

AGE CHANGES OF ANTIOXIDANT-PROOXIDANT BLOOD STATUSES IN RATS DURING PARACETAMOL POISONING AND THEIR CORRECTION WITH ENTEROSGEL

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Background

Liver damage is a widespread pathology, which is frequently the cause of death among people. Medicaments play a significant role in the etiologic structure of liver diseases. Drug-induced liver damage accounts for 10 % of all adverse reactions of macroorganism associated with the taking of pharmaceuticals [3].

The medications that can cause severe liver damage include even such widespread pharmaceuticals as paracetamol. Simultaneously, the advertisement and unrestricted sale of medication works towards its very wide application [4]. Uncontrolled use of paracetamol is associated with a high risk of complications. According to data from medical centres in the USA and the UK, overdoses of paracetamol is one of the main causes of acute hepatic failure, which results in the death of patients in many cases.

The toxicity of paracetamol is related to how it is metabolized. In therapeutic doses, paracetamol is mainly metabolized through conjugation with sulfuric acid and, especially, glucuronic acid (60%) in the liver of animals and humans. About 5-10% of this medication is oxidized through cytochrome P450 (CYP450)-dependent ways (typically, CYP2E1 and CYP3A4) to a toxic metabolite with electrophilic properties N-acetyl-*p*-benzoquinonimine (NAPQI) or reduced to the original paracetamol, or detoxicated with glutathione and excreted with urine or bile.

Under conditions of acute intoxication, the metabolic conjugation of paracetamol with sulfuric and glucuronic acids undergo dose-dependent saturation. As a result of which the portion and total amount of the medication oxidized by the cytochrome P-450 to the electrophilic metabolites increase. In this case, there occurs not only the formation of NAPQI in the two-electron oxidation reaction, but also the genesis of the free radical of NAPQI *via* single-electron oxidation. This substance is reduced by glutathione or NADP-H to paracetamol and oxidized by atmospheric oxygen to NAPQI to simultaneously form a superoxide anion. The NAPQI that did not undergo detoxication is capable of binding to the sulfhydryl groups of proteins and glutathione predominantly in the center-lobular regions of the liver to cause their damage and necrosis and can trigger lipid peroxidation [13].

Treatment and prevention of toxic liver damage by paracetamol is limited to date by application of acetylcysteine and methionine [14, 15]. Administration of these medications over 10-12 h after intoxication allows one to prevent severe liver damage. However, it is very difficult to diagnose drug induced hepatitis. This circumstance is related to several factors, among which are: the possibility of latent progress of disease, inadequate interpretation of clinical symptoms and clinical laboratory parameters, insufficient study of anamnesis [1]. Therefore, the significant disadvantage of these medications is the limited time period in which they can be used, and detoxifying only before the end of phase II of the metabolism process.

To date, enterosorption is increasingly frequently applied for the treatment of liver diseases of different genesis. Having comparative effectiveness, it is a simple, cheap, and safe method of treatment, which can be applied for a long period of time to decrease the toxic and metabolic load on liver [2]. At the same time, there exists only brief data on the application of enterosorbents in view of age-related properties of the body. The questions of change in the parameters of detoxication function under the influence of enterosorbents in the case of its pathology are disclosed insufficiently.

Aim

Therefore, the aim of the present work was to study the status of lipid peroxidation (LPO) and antioxidant system in white rats of different age groups in case of paracetamol liver damage and the possibility of correction of abnormalities using the enterosorbent Enterosgel.

The enterosorbent Enterosgel which is a hydrogel of methylsilicic acid, has a high bio- and hemocompatibility, does not damage the mucosa of digestive tract, is not absorbed in blood, and is easily excreted from the body. It absorbs toxic substances and products of incomplete metabolism with a molecular weight 70 to 100 Daltons from intestinal contents and blood plasma through membranes [12].

Materials and methods

Studies were performed on one-, six-, and twelve-month old male scrub rats. The animals were kept under standard ration animal quarters, and provided with basic food requirements. The rats were randomized in five groups: 6 animals per group.

Group I included intact animals; group II included control animals with acute paracetamol hepatitis; group III included animals with paracetamol hepatitis, to which Enterosgel was administered orally over 7 days before simulation of acute hepatitis; group IV included animals with paracetamol hepatitis, to which Enterosgel was administered orally for 7 days after simulation

of acute hepatitis; group V included animals with paracetamol hepatitis, to which Enterosgel was administered orally for 7 days before and after simulation of acute hepatitis.

Paracetamol was administered to animals intragastrically through gastric tubes in a dose of 1250 mg/kg (0.5 LD50) in the form of suspension in a 2% solution of starch gel once daily over 2 days [6]. Enterosgel was also administered intragastrically through gastric tubes in 14% suspension based on 650 mg per kg of body. Enterosgel was administered to the group IV and V animals one hour prior to administration of paracetamol.

The animals were decapitated upon thiopental narcosis. We used blood in the studies, the plasma allowed us to determine the level of primary and secondary LPO products, *viz.*, diene conjugates (DC) by spectrophotometry [11], malonic dialdehyde (MDA) by the method based on the formation of a stained complex in the reaction of MDA with thiobarbituric acid [5]; and the activities of superoxide dismutase (SOD) and catalase [8].

All animal studies were performed in accordance with "The rules for use of laboratory experiment animals" [7]. The digital data obtained was statistically processed [9].

Results and Discussion

Intragastric administration of paracetamol to the test animals in a dose of 1250 mg/kg was accompanied by intensification of free-radical processes, which was manifested in the rise of plasma levels of DC and MDA in white rats of different age groups. The analysis of experimental data showed that the most clear increase in the plasma levels of DC and MDA was observed in the one- and twelve-month white rats: the plasma level of DC and MDA increased 2.2- and 1.7-fold in the one-month old white rats and 2.5 and 1.8-fold in the twelve-month old rats, respectively, compared to those of the animals of the intact group (*table 1*). Administration of Enterosgel to the animals prior to and/or after damage with paracetamol decreased the intensity of free-radical processes. The application of the enterosorbent Enterosgel in the group IV and V of white rats of all age categories was the most efficient.

Table 1

Antioxidant-prooxidant blood parameters in rats of different age upon paracetamol intoxication and correction with Enterosgel ($M \pm m$, $n = 6$)

Animal age, months	Animal group	Index			
		DC, c.u.	MDA, $\mu\text{mol/L}$	SOD, %	Catalase, %
1	I intact	3.31 \pm 0.27	3.43 \pm 0.28	29.72 \pm 0.18	28.22 \pm 0.63
	II control	7.42 \pm 0.65*	8.52 \pm 0.62*	18.56 \pm 0.12*	17.7 \pm 0.11*
	III test	6.81 \pm 0.51	6.62 \pm 0.48 [#]	27.43 \pm 0.17 [#]	20.36 \pm 0.75 [#]
	IV test	5.94 \pm 0.37 [#]	4.95 \pm 0.32 [#]	22.35 \pm 0.13 [#]	25.2 \pm 0.54 [#]

	V test	5.35±0.42 [#]	3.68±0.25 [#]	20.18±0.12 [#]	27.91±0.46 [#]
6	I intact	5.45±0.42	7.09±0.42	44.34±3.28	47.95±3.57
	II control	9.28±0.84*	8.63±0.52	26.33±1.55*	28.83±1.47*
	III test	7.32±0.65 [#]	7.62±0.60	34.78±2.64	33.35±2.65
	IV test	6.78±0.52 [#]	7.15±0.59	38.71±2.87 [#]	39.82±2.06 [#]
	V test	6.02±0.48 [#]	6.86±0.51	42.52±3.08 [#]	44.6±2.77 [#]
12	I intact	6.23±0.41	8.33±0.52	38.34±0.77	44.82±3.45
	II control	10.81±0.62*	15.25±0.11*	22.27±1.14*	25.53±1.93*
	III test	8.85±0.54 [#]	10.02±0.61 [#]	25.06±0.71 [#]	31.94±2.56 [#]
	IV test	7.65±0.61 [#]	9.81±0.85 [#]	27.13±0.77 [#]	37.9±2.73 [#]
	V test	6.95±0.44 [#]	7.73±0.63 [#]	34.71±0.61 [#]	41.34±3.04 [#]

Probable differences in the studied plasma parameters in the group II animals compared to their plasma levels in the group I animals ($p < 0.05$);

* Probable differences in the studied plasma parameters in the group III, IV, and V animals compared to their plasma levels in the group II animals ($p < 0.05$);

In particular, the plasma levels of primary LPO products, *viz.*, DC, in the one-, six-, and twelve-month white rats of group V were 1.4-, 1.5-, and 1.6-fold less than those in the group II animals. The age-dependent features of the plasma level of the end LPO product, *viz.*, MDA, in white rats was observed upon administration of Enterosgel prior to and/or after simulation of paracetamol hepatitis. As is shown in the table data, the plasma level of MDA in white rats decreased in a greater degree in the group IV and V animals.

When analyzing the results obtained, one can state that the use of the enterosorbent Enterosgel after intoxication with paracetamol most efficiently decreases the LPO processes in the one- and twelve-month old white rats. The plasma level of MDA in the six-month old white rats of all groups under study did not change significantly compared to the one- and twelve-month old animals. This is evidence of high adaptive resources of adult organisms under the condition of influence of exogenous xenobiotics.

The analysis of experimental data given in the Table showed that upon intoxication with paracetamol, the activity of SOD in the blood plasma of white rats of all age groups decreased 1.6-1.9-fold and the activity of catalase decreased 1.6-1.8-fold compared to the control animals. Administration of Enterosgel to the white rats prior to and/or after inducing paracetamol hepatitis results in the recovery of the activity of said enzymes. This was best expressed in the group V animals of all age categories: the activities of SOD and catalase were approximately at the same level of parameters as it was in the intact animals.

Consequently, the paracetamol intoxication of one-, six-, and twelve-month old rats causes intensification of the LPO processes, which is manifested in the rise of the plasma levels of DC and MDA. The activities of antioxidant enzymes and catalase decreased with this pathology. The application of the enterosorbent Enterogel in animals prior to and after induced liver damage with paracetamol results in the recovery of prooxidant and antioxidant systems.

Conclusions

1. It is established that the application of the enterosorbent Enterogel after liver damage with paracetamol in the one, six-, and twelve-month old white rats, decreases the plasma levels of DC 1.25-, 1.37-, and 1.41-fold, respectively, the plasma level of MDA 1.72-, 1.21-, and 1.55-fold, respectively, compared to the damaged animals.
2. Upon paracetamol intoxication, there were 1.62-, 1.68-, and 1.89-fold decrease in the activity of SOD and 1.64-, 1.66-, and 1.82-fold decrease in the activity of catalase in the blood plasma of one-, six-, and twelve-month old white rats, respectively compared to the control animals.
3. The enterosorbent Enterogel shows a more efficient influence on the LPO parameters and activities of SOD and catalase in the blood plasma of white rats of all age categories upon its application prior to and after simulation of toxic hepatitis than in cases when it is applied alone for prophylactic or therapeutic purposes.
4. The results of these studies make it possible to use the enterosorbent Enterogel on a case-by-case basis and establish clinical practice in different age groups in case of toxin induced liver damage, which shows the prospectivity of such studies.

Recommended for publishing by the Bioethics Committee

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Received 22.09.08

**AGE CHANGES OF ANTIOXIDANT-PROOXIDANT BLOOD INDICES IN RATS
WITH PARACETAMOL POISOING AND THEIR CORECTION BY ENTEROSGEL**

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The results of the influence of enterosorbent "Enterogel" on the LPO and activities of antioxidant protection enzymes in the one, six-, and twelve-month white rats are given. The study was performed on the model of experimental paracetamol damage of the liver of white rats. It is established that the enterosorbent Enterogel has antioxidant properties, which were manifested in the decrease of plasma levels of diene conjugate (DC) and malonic dialdehyde (MDA), as well as in the recovery of activities of SOD and catalase in the blood plasma of experimental animals, especially, one- and twelve-month ones.

Key words: paracetamol, hepatitis, rats, lipid peroxidation.

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