Research Article

# Influence of Chronic Intoxication of Sulfur-Containing Gas on the Condition of Peroxidation of Lipids, Nitric Oxide and Induced No-Syntase

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**ABSTRACT**

Lipids directly participate in the immune and chemical processes, neurotransmission and contribute to the body energy reserve formation. That is why the topic is relevant and research was aimed to investigate the peculiarities of the condition of peroxidation of lipids in the bone marrow, blood plasma and erythrocytes, nitric oxide metabolites in blood serum and i-NOS content in rats at the stages of the postnatal ontogenesis under the conditions of the experimental influence of subtoxic doses of sulfur-containing gas. Experimental and theoretical methods, applied by the authors, have made it possible to research the issues thoroughly. The authors have conducted experimental research, targeted to investigating the chronic influence of subtoxic doses of sulfur-containing gas within the system of lipids peroxidation – antioxidant defense, as well as between the level of nitric oxide metabolites and induced NO-synthase concentration in the bone marrow and blood. It has been established that a long-term influence of sulfur-containing gas results in the shift in the system of proliferative processes in bone marrow cells, decreasing their germinative potential, causing degradation in the erythron system.

**Keywords**

Sulfur-Containing gas, Lipids Peroxidation, Nitric Oxide, i-NOS, Rats

## Introduction

Currently the role of microcirculation vessel endothelium in the regulation of haemodynamics, haemostasis, thromboresistance maintenance, etc. is generally recognized [1-5].

Particularly, endothelium is essential to maintaining vascular tone, producing various vasoactive substances, the most important of which being nitric oxide (NO) – the most powerful among the known vasodilators [6-9]. Simultaneously NO is able to cause different and even opposite effects at target-cells, depending on additional factors availability: oxidation-reduction and proliferative status and a set of other conditions. NO influences effector systems, controlling proliferation, apoptosis and cell differentiation, as well as their stress resilience [2].

In addition, NO can both facilitate lipid peroxidation processes (LPP) in cell membranes and serum lipoproteins and inhibit them; cause vasodilation, but it can also result in vasoconstriction, induce apoptosis or produce a defensive effect on apoptosis, induced by other agents; NO is able to modulate inflammatory reaction development [10,11].

and inhibit oxidative phosphorylation in chondriosomes and ATP generation [12].

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A prior problem with endothelium functional condition depends on the pathologic process localization, haemodynamic shifts availability, dominance of various humoral factors, damaging endothelium [13]. Various factors can be reasons for endothelium dysfunction [14] tissue hypoxia, age-related changes, free radical damage, impact of cytokines, hyperglycemia, hypertension, endogenous and exogenous intoxications, etc.

Hypoxia, developing under the long term influence of sulfur-containing gases [15-17], causes problems with endothelium functional activity [13,18,19] reduces the production of endogenous nitric oxide [20] as a result reflecting on the processes of blood-forming tissue regeneration and erythron regeneration, in particular.

The purpose of the work is to research the peculiarities of lipids peroxidation in the bone marrow, blood plasma and erythrocytes, nitric oxide metabolites in blood serum and i-NOS content in rats at the stages of the postnatal ontogenesis under the conditions of experimental influence of subtoxic doses of sulfur-containing gas.

## Materials and Methods

The experiment has been conducted on 40 white outbred male rats. Groups of two types were formed: I. control set; II. Exposed to sulfur-containing pollutants. Each type comprised four groups with 5 specimens in each, the animals being at the same stages of individual development with people throughout the postnatal ontogenesis **(Table 1).**

The table is compiled on the basis of the data, represented at works by [21,22]. A natural crude dehydrated gas from Astrakhan condensate field, obtained from U-121 plant from bore № 17 was used as a toxicant.

At the experiments there was used the natural gas concentration in the gas-air mixture chamber of 90 3 mg/m$^{3,}$ being measured by hydrogen sulfide, which is 30 times more than permissible exposure limit of hydrogen sulfide for working areas at the simultaneous hydrogen sulfides availability.

The hydrogen sulfide concentration at Kurliandskiy's inoculating chamber was measured with detector tubes by the Auer firm (Germany). Sulfur-containing gas inoculation was conducted for 4 hours in the autumn-winter seasons, following the static method, 5 specimens being at the chamber simultaneously, every day for 30 days, except for Sundays, strictly from 10 till 14, the chamber temperature being +22 ± 2°C. During the experiment the relative humidity rose from 53 ± 4% up to 66 ± 6%.

In the course of the experiment there were taken into consideration requirements for its conditions and the necessary gas concentration, reflected in the WHO publications: Principles and Methods of Assessing Chemicals Toxicity (1981), Rules of Performing Works, Using Experimental Animals (order of Ministry of Healthcare of the Russian Federation № 267, dated back to 19.06.2003) [23,24].

The condition of lipids peroxidation in the bone marrow, blood plasma was assessed according to the malondialdehyde concentration, measured according to the method by Vladimirov YA and Archakov AI [25], modified by Stalnaya ID and Garishvili TT [26], whereas in erythrocytes it was assessed according to the method of Ohkawa H, Ohishi N, Yagi K [27]. Nitric oxide metabolites in the blood serum were defined according to the method by Metelskaya VA and Gumanova NG [28]. Paraffin blocks were produced for the purpose of conducting an immune histochemical research of i-NOS content, cannon-bones having been fixed in 10% neutral buffered formaline. At the work there were used antibodies to Nitric Oxide Synthase, Inducible (iNOS) Rabbit Polyclonal .0.1 ml (cat. № E3740), diluted 1:100, the detection system by the *SpringBioscience* firm (the USA), glasses by the *Menzel-Glaser* firm (Germany) with poly-L-lysine cover by *Thermo Scientific* (the USA), ensuring fixation after long term staying at the humid chamber (for 3 days).

Quantity data, obtained in the course of the research, were analyzed with the variation statistics, correlation analysis and difference verification. During statistic processing the OpenOffice Calc utility from freely distributed *OpenOffice* (Ver. 3.0) software under the *Windows XP Home Edition* operation system (certificate OEM X12-53766).

**Table 1: Correspondence of experimental animals' development stages according to the human postnatal ontogenesis stages.**

| A Human | Lab rats | |
|---|---|---|
| **Stage** | **Stage** | **Age (ontogenesis day)** |
| infancy | Impuberal | 6-36 |
| adulthood, stage I | mature I | 368 - 398 |
| adulthood, stage II | mature II | 472-502 |
| Late adulthood | presenile | 700-730 |

## Results

Concern of programmed cell death activators – free radicals, the research of their content in the body liquids is rather complicated due to a short term of their activity, that is why our work was aimed at investigating the condition of the systems, participating in radicals metabolism – lipids and antioxidants peroxidation. According to the research results **(Table 2),** the most intense lipids peroxidation processes both in blood plasma and erythrocytes of intact animals were recorded at the ontogenesis young stage. By ontogenesis mature stage I the MDA content, reflecting the lipids peroxidation level, decreases, in the blood plasma it is highly statistically significant. Further the lipids peroxidation intensity in case of control set animals decreases gradually. In the blood plasma the process is statistically significant, but in erythrocytes it is of the tendency character, that probably reflecting a higher phylogenetic stability of blood cell elements.

Exposure to sulfur-containing gaseous pollutants causes statistically significant lipids peroxidation intensification in rats' bone marrow at all considered ontogenesis stages but to a different extent. Due to the initial high MDA concentration, after the altering influence the most intensive lipids peroxidation is registered in the bone marrow of presenile rats, despite its relatively small external activation, compared to other investigated ontogenesis stages. In terms of this factor, even taking into account the initial low MDA concentration, rats of a young age are ranked second as a result of extremely high lipids peroxidation external activation. Probably, the phenomenon is caused by the developmental deficiency of their antioxidant defense system. The antioxidant defense system of rats' bone marrow of ontogenesis mature stages are the most resilient to the external lipids peroxidation activating influence. It is related to both sufficiently low initial level of lipids peroxidation and its moderate activation as a result of exposure to gaseous sulfur-containing pollutants.

Subtoxic influence of gaseous sulfur-containing pollutants radically alters the above described lipids peroxidation condition in blood plasma and erythrocytes, intensifying it energetically. Herewith toxic influence effects overlap with the ontogenesis standard. The level of lipids peroxidation raises most of all as a result of gaseous sulfur-containing pollutants activity in young rats' plasma and erythrocytes. In terms of this factor rats of mature I stage development are the most resilient. Further, the blood antioxidant system resilience starts to gradually decrease but even at the experimental animals' ontogenesis presenile age it does not reach such a low level as at the young age of development.

Our work has also been aimed at investigating metabolites of nitric oxide concentration in the blood serum. It has been discovered that the greatest nitric oxide concentration is registered in groups of young animals and animals at the presenile stage, nitric oxide metabolites concentration in mature stages I and II is accurately lower. At the background of sulfur-containing pollutants activity there has been recorded nitric oxide produce activation that can be a normal adaptation response to the exogenous provocative activity. Herewith the greatest nitric oxide metabolites concentration is registered in young rats' blood serum, then in the group of the ontogenesis presenile age, then in mature II group and the least one – in mature I group. Thus, the increased nitric oxide produce acts as an adaptation mechanism but, taking into consideration possible effects of nitric oxide in excess, it can become a provoking factor to activate apoptosis in cells of red bone marrow.

For the purpose of confirming the hypothesis about the induced NO-syntase concentration growth in cells of red bone marrow, we have investigated i-NOS content in various age groups in the normal condition and under the subtoxic

**Table 2: Malondialdehyde concentration in the rats' bone marrow, blood plasma and erythrocytes at the ontogenesis stages normally, having been exposed to the sulfur-containing gas (M ± m, nMole/mg).**

| Ontogenesis stage | | Young | mature I | Mature II | Presenile |
|---|---|---|---|---|---|
| Bone marrow | control | 58.9 ± 1.7 | 59.2 ± 1.6 | 60.3 ± 1.8 | 76.7 ± 1.6* |
| | gas | 74.3 ± 2.3* | 70.4 ± 2.2* | 72.4 ± 2.1* | 82.6 ± 2.4* |
| Blood plasma | control | 1.86 ± 0.02 | 1.13 ± 0.03 | 1.32 ± 0.04 | 1.54 ± 0.03 |
| | gas | 3.57 ± 0.03* | 2.44 ± 0.04* | 2.62 ± 0.03* | 2.83 ± 0.05* |
| erythrocytes | control | 7.03 ± 0.68 | 6.23 ± 0.57 | 6.32 ± 0.56 | 6.64 ± 0.53 |
| | gas | 11.71 ± 0.64* | 9.34 ± 0.58* | 10.03 ± 0.53* | 10.68 ± 0.61* |

* - the difference with control is accurate (p<0,05)

influence of sulfur-containing pollutants with the help of immune histochemical method.

The positive i-NOS albumen reaction in both mature subgroups and a subgroup of young animals, not discovered in our research, lets us claim the absence of external (exogenous) and internal (endogenous) endothelial cells stimulation **(Figure 1).** In the animals' presenile group there has been noticed a weakly positive i-NOS reaction mainly in the internal part of the compact substance, the positive i-NOS albumen reaction has not been noticed in the trabecular substance and in other subgroups.

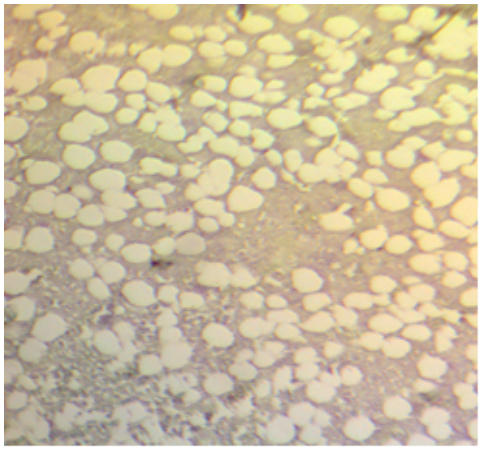

**Figure 1:** The negative immune histochemical i-NOS albumen reaction in the structures of animals young subgroup's red bone marrow. 20-fold magnified.

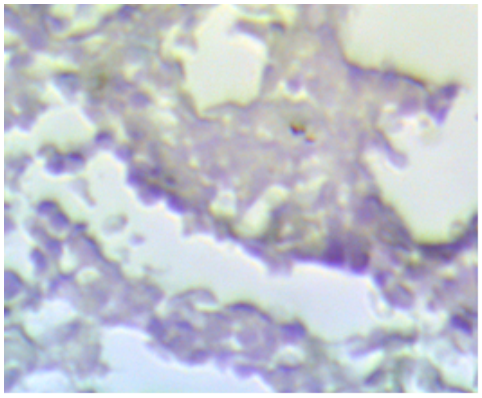

**Figure 2:** i-NOS-positive immune histochemical reaction of red bone marrow endotheliocytes (depicted with arrows) at the background of subtoxic influence of sulfur-containing-containing gas. 40-fold magnified.

At the background of subtoxic influence of sulfur-containing pollutants there are discovered positively dyed regions of red bone marrow. The weakest reaction is defined in animals' mature I subgroup, i-NOS-positive endotheliocytes are defined in bone trabeculae vessels. In mature II subgroup i-NOS-positive reaction is demonstrated not only by endotheliocytes of the compact substance but also singular ones in the trabecular substance. The highest grade reaction is observed in lab animals' young subgroup. I-NOS-positive endotheliocytes are defined in the bone marrow sinous capillaries **(Figure 2).**

It can be the result of insufficient maturity of enzyme systems, producing the induced NO-syntase in response to exogenous stimulation with sulfur-containing pollutants.

## Conclusion

Based on the obtained results it can be claimed that chronic influence of sulfur-containing gas causes alterations in the regulation of proliferation of bone marrow cells and reduces their germinative potential. As in the red bone marrow, as the central blood-forming body, there are permanent processes of restoration with young cells, alongside with damage processes, adaptation processes are formed. Under the long term exposure to subtoxic doses of sulfur-containing gases this mechanisms found to be ineffective. As a result, degradation in the erythron system occurs and its sensitivity for external influences reduces. The authors have not discovered i-NOS-positive reaction in different animals' subgroups. The experiment testifies to the absence of external (exogenous) and internal (endogenous) endothelial cells.

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