood. By the end of the experiment, sexually immature rats showed a maximum increase in the content of TBK-active products in all organs (in the liver – 3.3 times, in the kidneys – 3.6 times, in the lungs – 4.3 times, myocardium – 6.0 times). At the end of the experiment, the content of methemoglobin in the blood of young animals after exposure increased significantly (2.6 times). The simultaneous effect of sodium nitrite and tobacco smoke causes disturbances in the antioxidant system. Suppression of superoxide dismutase activity in the blood serum and liver of rats of all age groups was detected. Suppression of superoxide dismutase activity was detected in the blood serum and liver of rats of all age groups. By the end of the experiment, ceruloplasmin content had increased in the group of sexually immature rats, exceeding the level in control animals by 2.4 times at the end of the experiment. Glutathione levels decreased in the liver, lungs, kidneys, and myocardium of rats of all age groups, most significantly in sexually immature rats. At the same time, nitrooxidative stress developed, as evidenced by an increase in nitrite ion levels in all organs during the experiment. After exposure to sodium nitrite, rats of different ages that had been poisoned with tobacco smoke for 45 days showed a significant increase in enzyme activity in their blood serum (alanine aminotransferase activity in blood serum increased 7.7 times, aspartate aminotransferase 5.0 times, lactate dehydrogenase by 1.8 times, gamma-glutamyl transpeptidase by 4.1 times, and alkaline phosphatase by 3.6 times. Secondary endogenous toxins accumulated in the affected organism – medium-weight molecules, the content of which in blood serum was highest at the end of the experiment in sexually immature rats. The myocardium of elderly rats was most sensitive to the action of toxicants. In the lungs of sexually immature and aged rats, cytochrome oxidase activity decreased 1.4 times at the end of the experiment.

Microscopic examination of organs revealed that structural reorganization of lungs, liver, kidneys, myocardium of the animals of all age groups was characterized by changes in vascular bed, main morpho functional components (acini, hepatocytes, nephrons and muscle fibers of the myocardium).

Conclusions: Exposure of rats of all age groups to sodium nitrite and tobacco smoke causes intensification of free radical oxidation processes in the rats' bodies, manifested by the occurrence of oxidative and nitrooxidative stress, activation of lipoperoxidation and oxidative modification of proteins, suppression of the antioxidant system, energy supply processes, formation of endogenous intoxication, and increased intensity of inflammatory processes in the body. The most pronounced metabolic changes with underlying nitrite-tobacco intoxication were evidenced in the immature rats that were proved by the experimental data. The identified violations lead to severe intoxication of the body, which requires the additional introduction of corrective factors.

KEY WORDS: metabolic disorders, endogenous intoxication, heart, lungs, liver, blood vessels, sodium nitrite, tobacco smoke

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INTRODUCTION

In everyday life, people are exposed to a number of toxic factors, which leads to general poisoning of the body [1, 2, 3]. Harmful habits such as smoking, alcohol, and medication abuse play a significant role in the development of pathology. Active and passive smoking can cause the formation of active forms of oxygen, which activate free radical oxidation processes in the body [4]. At the same time, the components of cigarette smoke cause mitochondrial oxidative stress in various types of

cells. Reactive oxygen species cause a complex pro-inflammatory response in the body, resulting in increased production of pro-inflammatory cytokines [3, 5].

At the same time, a significant environmental and medical-biological problem in the agricultural and industrial regions of Ukraine is the combined effect of inorganic nitro compounds on the human and animal organism, accompanied by nitrate-nitrite intoxication [6, 7]. The intake of nitrates and nitrites into the body leads to the formation of excessive amounts of nitrogen oxide, which can initiate chain free radical reactions [8]. This creates the conditions for the formation of other active forms of nitrogen, which can cause hypoxic and free radical necrobiosis.

The increase in the number of diseases and pathological conditions, in which disturbances in oxidative processes, immune and inflammatory responses play a significant role, leads to a deepening of the manifestations of endogenous intoxication.

Thus, given the prevalence of smoking and the active use of nitrites in the national economy, it is important to study the mechanisms of the simultaneous effect of toxicants on the body, taking into account the age aspect.

AIM

To determine the characteristics of metabolism in rats of different ages exposed by sodium nitrite with underlying tobacco intoxication.

MATERIALS AND METHODS

The experiments were conducted on male white rats kept on a standard vivarium diet. The rats were divided into three age groups: sexually immature with a body weight of 60–80 g (3 months old), sexually mature with a body weight of 180–200 g (12 months old), and elderly with a body weight of 300–350 g (18 months old).

The animals received a single intragastric dose of sodium nitrite via a probe in the form of an aqueous solution at a dose of 45 mg/kg body weight, which is 1/4 of the LD50. The study was conducted 24 and 72 hours after exposure to this toxicant. A model of chronic tobacco smoke exposure was created using a sealed chamber with a volume of 30 liters, exposing the animals to the toxicant daily through appropriate openings. Six animals were placed in the chamber simultaneously for 6 minutes. After 15, 30, and 45 days from the start of exposure to tobacco smoke, the animals were removed from the experiment. With simultaneous exposure to both toxicants on the 30th day of tobacco intoxication (24 and 72 hours before the specified date), rats were administered sodium nitrite intragastrically. Similarly, sodium nitrite exposure was modeled on the 45th day of tobacco smoke poisoning in rats.

The study involved 792 rats. For biochemical studies, 720 rats were used, divided into groups depending on the duration of the study, with 36 animals in each group. For morphological studies, 72 rats were used.

The study material consisted of homogenates of the liver, heart, lungs, kidneys, whole blood, and blood serum.

Two series of studies were conducted. In the first series of experiments, the following indicators were determined: the content of active oxygen species (AOS), TBK-active products, oxidative modification of proteins (OMP), carboxyhemoglobin (HbCO), superoxide dismutase activity (SOD), catalase activity (CAT), and the erythrocyte intoxication index (EII). In the second series of experiments, the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), γ-gamma-glutamyltranspeptidases (GGTP), alkaline phosphatase (ALP), succinate dehydrogenase activity (SDH), cytochrome oxidase activity (CO), C-reactive protein (CRP) content, interleukin-4 (IL-4) content, and interleukin-6 (IL-6) content.

RESULTS

Tobacco smoke intoxication caused the generation of ROS (O_2, OH-, H_2O_2), which led to oxidative stress in the body, accompanied by the activation of lipid peroxidation (LPO) and oxidative modification of proteins. The intensification of free radical oxidation processes under the action of AFO led to the destruction of nucleic acids and carbohydrates, causing structural and metabolic disorders in cells.

The content of TBK-active products in the blood serum of rats affected by both toxicants simultaneously significantly exceeded the content recorded after the separate action of each factor.

The highest content of TBK-active products was found in the liver of young and mature rats after poisoning, which gradually increased and reached its maximum value at the end of the study. Similar activation of LPO processes after sodium nitrite exposure against the background of tobacco intoxication was observed in the kidneys of rats of different ages. By the end of the experiment, the content of TBK-active products had increased significantly in the kidneys of sexually immature rats by 3.6 times, in old rats by 3.5 times, and in sexually mature rats by 2.4 times ($p \leq 0.05$).

The lungs and myocardium are the targets of the toxic effects of tobacco smoke: the lungs of sexually immature rats, in which the content of TBK-active products was highest ($p \leq 0.05$) on both the 15th day

of intoxication and the 45th day of TS damage (24 and 72 hours after exposure to sodium nitrite against this background).

A study of the content of TBK-active products in the myocardium of rats after exposure to toxicants showed an increase in all age groups during the experiment ($p \leq 0.05$). The most pronounced changes in the content of TBK-active products were recorded in the myocardium of sexually immature animals. The content of TBK-active products in the myocardium of young rats increased after exposure depending on the duration of the study and at the end of the experiment was 12.3 times higher than that of control animals. This may be due to the increased sensitivity to stress of this particular group of animals. One of the reasons for this condition is the increased release of catecholamines into the blood in response to a stress factor (smoke), in particular adrenaline, which, when increased, has a toxic effect on the myocardium, further activating the processes of lipoperoxidation and increasing the content of its intermediate products in the heart.

During the study of OMP in rats, an increase in the content of 2,4-dinitrophenylhydrazones (2,4-DPH) was observed as both basic (λ=430 nm) and neutral (λ=370 nm) in the blood serum and organs of animals of all age groups after exposure to sodium nitrite against the background of tobacco smoke intoxication.

When studying the content of neutral OMP products in the lungs of rats, it was found that the most sensitive to the action of toxicants were sexually immature animals, in which this indicator increased by 2.4 times ($p \leq 0.05$) by the end of the experiment compared to the group of control animals.

The kidneys of sexually immature rats were the most sensitive to the effects of toxicants, in which 72 hours after the administration of sodium nitrite against the background of 45-day TS intoxication, the content of neutral OMP products increased 2.9 times ($p \leq 0.05$) compared to the control animals.

A similar increase in basic 2,4-DHFG was observed in the blood serum and organs of rats after exposure to toxicants.

Another indicator that characterizes pathological changes in the body of animals after exposure to tobacco smoke and indicates the deepening of hypoxia is carboxyhemoglobin. By the end of the experiment, the HbCO content in the blood of sexually immature rats (under the action of both toxicants) increased the most – 3.0 times higher than the control level, in sexually mature rats – 1.5 times higher, and in elderly rats – 2.3 times higher than in the control group.

Under conditions of sodium nitrite poisoning against a background of tobacco intoxication, superoxide dismutase activity was studied in the blood serum and liver of rats of all age groups. The latter in the blood serum of sexually immature rats decreased 2.5 times by the end of the experiment when poisoned with two toxicants, in sexually mature rats – 1.6 times, and in elderly rats – 1.9 times ($p \leq 0.05$) compared to the control group. The study of SOD activity in the liver confirmed its decrease at all stages of the study in all age groups. The enzyme activity decreased most significantly during the experiment in the liver of sexually immature animals.

Catalase activity in the blood serum and organs of rats of all age groups after exposure to toxicants was studied. It was found that CAT in the blood serum of rats of all age groups decreased throughout the experiment, which could lead to hydrogen peroxide intoxication of the body. The lowest CAT was recorded in the blood serum of sexually immature rats, which at the end of the experiment was 1.9 times lower than the control level, in sexually mature rats – 1.6 times lower, and in elderly animals – 1.4 times lower ($p \leq 0.05$).

A decrease in this indicator was observed in the liver of rats affected by toxicants, and it was lowest at the end of the study. The greatest decrease in CAT was observed in the liver of elderly rats.

CAT in the lungs of rats of different ages decreased depending on the duration of the experiment, and by the end of the study, it was 2.4 times lower than the control in young animals, 2.7 times lower in mature animals, and 2 times lower in elderly rats.

A pronounced decrease in CAT was recorded in the kidneys of rats of all age groups after sodium nitrite exposure against the background of tobacco intoxication. After 15 days of mildronate administration, catalase activity significantly increased in sexually mature and elderly animals.

The myocardium of elderly rats was most sensitive to the effects of both toxicants, with CAT decreasing 2.2 times compared to the control animals at the end of the study.

To assess the functional state of erythrocyte cytoplasmic membranes, it is important to determine their permeability percentage. Fifteen days after tobacco smoke poisoning and 72 hours after sodium nitrite poisoning, EII increased by 38.7% in the blood of sexually immature rats, after 30 days of tobacco intoxication and 72 hours of sodium nitrite poisoning, it increased by 31.6%, and at the end of the experiment, it exceeded the level of control animals by 55.6%. In sexually mature rats, this indicator exceeded the control values by 38.0% at the end of the experiment, and in elderly animals – by 54.1%.

When studying the functional state of the liver in rats affected by sodium nitrite against the background of

tobacco intoxication, cytolytic syndrome was diagnosed based on ALT and AST activity indicators, and cholestatic syndrome was diagnosed based on γ-glutamyltranspeptidase and alkaline phosphatase activity.

Sodium nitrite poisoning in rats against the background of tobacco intoxication led to an increase in serum ALT (a marker enzyme of the liver) activity in all age groups. By the end of the experiment, serum ALT activity in sexually immature rats increased 7.7 times compared to the control level, and in the other two age groups — 5.3–5.4 times ($p \leq 0.05$).

When determining ALT activity in the liver, the changes were reversible. Enzyme activity decreased in all groups of rats at all study periods, which was progressive in nature.

By the end of the experiment, ALT activity in the liver of sexually immature rats decreased by 73%, i.e., it was only 27% of the level in control animals.

A decrease in ALT activity in the lungs of affected rats was observed at all study periods. A more pronounced decrease was observed in the lungs of elderly rats, in which enzyme activity was 3.5 times lower than the control level at the end of the study. In sexually immature and elderly rats, this indicator decreased in the lungs by 2.4 and 2.3 times, respectively.

The myocardium of elderly rats was most sensitive to the effects of toxicants. After 72 hours of sodium nitrite poisoning against a background of 45-day intoxication, ALT activity in this group of animals decreased 4.5 times. During the same period, ALT activity was 4.3 times lower than the control in the myocardium of sexually immature rats and 3.7 times lower in sexually mature rats.

A study of enzyme activity in the kidneys of rats of different ages showed that the most sensitive to the simultaneous action of sodium nitrite and tobacco intoxication were elderly rats, in which the studied indicator decreased by 5.7 times, in sexually immature rats it decreased 3.0 times, and in sexually mature rats it decreased 2.3 times.

The highest AST activity was recorded in the blood serum of elderly rats after exposure to sodium nitrite against the background of tobacco intoxication. The experimental indicator increased during the experiment and reached its highest level at the end of the study (8.2 times higher than in control animals). In sexually immature rats during this period, it increased 5.0 times, and in sexually mature rats, 6.9 times compared to the control.

The most pronounced changes in AST activity after exposure to toxicants were observed in the liver of sexually immature rats, in which this indicator decreased by 4.8 times compared to the level of control animals at the end of the study. During the same period, enzyme activity decreased by 2.7 and 3.7 times, respectively, in the livers of sexually mature and elderly rats.

Considering that increased AST activity in the blood serum of rats of all age groups is a test for the degree of myocardial damage, this indicator was studied specifically in the myocardium. After poisoning rats with sodium nitrite against the background of 45-day tobacco intoxication, AST activity in the myocardium of rats of different ages decreased. In the myocardium of sexually immature and elderly rats, the indicator decreased depending on the duration of the study. By the end of the experiment, enzyme activity had decreased by 3.8 and 3.9 times, respectively, compared to the level in the control group. A study of AST activity in the kidneys of rats of different ages after exposure to sodium nitrite showed a similar decrease ($p \leq 0.05$) during the experiment.

To confirm the development of cytolytic syndrome in the affected organism, the activity of another organ-specific enzyme, LDH, was studied. Its activity in the blood serum of rats of all age groups increased and reached its highest value at the end of the experiment in sexually immature animals. After 45 days of tobacco smoke poisoning and 72 hours from the moment of sodium nitrite exposure, the enzyme activity in this group of animals was 1.8 times higher than in the control group, and 1.7 times higher in sexually mature and elderly rats.

The study of LDH activity in the myocardium showed its decrease in rats of all experimental groups. The lowest enzyme activity after exposure of rats to both toxicants was recorded in the myocardium of sexually immature and elderly rats at the end of the experiment – 1.5 times lower. The lowest LDH activity was recorded in the liver of sexually immature and elderly rats as a result of exposure to toxicants. In the lungs of affected sexually immature rats, LDH activity was lowest (1.6 times lower than the control level) at the end of the study.

Exposure of rats of all age groups to sodium nitrite against the background of 45-day tobacco smoke intoxication led to a decrease in LDH activity in their kidneys. The most pronounced changes were observed in the kidneys of young and elderly rats, in which the indicator was 1.7 times lower than the control level.

GGTP activity in the blood serum of rats increased after exposure to both toxicants simultaneously. In the blood serum of sexually immature rats, GGTP activity increased after exposure in the last term the most – 4.1 times compared to control animals. During this period, the indicator increased 1.6 times in the blood serum of sexually mature rats and 2.3 times in elderly animals.

A study of GGT activity in the liver showed a decrease

after injury in all experimental groups of animals. The greatest decrease in the activity of this enzyme was observed in the liver of sexually immature rats. In these animals, the experimental indicator decreased continuously depending on the duration of the experiment and at the end of the study reached 48% of the control.

Damage to the membrane structures of hepatocytes is evidenced by the results of studies of the organ-specific enzyme (a marker of cholestasis) – alkaline phosphatase in blood serum. The LP study showed a statistically significant increase in serum during the experiment in all experimental groups of animals. The enzyme activity in serum ($p \leq 0.05$) increased linearly and reached its highest value at the end of the study. ALP activity in the blood serum of sexually immature rats at the end of the study increased 3.6 times, in sexually mature rats – 1.6 times, and in elderly rats – 3.4 times after their damage to the NN against the background of tobacco intoxication.

In the kidneys of sexually immature rats, this indicator decreased by 2.7 times, in mature rats by 1.5 times, and in elderly rats by 2.5 times.

Similar results were obtained when studying the activity of LF in the liver of intoxicated rats. The activity of LF decreased the most after poisoning with NN against the background of tobacco intoxication in the liver of sexually immature animals (by 76% relative to the control) at the end of the experiment.

Under conditions of simulated pathology in the tissues of the liver, lungs, and myocardium, the indicators of the mitochondrial electron transport system decreased at the beginning of the study, with maximum energy deficiency in cells at the end of the experiment.

In the liver of rats of all age groups, SDG activity decreased sharply depending on the duration of the experiment. The greatest decrease in enzyme activity was achieved in the liver of sexually immature rats at the end of the study (45 days of TD and 72 hours from the moment of NN poisoning) – 2.2 times ($p \leq 0.05$) below the level of control rats.

After exposure to toxicants, a decrease in SDG activity was observed in the lungs of rats of all age groups throughout the experiment. The decrease was significantly more pronounced than when poisoned with each of the toxicants separately. By the end of the study, SDG activity in the lungs of sexually immature rats decreased by 2.1 times, in sexually mature rats by 1.9 times, and in elderly rats by 1.7 times ($p \leq 0.05$) compared to the control group.

The study of SDG activity in the organs of rats poisoned with sodium nitrite against the background of tobacco intoxication and the detected decrease in its activity created the prerequisites for studying CO activity in animals of different ages under such pathological conditions. The greatest decrease in CO activity was observed in the liver of sexually immature rats – by the end of the experiment, it had decreased 2.7 times compared to the control.

A similar decrease in CO activity after damage was observed in the myocardium of rats of different ages. The myocardium of elderly rats was the most sensitive to the action of toxicants: enzyme activity decreased throughout the study period. By the end of the experiment, it was 2.2 times lower than that of the control group.

Damage by both toxicants led to a profound disruption of CO activity in the lungs of rats. In sexually immature and elderly rats, this indicator decreased by 60.0% at the end of the experiment, and in sexually mature rats, by 50.0%.

A decrease in CO activity in the mitochondria of various organs when exposed to toxicants can be explained by a restriction in the flow of electrons from the substrate link of the respiratory chain through cytochromes b-c. Inhibition of CO activity may also occur due to the binding of free oxygen radicals to metal atoms present in the enzyme under study.

The study of C-reactive protein content is one of the most acceptable markers for early diagnosis and monitoring of inflammatory diseases. The highest content of this indicator was recorded in the blood serum of elderly rats poisoned with both toxicants, in which it increased 3.3 times by the end of the experiment. Similar changes in CRP content were observed in the blood serum of sexually immature and sexually mature rats after injury and the application of corrective factors. Sexually mature rats were more resistant to such changes, with the smallest increase in this indicator after exposure. It is known that CRP synthesis and secretion occurs in the liver and is regulated by pro-inflammatory cytokines, primarily IL-6, but it can also be produced by macrophages and lymphocytes.

Exposure of rats to sodium nitrite against the background of 45-day tobacco smoke intoxication led to an increase in the content of pro-inflammatory cytokine IL-6 in the blood serum of rats in all experimental groups. The highest IL-6 levels were observed in the serum of sexually immature rats, which had already increased significantly at the beginning of the study. By the end of the experiment, after exposure to both toxicants, the content of pro-inflammatory cytokine in the blood serum of sexually mature rats increased 3.5 times, and in elderly animals – 2.6 times compared to the control group.

The basis for the development of the inflammatory process is the launch of a cytokine cascade, which

includes pro-inflammatory cytokines on the one hand and anti-inflammatory mediators on the other. The balance between the two oppositely acting groups of cytokines largely determines the course and outcome of the disease. It is known that IL-4 has a powerful anti-inflammatory effect and plays a key role in the onset of the inflammatory response.

The experimental indicator progressively decreased in all age groups of animals depending on the duration of intoxication. By the end of the study, the content of anti-inflammatory cytokine decreased most significantly in the blood serum of sexually immature rats – by 2.2 times, while in mature and elderly rats – by 1.7 and 1.8 times ($p \leq 0.05$), respectively (compared to the control).

Oxidative stress and the accumulation of toxic products of exogenous and endogenous origin in the bodies of affected rats led to the development of inflammatory processes, which intensified depending on the duration of the experiment and the age of the animals. This was confirmed by an imbalance of pro- and anti-inflammatory cytokines and an increase in acute phase protein in the blood serum.

To confirm the results obtained, morphological studies of the organs of rats of different age groups after exposure to sodium nitrite were conducted.

Microscopic studies of the internal organs of white rats affected by sodium nitrite against the background of tobacco intoxication showed that the structural organization of the lungs and heart of animals of all age groups is characterized by changes in the vascular bed and the main morphofunctional components.

The most pronounced changes occur in the lungs of sexually immature and elderly animals. In the respiratory section of the lungs, the alveolar area is significantly increased compared to the control group of animals. There is a thickening of the interalveolar septa, blood filling of the hemocapillaries, and lymphoid infiltration under conditions of toxicant exposure.

The results of the studies allow us to conclude that when rats of different ages are exposed to sodium nitrite and tobacco smoke, oxidative and nitrooxidative stress develops in the body, which is exacerbated by the simultaneous use of toxicants. Sexually immature rats were the most sensitive to the effects of the xenobiotics we used.

DISCUSSION

Poisoning rats with sodium nitrite against a background of chronic tobacco intoxication is accompanied by hyperproduction of aggressive ROS in the affected organism, leading to the development of oxidative stress. This is indicated by the intensification of lipoperoxidation and oxidative modification of proteins after damage and a decrease in the activity of the antioxidant system components [9]. Sodium nitrite causes increased methemoglobin formation through a free radical mechanism. The primary reaction of tobacco smoke on the body is the formation of carboxyhemoglobin. Both hemoglobin derivatives are unable to transport oxygen to organs and tissues. It is known that the entry of sodium nitrite into the body is accompanied by the development of hemic hypoxia, as indicated by the increased content of methemoglobin in the blood of poisoned rats. After exposure to both toxicants, sexually immature rats were found to be the most sensitive, with this indicator progressing and reaching its highest level by the end of the experiment – 3.3 times higher than in control animals.

Thus, nitrite-tobacco toxicosis leads to tissue hypoxia, which is confirmed by a decrease in the activity of mitochondrial enzymes, resulting in the suppression of energy production processes [5, 10]. The above factors cause the destruction of plasma and cytoplasmic membranes and changes in their permeability. At the same time, the activity of organ-specific enzymes in the blood serum increases and decreases in the organs of animals. The identified disorders are accompanied by a syndrome of endogenous intoxication caused by the accumulation of degradation products of lipid and protein molecules, in particular MSM, in the body. A significant amount of endogenous toxins, as well as exogenous toxins that enter the body with tobacco smoke, leads to the activation of inflammatory processes. Under conditions of nitrite-tobacco toxicosis, an imbalance occurs in the content of pro- and anti-inflammatory cytokines. Prolonged hypoxia caused by sodium nitrite and tobacco smoke leads to increased formation of nitric oxide under the action of inducible NO synthase, which is activated in pathological conditions. Nitrooxidative stress occurs in the affected organism, confirmed by the suppression of endothelial NO synthase activity and the accumulation of nitrite ions in the organs of animals.

The proposed scheme of development of nitrite-tobacco toxicosis suggests that the primary response to the entry of these toxicants into the body is the activation of free radical oxidation processes, resulting in the development of oxidative and nitrooxidative stress, deepening endogenous intoxication, and activation of inflammatory processes, disruption of energy supply processes, and changes in the body's defense systems [11].

It has been found that after rats are exposed to toxicants, there is hyperproduction of active forms of oxygen, which leads to the activation of free radical oxidation processes (lipoperoxidation and oxidative modification of proteins) in the animals' bodies [12]. Under these conditions, hypoxia occurs due to the significant formation of methemoglobin and carboxyhemoglobin. The toxic products formed provoke the destruction of biomembranes and the release

of intracellular components into the blood. Secondary endogenous toxins (medium-weight molecules) accumulate in the body, and their content increases in the blood serum of rats of all age groups. Increased endogenous intoxication leads to changes in the antioxidant system (inhibition of enzymatic and non-enzymatic links).

The inhibition of mitochondrial oxidation system enzymes is more pronounced when both toxicants are present simultaneously. The development of inflammatory processes has been observed, as evidenced by an imbalance of pro- and anti-inflammatory cytokines and an increase in serum C-reactive protein levels. There are disturbances in the functioning of the NO system and nitrooxidative stress occurs, manifested by an increase in the activity of inducible NO synthase and a decrease in the activity of its endothelial isoform.

Given that LF is an organ-specific liver enzyme, an increase in its activity is a typical sign of cholestasis, and the results obtained should be considered as confirmation of hepatocyte damage with manifestations of inflammatory processes, cytolysis, and bile stasis in the bile capillaries and ducts. All this together contributes to the overall endogenous toxicosis, which manifests itself in an increase in MSM – markers of toxic syndrome.

Poisoning rats with sodium nitrite against the background of tobacco intoxication leads to oxidative stress in the body with the formation of a significant amount of AFO, accompanied by the development of destructive processes and cytolytic syndrome, as well as a change in the permeability of cell membranes with subsequent disruption of the functions of intracellular organelles. Irreversible disturbances in the structure and functioning of mitochondria caused by the action of excessive amounts of ROS lead to a shift in energy metabolism towards increased glycolysis and inhibition of oxidative phosphorylation [13, 14].

Sodium nitrite poisoning causes nitrooxidative stress in rats due to the formation of significant amounts of nitric oxide. Tobacco smoke contains nitrogen dioxide and nitrogen oxide, which, when ingested, are toxic due to the formation of peroxynitrite, nitrite, and nitrate ions that initiate LPO reactions. Thus, nitrooxidative stress developed in the body along with oxidative disorders.

Research results have shown that simultaneous exposure to sodium nitrite and tobacco smoke leads to significant activation of nitrite ion formation processes in the bodies of rats of different ages. In the blood serum of sexually immature rats after exposure to both toxicants, the nitrite ion content increased 2.3 times at the end of the study, which can cause significant formation of endogenous nitric oxide and the development of nitrooxidative stress. At the same time, the indicator exceeded the control level by two times in the blood serum of sexually mature and elderly animals. The maximum formation of nitrite ions occurs at the end of the study.

After exposure to toxicants, the nitrite ion content increased in the liver, myocardium, and kidneys of rats of all age groups. Sodium nitrite poisoning of animals against the background of 45-day tobacco smoke intoxication caused an increase in the nitrite ion content in the lungs of rats in all experimental groups. In the lungs of sexually immature animals, this indicator increased 3.7 times by the end of the experiment, in sexually mature animals – 3.2 times, and in elderly rats – 2.7 times compared to the control group, which confirms the tropism of tobacco smoke to this organ.

Excessive accumulation of nitrite ions in the organs of rats after injury can cause increased formation of nitric oxide.

It has been proven that after rats were injured with sodium nitrite against a background of tobacco intoxication, iNOS activity progressively increased in the blood serum in all age groups. iNOS was most active in the blood serum of sexually immature rats, increasing 3.3 times ($p \leq 0.05$) by the end of the experiment after exposure to toxicants.

A study of iNOS activity in the liver of rats of different ages after exposure to toxicants showed that it was most active in sexually immature animals. At the beginning of the experiment, the enzyme activity in the liver of this group of rats increased 3.0 times ($p \leq 0.05$), and by the end of the experiment, it exceeded the level of control animals by 4.3 times ($p \leq 0.05$).

The activity of eNOS in the blood serum and liver of rats of different age groups after exposure to toxicants was studied. The enzyme activity in the blood serum of sexually immature rats decreased almost equally at all stages of the study and at the end of the experiment was 56.0% lower than the control. In aged rats, this indicator decreased by 56.0%, and in sexually mature rats, by 50.0% ($p \leq 0.05$).

Exposure to both toxicants simultaneously led to a decrease in eNOS activity in the liver of animals of all age groups. The lowest eNOS activity was recorded in the liver of sexually immature rats at the end of the experiment; it decreased 5.1 times after exposure compared to the intact control group of the same age. In sexually mature rats, this indicator decreased by 3.0 times, and in elderly animals, by 3.7 times compared to the control group at the end of the study.

Sodium nitrite poisoning against the background of tobacco intoxication caused oxidative and nitrooxidative stress in the animals, which determined the severity of the pathological process.

The results obtained allow us to conclude that NO hyperproduction, which is associated with an increase in nitrite ion content after injury and iNOS activity, along with the activation of oxygen free radical reactions, is

one of the key links in toxic damage to the body, particularly in cases of sodium nitrite poisoning against the background of tobacco intoxication.

CONCLUSIONS

Exposure of rats of all age groups to sodium nitrite and tobacco smoke causes intensification of free radical oxidation processes in the rats' bodies, manifested by the occurrence of oxidative and nitrooxidative stress, activation of lipoperoxidation and oxidative modification of proteins, suppression of the antioxidant system, energy supply processes, formation of endogenous intoxication, and increased intensity of inflammatory processes in the body. The most pronounced metabolic changes with underlying nitrite-tobacco intoxication were evidenced in the immature rats that were proved by the experimental data. The identified violations lead to severe intoxication of the body, which requires the additional introduction of corrective factors.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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