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FREE RADICALS AND INFLAMMATION IN RATS OF DIFFERENT AGE IN CASES OF SODIUM NITRITES AND TOBACCO SMOKE POISONING

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Background. *Due to the wide use of nitrate fertilizers in agriculture and their migration into groundwater and food, the spreading of nitrate poisoning has become epidemic. However, people in the process of life get into bad habits: smoking, alcohol, drugs abuse. All these factors affect health and can cause fatal outcome. In real life, people are often exposed to more toxic factors that lead to general poisoning of the body and damage of many organs.*

Objective. *The research was aimed to study the activity of free radicals and inflammation in rats of different age in cases of sodium nitrite affection with underlying 45-day tobacco intoxication.*

Methods. *The content of nitrite ion (NO_2^-) was evaluated by Gris reaction. The level of pro-inflammatory (interleukin 6 (IL-6) and anti-inflammatory (interleukin 4 (IL-4) cytokines was determined in serum by ELISA method using test kits.*

Results. *It was proved that in rats of different age affected by sodium nitrite with underlying 45-day tobacco smoke intoxication, the content of nitrite ion in serum, liver, lungs and myocardium is increased. After poisoning the animals with the studied toxicants, inflammation was activated in the body that was evidenced by the increased pro-inflammatory cytokine IL-6 and decreased inflammatory cytokine IL-4 in serum.*

Conclusions. *The nitrite ion content in organs was the most significant and inflammation was manifested in the immature rats. In these animals the content of anti-inflammatory cytokines was the lowest.*

KEY WORDS: **tobacco smoke; sodium nitrite; rats of different age; nitrite ions; cytokines.**

BIOMEDICAL SCIENCES

Introduction

The wide spread smoking is a global problem of humanity; many scientists and experts make efforts to solve it. Smoking has a negative impact on the health of the smokers, but also on the health state of people, who do not smoke but are exposed to harmful effects of pollutants entering the atmosphere with tobacco smoke [1]. Numerous scientific studies clearly highlighted the negative influence of smoking on the development of many diseases of different organs and systems of the human body [2, 3].

In recent years, the influence of smoking on free radical processes on the body has been extensively studied. It is established that smoking causes depletion of vitamins C and A, decreases serum levels of other antioxidants, which leads to tissue damage by free radicals [4, 5]. Study of the oxidation-antioxidant status in passive smokers revealed similarity of such changes in active smokers [6].

Due to the increasing technogenic and anthropogenic pollution, the study of combined effects on the body of the most common xenobiotics: heavy metals, nitrates and nitrites, tobacco smoke, medications, is urgent. Considerable attention is attracted to intoxication mechanism of nitrite and nitrate. This happens because of the intensive application of chemicals in the industry, lack of efficient methods for purifying drinking water, high levels of pollution caused by nitrates and nitrites number in foods, especially early fruits and vegetables. Hemic hypoxia, caused by nitrates that penetrate into the body, cause functional and morphological changes in many organs, including kidneys and liver, which can lead to further development of comorbidity [7, 8].

Because of the summation of environmental risk factors, chronic inflammation may develop, which involves all organs and tissues.

Recently, the attention of researchers has been attracted to mediators of immune re-

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sponses – cytokines. Cytokines are produced by all body cells for cell-to-cell interaction and regulation of biochemical processes in cell [9]. Imbalance of cytokines is important for the pathogenesis of toxic affection of the body.

The aim of this research was to study the activity of free radicals and inflammation in rats of different age in cases of sodium nitrite affection with underlying 45-day tobacco intoxication.

Methods

The experiments were conducted on white outbred male rats that were kept on a standard diet at the vivarium of Ternopil State Medical University. The rats were divided into three age groups: the first – immature, weighing 60-80 g, the second – mature, weighing 180-200 g, the third – senile rats, weighing 300-320 g; each age group consisted of two subgroups: an intact control and experimental group. Rats study groups were affected with tobacco smoke for 45 days. The test animals were divided into 3 groups. One of them was administered with sodium nitrite, dose 45 mg/kg 24 hours before the end of the experiment; the second – with sodium nitrite 72 hours before euthanasia. The third group of rats was subjected to toxic affection with tobacco smoke only. Depending on the chronic smoke effect, the model was simulated using a sealed chamber 30 litres in volume, allowing fumigating the animals in free behaviour. Tobacco smoke, formed by combustion of 6 cigarettes "Prima sribna (blue)", containing 0.6 mg of nicotine and 8 mg of tar, was served into it through openings in the chamber. The camera was located 6 animals both within 6 minutes. The animals in the control group were well for 6 minutes in a sealed chamber, but were not subject to smoke.

In 45 days after the beginning of the affection of the animals with tobacco smoke they were taken out of the experiment by euthanasia under thiopental anaesthesia.

Blood, serum, liver, lungs and myocardium of the animals were used for the study. The experimental tissues were used to prepare 10% homogenates using saline.

The content of nitrite ion (NO-2) was determined by Gris reaction [10].

Pro-inflammatory (interleukin 6 (IL-6) and anti-inflammatory (interleukin 4 (IL-4) cytokines levels in blood serum were determined by ELISA methods using test kits. Quantitative assessment of serum concentration in peripheral blood of these cytokines was performed by chemiluminescent enzyme-linked immunosorbent assay using ELISA analyser RT-2100C. Test systems and control serum IL-4, IL-6, Russia, were used according to the test systems protocols. The results of the reaction were determined by a spectrophotometer ULAB-108UA, wavelength 450 nm. These cytokines concentration was evaluated using a calibration curve in picogram per 1 ml (pg/ml) [11].

The research was performed according to the general principles of animal experiments approved by the National Congress on Bioethics (2001, Kyiv, Ukraine) and is consistent with the regulations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, France, 1985) [12]. Statistical analysis of the data was performed using STATISTICA 6.0, parametric Student's t-test and nonparametric Wilcoxon criterion for related samples. Changes considered significant at $p \leq 0.05$ [13].

Results

After rats poisoning with sodium nitrite (SN) during a 45-day tobacco smoke (TS) toxicity we have noticed the increase in content of nitrite ion in serum and organs (Table 1).

In immature rats nitrite ion content in serum increased by 18% till 45 day of TS toxicity. After the poisoning with sodium nitrite (24 hours before the end of the experiment on the 45th day) the content of this indicator increased by 69%, and after the application of toxicants in 72 hours before the study deadline the content of nitrite ion increased by 132%.

A similar increase in nitrite ion content was evidenced in serum of mature and old rats. Using of both toxicants led to increase in its content in 2 times (72 hours after 45-day SN poisoning of TS animals).

In liver of rats of all age, nitrite ion content increased after the affection with tobacco smoke. The use of sodium nitrite as an additional toxicant contributed to the formation of nitrite ion. In liver of the immature rats the rate increased in 3 times, in mature – in 3.5 times. The least sensitive liver was evidenced in the senile animals which nitrite ion content increased in 2.2 times in the final period of the study.

Similar dynamics was observed in lungs of rats of different age. The highest content of nitrite ion was in the groups of rats with underlying 45 day tobacco intoxication which 72 hours before the end of the experiment were administered with sodium nitrite. This rate

Table 1. The content of nitrite ion in serum (nmol/L) and organs (nmol/kg) of rats of different age affected by sodium nitrite with underlying 45 day tobacco smoke toxicity (M±m; n=72)

Research time, days	Groups of experimental animals		
	immature rats	mature rats	senile rats
	blood serum		
intact rats	10.00±0.46	8.20±0.74	8.80±0.56
45 day affection with tobacco smoke	11.80±0.44*	10.20±0.18	10.70±0.46
45 day affection with tobacco smoke + 24 hours sodium nitrite poisoning	16.90±0.87*	15.30±0.22*	15.80±0.29*
45 day affection with tobacco smoke + 72 hours sodium nitrite poisoning	23.20±0.37*	16.40±0.25*	17.90±0.18*
	Liver		
intact rats	7.60±0.22	3.20±0,16	9.90±0.39
45 day affection with tobacco smoke	10.20±0.61*	5.40±0.51*	11.40±0.43*
45 day affection with tobacco smoke + 24 hours sodium nitrite poisoning	15.60±0.21*	11.00±0.29*	16.10±0.79*
45 day affection with tobacco smoke + 72 hours sodium nitrite poisoning	23.00±0.59*	11.30±0.48*	21.50±0.73*
	Lungs		
intact rats	1.30±0.14	1.00±0.11	1.70±0.19
45 day affection with tobacco smoke	2.90±0.28*	1.60±0.14*	2.60±0.23*
45 day affection with tobacco smoke + 24 hours sodium nitrite poisoning	3.40±0.31*	1.90±0.11*	3.10±0.17*
45 day affection with tobacco smoke + 72 hours sodium nitrite poisoning	4.80±0.21*	3.20±0.13*	4.60±0.32*
	Lungs		
intact rats	2.20±0.14	1.60±0.15	1.80±0.15
45 day affection with tobacco smoke	4.00±0.26*	2.70±0.14*	3.40±0.14*
45 day affection with tobacco smoke + 24 hours sodium nitrite poisoning	4.40±0.21*	2.90±0.17*	3.70±0.20*
45 day affection with tobacco smoke + 72 hours sodium nitrite poisoning	4.90±0.16*	3.20±0.13*	4.10±0.13*

Note: here and in the following tables * – significant changes between intact rats and rats affected with tobacco smoke ($p \leq 0.05$).

increased in the immature rats in 3.7 times, in the mature – in 3.2 times, and in the senile – in 12.7 times compared with the intact control group.

During the study of myocardium of the smoke intoxicated rats almost the same increase in the content of nitrite ion in all age groups (in 1.7-1.9 times higher than normal) was noted. In the rats poisoned with both toxicants this rate also increased: in the immature and old rats it exceeded the intact control in 2.2-2.3 times respectively. In the mature rats the rate increase in myocardium exceeded 2 times.

Thus, the poisoning of rats for 45 days with tobacco smoke caused a moderate increase in nitrite ion content in blood serum and organs of rats of different age. After an additional affection of rats with sodium nitrite, this rate significantly increased ($r \leq 0.05$) and was considerably higher than in the intact animals. This increase in the content of nitrite ion may be caused by activation of free radical processes in the affected organism.

We determined the content of pro- and anti-inflammatory cytokines in blood serum of rats after simultaneous affection with sodium nitrite and tobacco smoke. The results of the study of pro-inflammatory cytokine (IL-6) content in blood of rats of different age groups are presented in Table 2.

In old rats inflammation development was less significant, as evidenced by the increase of IL-6 content in serum after the affection with tobacco smoke in 2.4 times, and after the application of both toxicants in 2.6 times (at the end of the experiment).

Most inflammation was activated in the immature rats. Poisoning of the young animals with tobacco smoke resulted in pro-inflammatory cytokine increase in 3.4 times. In the smoke affected animals, which 72 hours before

Table 2. The content of pro-inflammatory cytokines (IL-6) in serum (ng/L) of rats affected with sodium nitrite with underlying 45-day tobacco smoke toxicity (M±m; n=72)

Research time, days	Groups of experimental animals		
	immature rats	mature rats	senile rats
intact rats	1.91±0.28	3.00±0.30	4.14±0.17
45 day affection with tobacco smoke	6.43±0.21*	8.31±0.19*	9.97±0.29*
45 day affection with tobacco smoke + 24 hours sodium nitrite poisoning	7.74±0.35*	9.35±0.32*	10.94±0.21*
45 day affection with tobacco smoke + 72 hours sodium nitrite poisoning	8.87±0.21*	10.50±0.26*	10.90±0.31*

Note: here and in the following tables * – significant changes between the intact rats and the rats affected with tobacco smoke ($p \leq 0.05$).

the end of the experiment end (45th day of tobacco affection) were administered with sodium nitrite, the content of IL-6 increased in 4.6 times.

Thus, the immature rats were the most sensitive to smoke and both toxins, inflammation in them was developed the fastest and most actively.

The research of anti-inflammatory cytokines (IL-4) content in the studied rats affected with xenobiotics was useful. The results are presented in Figure 1.

The rate of inflammatory cytokines activity is the lowest in the immature rats, in the simul_taneous use of sodium nitrite and smoke the content of IL-4 reaches 45% compared to the norm (on 45th day of smoke intoxication and 72nd hour of sodium nitrite poisoning), as presented in Figure 1.

In the immature and senile rats the content of this indicator after the affection with tobacco smoke was on the same level, 68% of the intact animals' rate. The use of sodium nitrite as an additional toxicant contributed to the disorders in the system of anti-inflammatory cytokines formation. In the old rats after sodium nitrite application, the IL-4 content in tabaco smoke intoxicated rats decreased by 45% and was 55% of norm, in the mature rats in this period it was 59%.

Therefore, sodium nitrite poisoning of rats with underlying tobacco intoxication leads to the imbalance in the system of pro-/anti-inflammatory cytokines, pro-inflammatory indicators are predominant.

Discussion

It is established that after getting into the human body nitrates are restored to nitrites, which lead to the activation of methaemoglobin formation that causes tissue hypoxia. According to literature [7], sodium nitrite in contact with oxyhaemoglobin is a powerful generator of active radicals: HO_2, O_2 OH, NO_2. These metabolites damage biological systems, have a significant cytotoxic action, initiate the process of lipid peroxidation, can reveal a strong oxidizing action interacting with SH-groups of proteins, reduced forms of nucleotides, physiologically active compounds [14].

In the pathogenesis of various diseases, inflammation caused by immune mechanisms is of great importance that is confirmed by the numerous experimental studies [15]. Inflammation is developed in response to damage and penetration into tissues of pathogens with

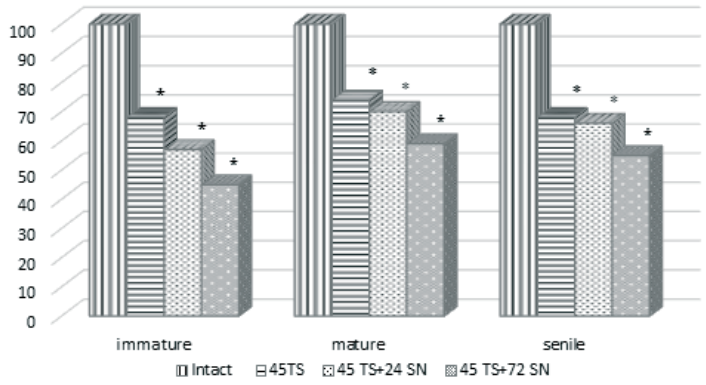

Fig. 1. The content of anti-inflammatory cytokines (IL-4) in blood serum of the rats of different age groups affected by sodium nitrite with underlying 45-day tobacco intoxication, %.

participation of pro-inflammatory cytokines: IL-1, TNF-α, IL-6, IL-8 chemokines. In case of local reactions violation, protective inflammatory response is intensified, synthesis of cytokines is increased; they get into the bloodstream and effect at a system level. In this case pro-inflammatory cytokines affect almost all organs and body systems. [15]

The cytokine imbalance is important for the initiation and progression of inflammation in the body, but the role of cytokines during chronic inflammatory reactions still remains to be determined.

Conclusions

The affection of rats of different age with sodium nitrite with underlying tobacco intoxication leads to nitrite ion accumulation in serum and organs of animals that may be caused by activation of free radical processes in the body. The violation in ratio pro- and anti-inflammatory cytokines was determined. The most significant increase of IL-6 in the immature rats with underlying decrease of anti-inflammatory IL-4 was proved; that is one of the causes of decreased activity of protective systems after injury.

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