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Experimental study of the toxic effects of tobacco smoke and nitrites on the body of immature and mature rats

Topicality. In the context of the progression of man-made pollution of the environment a priority for Toxicology and Medicine is the study of the characteristics and mechanisms of the combined effect of xenobiotics – risk factors for many environmentally related diseases.

The aim of these studies was to investigate the cytotoxicity and markers of endogenous intoxication when exposed to the body of immature and mature rats sodium nitrite poisoning on against the background of 15 days intoxication with tobacco smoke.

Material and methods. Experiments were performed on immature and mature rats, which during the 15 days exposed to tobacco smoke. 24 hours before the end of the experiment one animal group was treated with sodium nitrite at a dose of 45 mg/kg body weight, sodium nitrite second was administered 72 hours before euthanasia. The rats were taken out of the experiment under thiopental anesthesia.

In the serum and organs of animals determined by the content of nitrite ion, in the blood – methemoglobin content and erythrocyte index of intoxication.

Results and discussion. Experimental results confirmed that the complication of exposure to tobacco smoke by introducing into the body of sodium nitrite in rats causes a marked change in the nitrite ion content of methemoglobin and permeability of erythrocytею

Conclusions. It was found that the most sensitive to the effects of tobacco smoke and sodium nitrite are immature rats.

***Key words**: tobacco smoke; sodium nitrite; rats; nitrite-ion; methemoglobin*

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Експериментальне вивчення токсичного впливу тютюнового диму та нітритів на організм статевонезрілих та зрілих щурів

Актуальність. В умовах прогресування техногенного забруднення довкілля одним із пріоритетних напрямків токсикології та медицини залишається вивчення особливостей та механізмів комбінованої дії ксенобіотиків – факторів ризику багатьох екологічно залежних хвороб.

Метою роботи було дослідити маркери цитотоксичності та ендогенної інтоксикації за умов впливу на організм статевонезрілих та зрілих щурів натрію нітриту на тлі 15-денного отруєння тютюновим димом.

Матеріали та методи. Експерименти проведені на статевонезрілих та зрілих щурах, які протягом 15 днів піддавались впливові тютюнового диму. За 24 год до закінчення експерименту одній групі тварин вводили натрію нітрит у дозі 45 мг/кг маси тіла, другій групі натрію нітрит вводили за 72 год до евтаназії. Щурів виводили з експерименту під тіопенталовим наркозом.

У сироватці крові та органах тварин визначали вміст нітрит-іону, у крові – вміст метгемоглобіну та еритроцитарний індекс інтоксикації.

Результати та їх обговорення. Експериментальні дані підтвердили, що ускладнення впливу тютюнового диму введенням в організм щурів натрію нітриту викликає більш виражені зміни вмісту нітрит-іону, метгемоглобіну та проникності еритроцитарних мембран.

Висновки. Встановлено, що найбільш чутливими до дії тютюнового диму та нітриту натрію є статевонезрілі щури.

Ключові слова*: *тютюновий дим; нітрит натрію; щури; нітрит-іон; метгемоглобін

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Экспериментальное изучение токсического влияния табачного дыма и нитратов на организм половонезрелых и зрелых крыс

Актуальность. В условиях прогрессирования техногенного загрязнения окружающей среды одним из приоритетных направлений токсикологии и медицины остается изучение особенностей и механизмов комбинированного действия ксенобиотиков – факторов риска многих экологически зависимых болезней.

Целью работы было исследовать маркеры цитотоксичности и эндогенной интоксикации при воздействии на организм неполовозрелых и зрелых крыс натрия нитрита на фоне 15-дневного отравления табачным дымом.

Материалы и методы. Эксперименты проведены на неполовозрелых и зрелых крысах, которые в течение 15 дней подвергались воздействию табачного дыма. За 24 часа до окончания эксперимента одной группе животных вводили натрия нитрит в дозе 45 мг/кг массы тела, второй группе натрия нитрит вводили за 72 ч до эвтаназии. Крыс выводили из эксперимента под тиопенталовым наркозом.

В сыворотке крови и органах животных определяли содержание нитрит-иона, в крови – содержание метгемоглобина и эритроцитарный индекс интоксикации.

Результаты и их обсуждение. Экспериментальные данные подтвердили, что осложнение влияния табачного дыма введением в организм крыс натрия нитрита вызывает более выраженные изменения содержания нитрит-иона, метгемоглобина и проницаемости эритроцитарных мембран.

Выводы. Установлено, что наиболее чувствительными к действию табачного дыма и нитрита натрия являются неполовозрелые крысы.

***Ключевые слова*:** *табачный дым; нитрит натрия; крысы; нитрит-ион; метгемоглобин*

INTRODUCTION

The problem of the influence of chemical pollution on the human body is a priority and is not fully understood. Among pollutants a significant threat represent heavy metals, nitrate fertilizers, industrial waste, among which significant place belongs to oxygen nitrogen compounds (NOз), nitrite (NO_2), nitric oxide (NO) and its various forms (N_2O, N_2O_3), nitrogen dioxide (N_2O_5). In connection with the extensive use of nitrate fertilizers in agriculture and their migration into groundwater and food products distribution of nitrate poisoning acquired an epidemic value [1, 2].

Nitradmin compounds are strong oxidizing agents, show the influence hematological parameters, converting ferrous iron heme in the ferric and forming a pathological form of hemoglobin, methemoglobin or hemeglobin, which is capable of reversibly up oxygen, causing further hypoxia, and is a major marker of the severity of intoxication by nitrogen compounds [3, 4, 5].

At the same time, people in the course of their life attach to unhealthy habits – smoking, alcohol, abuse of drugs. All these factors negatively affect the health and can cause fatal consequences [6, 7].

It is known that the basis of the pathogenic action of contaminated air by polutants or tobacco smoke lies the oxidative aggression on the mucosa of the respiratory tract reactive oxygen species, the dioxides of nitrogen and sulfur, other free radicals, which leads to activation of free radical oxidation and damage biological membranes [8]. As a result, the accumulation in tissues of toxic products occurs damaging the structure and disrupt function of cell membranes [9].

The aim of our work was to investigate the cytotoxicity and markers of endogenous intoxication in terms of the effects on the body of immature and mature rats of sodium nitrite on the background of 15 days toxicity of tobacco smoke.

MATERIALS AND METHODS

For research there were used white outbred rats-males, which were kept in standard vivarium ration in Ternopil State Medical University. Rats were divided into two age categories: the first is immature, weighing 60-80 g, the second adult with body weight of 180-200 g. Each age group consisted of two subgroups – intact control (C) and experimental group (E). Rats of experimental groups for 15 days were exposed to tobacco smoke. Experienced animals were divided into 3 groups. One of them for 24 hours before the end of the experiment were administered sodium nitrite in the dose of 45 mg/kg of body weight, the second sodium nitrite was administered 72 hours before euthanasia. In the third group, rats were subjected to the toxic influence of tobacco smoke. The model is based on chronic exposure to tobacco smoke created by using a sealed chamber volume of 30 liters, which allowed fumigating animals in free behavior. Tobacco smoke generated from burning of 6 cigarettes "Prima Sribna (blue)" (containing 0.6 mg of nicotine and 8 mg of tar), through the holes in the chamber moved inside it. In the chamber there were 6 animals for 6 minutes. Control animals were also kept for 6 minutes in a sealed chamber, but not exposed to tobacco smoke.

In 15 days from the beginning of the lesion of animals by tobacco smoke they were withdrawn from the experiment by euthanasia under general thiopental anesthesia.

For the study we took blood, blood serum, liver, lungs, kidneys and myocardium of animals. Research tissues were prepared 10 % homogenate in physiological solution.

The content of nitrite-ion (NO^-_2 was determined by the reaction of Gries [10], the content of methemoglobin (MetHb) was evaluated in reaction with acetonate [5], cytolysis of erythrocyte membranes was studied according to erythrocyte toxicity index (ETI) [9].

When conducting research, we used the General principles of animal experiments approved by the National Congress on Bioethics (Kyiv, Ukraine, 2001) and consistent with the provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, France, 1985) [11]. Statistical processing of obtained data was performed using the STATISTICA 6.0 program using parametric Student-t test and nonparametric Wilcoxon test for related samples. Changes were considered reliable at $p \leq 0.05$ [12].

RESULTS AND DISCUSSION

It is known that nitrates are characterized by a wide range of toxic effects, affecting the body at different bio levels. The universality of their toxic action is due to free radicals NO⍰.

Poisoning of rats with nitrite (NI) on the background of the 15-day tobacco intoxication (TI) led to accumulation of nitrite-ion in all organs of the affected animals (Tab. 1).

In the serum of immature rats significant changes ($p \leq 0.05$) content of nitrite-ion was noted at 24-th and 72-nd hour of NI on the background of the 15 day of defeat by tobacco smoke (its content was increased on 20 %). Rats that were influenced only by tobacco smoke, the content of this index was increased on 11 % and the change proved to be unreliable. In sexually mature rats, the con-

Table 1

THE CONTENT OF NITRITE ION IN THE BLOOD SERUM (NMOL/L) AND TISSUES (NMOL/KG) OF RATS, AFFECTED BY NITRITE OF SODIUM ON THE BACKGROUND OF POISONING BY TOBACCO SMOKE (M ± m; n = 48)

Tissues	Immature rats				Sexually mature rats			
	Groups of animals				Groups of animals			
	Intact rats	15 TI day	15 TI day + 24 hours NI	15 TI day + 72 hours NI	Intact rats	15 TI day	15 TI day + 24 hours NI	15 TI day + 72 hours NI
Serum	10.00 ± 0.46	11.10 ± 0.24	12.00 ± 0.24	11.90 ± 0.45	8.20 ± 0.74	9.4 ± 0.24	10.30 ± 0.33	11,20 ± 0.51
Liver	7.60 ± 0.22	10.2 ± 0.56	14.9 ± 0.57	17.00 ± 0.77	3.20 ± 0.16	5.30 ± 0.23	10.30 ± 0.30	13.30 ± 0.98
Lungs	1.30 ± 0.14	2.90 ± 0.24	3.70 ± 0.17	4.20 ± 0.21	1.00 ± 0.11	1.90 ± 0.12	2.30 ± 0.18	2.40 ± 0.11
Kidneys	8.50 ± 0.29	11.30 ± 0.30	14.50 ± 1.30	17.50 ± 0.74	10.00 ± 0.29	10.90 ± 0.10	11.60 ± 0.21	15.00 ± 0.80
Myocardium	2.20 ± 0.14	2.80 ± 0.21	3.40 ± 0.15	3.80 ± 0.20	1.60 ± 0.16	1.90 ± 0.19	2.20 ± 0.11	2.40 ± 0.17

tent of nitrite ion significantly increased only in the last period of the study (the 15-th day of intoxication by smoke and the 72-nd hour of lesion by sodium nitrite) and on 36 % it exceeded the level of intact control.

In the liver of rats of both age groups there was a significant increase of this index in all experimental groups at the end of the study in immature animals it increased in 2.2 times in sexually mature rats – in 4.2 times.

Similar changes were noted in the lungs of affected rats. In immature rats the content of nitrite-ion was increased in 3.2 times compared with normal, sexually mature rats – in 2.4 times by the end of the experiment.

In the kidneys of immature rats at 72-nd hour of nitrite poisoning on the background of 15-day intoxication by tobacco smoke, the figure was 2.1 times higher than normal, sexually mature in 1.5 times.

In the myocardium of young rats there was observed a significant increase in the content of nitrite-ion in the groups of rats, which simultaneously affected the two toxicants, and in the last period of investigation, the content of nitrite ion exceeded the level of the intact control in 1.7 times. In sexually mature rats there was a significant increase only in 72 hours after contact with sodium nitrite in the organism of animals toxicomanic smoke and its content is 50 % larger than the normal level.

The toxic effect of nitrates and products of their recovery of nitrite is hypoxia, which develops due to violation of the transport of blood oxygen, as well as in the inhibition of activity of enzyme systems involved in tissue respiration. Nitrate toxicity leads to disruption of the processes of oxidative phosphorylation, due to the action themselves nitrates and nitrites. The result is a high level of methemoglobin in the blood [4].

We investigated the content of methemoglobin in the blood poisoned by the toxic rats. The results are shown in the Tab. 2.

From immature rats, the content of methemoglobin was significantly increased in all experimental groups. The highest value it reached in the animal, against the background of the 15-day nitrite intoxication that received sodium nitrite and studies were carried out in 72 hours after its ingestion. This index increased in 2.7 times.

In sexually mature rats, a significant increase ($p \leq 0.05$) of this parameter was recorded only in cases of poisoning by both toxicants. After 72 hours of poisoning with sodium nitrite and 15 days of intoxication by tobacco smoke the content of methemoglobin in the blood of rats was increased in 2.1 times.

Given the nature of the toxic effects of nitrite and the data obtained it can be concluded that the most sensitive to toxicants are rats of first months of life. They noted a fairly high content of methemoglobin after the defeat of the sodium nitrite on the background of TI indicating an active development of gemona hypoxia.

Table 2

THE CONTENT OF METHEMOGLOBIN (G/L) AND ERYTHROCYTE INDEX OF INTOXICATION (%) IN BLOOD OF RATS AFFECTED BY NITRITE OF SODIUM ON THE BACKGROUND OF POISONING BY TOBACCO SMOKE (M ± m; n = 48)

Tissues	Immature rats				Sexually mature rats			
	Groups of animals				Groups of animals			
	Intact rats	15 TI day	15 TI day + 24 hours NI	15 TI day + 72 hours NI	Intact rats	15 TI day	15 TI day + 24 hours NI	15 TI day + 72 hours NI
Blood	Methemoglobin							
	1.53 ± 0.14	2.05 ± 0.09	3.09 ± 0.12	4.16 ± 0,28	1.50 ± 0.15	1.65 ± 0.46	2.66 ± 0.11	3.09 ± 0.11
Blood	Erythrocyte index of intoxication							
	20.83 ± 3.09	26.25 ± 3.08	52.71 ±2.47	59.58 ±2.36	17.29 ± 2.29	33.33 ± 2.55	47.50 ±1.12	50.00 ± 2.16

Obviously, the reason for this can be several factors: embryonic hemoglobin in newborns is much easier oxidized by nitrate than by hemoglobin [4, 5].

Destruction of rats by sodium nitrite on the background of tobacco intoxication is accompanied by a pronounced embryotoxic effect. We note the percentage increase in the permeability of the erythrocyte membrane in animals of both age groups. In cases of poisoning by tobacco smoke from immature rats EII increased on only 5.4 %, which was not reliable. In sexually mature rats, the figure increased on 16 %.

After 24 hours of injection of intoxicated smoke in young animals of sodium nitrite EII was increased on 32 %, in 72 hours – 39 % ($p \leq 0.05$). At the same time, studies in sexually mature rats, this figure was higher than the level of intact on 30 % and 32 %, respectively.

Thus, in the blood of immature rats the content of methemoglobin and the percentage of the permeability of the erythrocyte membrane by poisoning them with both toxicants were significantly higher.

CONCLUSIONS

It is established that the lesion of immature and sexually mature rats by sodium nitrite on the background of tobacco intoxication leads to a significant increase in the content of nitrite-ion in blood serum, liver, lungs, kidneys and myocardium after poisoning, which, obviously, causes activation of free radical oxidative processes in the affected organism. Toxic products, which are formed by this cause membrane destructive effect on erythrocyte membrane and increased levels of methemoglobin in the blood. More sensitive to these indicators were immature animals.

Conflicts of Interest: authors have no conflict of interest to declare.

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