Antioxidant System in Experimental Toxic Hepatitis under the Influence of Enterosorption, Hyperbaric Oxygen Therapy and their Association

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Abstract

The experiments carried out on 13 white rats focused on the study of the influence of enterosorption, hyperbaric oxygen therapy and their association upon the intensity of the antioxidant system in the liver in acute toxic hepatitis induced by tetrachlormethane. Analysis of the results revealed that acute toxic hepatitis leads to some insignificant disorders of the antioxidant system in the liver which manifest as a reduction in the level of superoxide-dismutase (SOD), catalase, gamma-glutamyl transpeptidase and an increase in the amount of glutathione-reductase. The medicinal charcoal and Medicas E enterosorbent contribute to restoration of the above mentioned disturbances, mainly when used for 14 days. Enterosorption in association with hyperbaric oxygen therapy reduces the activity of gamma-glutamyl transpeptidase which is accompanied by indirect changes of SOD, catalase and glutathione-reductase activity.

Key words: enterosorption, hyperbaric oxygen therapy, toxic hepatitis, antioxidant system.

Антиоксидантная система при экспериментальном токсическом гепатите под влиянием энтеросорбции, гипербарической оксигенации и их ассоциации

В опытах, осуществляемых на 130 белых крысах, было изучено влияние энтеросорбции, гипербарической оксигенации и их комбинации на интенсивность антиоксидантной системы в печени при остром токсическом гепатите индуцированным тетрахлорметаном. В результате анализа полученных данных было установлено, что острый токсический гепатит приводит к несущественным нарушениям антиоксидантной системы в печени, которые проявляются снижением уровня супероксиддисмутазы, каталазы, гамма-глутамилтранспептидазы и увеличением глютатионредуктазы. Активированный уголь и энтеросорбент Медикас Е способствуют восстановлению обнаруженных нарушений, в частности, при использовании в течение 14 дней. Энтеросорбция в сочетании с гипербарической оксигенацией приводят к снижению активности гамма-глютамилтранспептидазы, которая сопровождается непрямыми изменениями активности супероксиддисмутазы, каталазы и глютатионредуктазы.

Ключевые слова: энтеросорбция, гипербарическая оксигенация, токсический гепатит, антиоксидантная система.

Introduction

Oxidative stress represents metabolic disturbances by free oxygen radicals both on the cellular and systemic level. It manifests as an imbalance in the pro- and antioxidant systems, either through excessive formation of free radicals or consumption of the antioxidant capacity of the body. The majority of pathological processes are characterized by involvement of the pro- and antioxidant system, thus further research of these processes is justified in attempts to understand natural physiological recovery as well as therapeutic antioxidant remedies. The liver, due to its role in metabolizing exogenous and endogenous compounds, is subjected to a continuous oxidative stress which results in metabolic and structural changes of the hepatic tissue with marked impairment of function [2, 3, 4, 5, 7].

Stabilization of balance between generation of reactive oxygen species (ROS) and antioxidant protection represents a crucial factor in prophylaxis of a disease with chronic evolution. Antioxidants inhibit oxidative processes and neutralize free radicals. They can block initiation and maintenance of oxidative processes, as well as interfere with the chain of reactions through which new free radicals are produced (for example, peroxidation of cellular membrane lipids or of lipoproteins).

There is a negative correlation between the content of the secondary products of ROS and enzymatic activity of the antioxidant system. Disturbance of the above mentioned balance leads to acceleration of the reaction of peroxide oxidation of lipids in biologic membranes and represents one of the fundamental factors in pathogenesis of multiple pathological conditions: hepatotoxicity, hyperoxia, hypoxia, hyper- and hypothermia, ischemia, inflammations etc. [8].

Due to various direct and indirect mechanisms, enterosorption has a wide use in medical practice, including complex treatment of hepatic diseases. Toxic diseases of the liver, caused by xenobiotics (industrial toxic substances, medicines etc.) have a considerable impact on occurrence of hepatic diseases. Carbon tetrachloride (CCl_4) represents one of the most widely spread harmful substances occurring in different domains of industry which can cause hepatic lesions in people exposed to it. It has been established that through administration of carbon tetrachloride in toxic hepatitis, enterosorption contributes to a considerable reduction of the intermediate and final products of lipid peroxidation (dienic conjugates and malonic dialdehyde), as well as to the correction of the activity of superoxide-dismutase, catalase, glutathione-reductase, glutathione peroxidase, phospholipid

concentration, alpha-tocopherol, ceruloplasmin, ascorbic acid and retinol [8].

Enterosorbents including activated charcoal have proved their importance in the complex treatment of both experimental and clinical hepatitis of various etiologies. (V. Gonciar, 2008, L. Baxan, 2006).

The purpose of the work is to assess the influence of enterosorption, hyperbaric oxygen therapy (HBOT) and their association in evolution of the antioxidant system in the liver in acute toxic hepatitis.

Material and methods

Experiments have been carried out on 130 white rats, 10 in each group, with a body weight of 180-220 gr. Toxic hepatitis was induced by subcutaneous administration of carbon tetrachloride during 4 days in dosage of 0.4 ml/100gr. Enterosorbents - medicinal charcoal (MC) and Medicas E sorbent were used in dosage of 50 mg/100gr, administered intragastric, for 7 and 14 days. Animals were divided into 13 groups: 1 - control, 7 days; 2 - CCl_4, 7 days; 3 - CCl_4, 14 days; 4 - CCl_4 + medicinal charcoal, 7 days, 5 - CCl_4 + medicinal charcoal, 14 days; 6 - CCl_4 + Medicas E, 7 days; 7 - CCl_4 + Medicas E, 14 days; 8 - HBOT (2026 kPa - 60min.) – sessions; 9 – HBOT (2026 kPa – 60 min.) – 10 sessions (14 days); 10 - HBOT (2026 kPa – 60 min.) 7 sessions + CM 7 days; 11 – HBOT (2026 kPa – 60 min.) 10 sessions + MC, 14 days; 12 - HBOT (2026 kPa – 60 min.), 7 sessions + Medicas E, 7 days; 13 - HBOT (2026 kPa – 60 min.), 10 sessions + Medicas E, 14 days. The rats were euthanized on the 8th and 15th day, and their liver was collected for biochemical studies. The results of investigations were subjected to statistical analysis, using "t-Student".

Activity of the following enzymes has been determined in the collected liver: superoxide-dismutase (SOD), catalase (CT), gamma-glutamyl transpeptidase (y-GTP) and glutathione reductase (GR) using the immunoenzymatic method.

Results and discussions

The activity of the antioxidant system: Superoxide-Dismutase (SOD), catalase (CT), gamma-glutamyl transpeptidase (y-GTP) and glutathione reductase (GR) is shown in the table. After injecting CCl_4 for 4 days, on the 7th day a reduction of SOD activity was seen from 9.06 ± 0.53 to 8.41 ± 3.78 (with 7,18%, $p > 0.005$), but it increased again on the 14th day from 9.06 ± 0.53 to 13.15 ± 1.02 (with 45.1%, $p < 0.05$). Similar data concerning reduction of SOD activity in the liver were reported by Lee M. K. et al. (2009); Sindhu E. R. et al. (2010); Akindele A. J. et al., (2010); Murzaahmedova A. A. et al. (2010).

Use of MC in animals with toxic hepatitis for one week did not reveal essential changes of SOD activity compared with the control group, while use of activated charcoal for 14 days contributed to restoring the SOD enzyme levels in rats, as compared to the control group (tab. 1). Administration of Medicas E sorbent during 7 days reduced SOD activity from 9.06 ± 0.53 to 7.06 ± 1.02 (22.1%, $p > 0.05$), but on the 14th day an increase was again seen from 9.06 ± 0.53 up to 12.68 ± 0.96 (14%, $p < 0.05$).

Administration of hyperbaric oxygen therapy for 7-10 sessions in animals with acute toxic hepatitis showed a 3.5-5 fold intensification of SOD activity. It is relevant that association of HBOT with MC and Medicas E causes an increase of activity of the respective enzyme both at early and late stages of the hepatic disease. We can conclude that proper use of hyperbaric oxygen enhances the activity of the enzymatic antioxidant system both for annihilation of ROS induced by hepatotoxic substances and for neutralizing superoxide anion formed from HBOT effect.

Experimental hepatitis does not change significantly the activity of catalase in the liver. Similar findings have been described by Lee M. K. et al. (2009); Login C.(2009); Sindhu E. R. et al. (2010); Akindele A. J.et al., (2010). Administration of CCl_4 on the 8th day revealed an intensification of GR activity in the liver from 3.56 ± 0.15 to 4.59 ± 0.34 (29%, $p < 0.05$), subsequently it returned to the baseline level of the control animals (day 14). Collected data are in accordance with the data from the literature [3]. Under these conditions a reduction of the catalase/glutathione reductase ratio (CT/GR) was seen from 0.23 to 0.15 at early stage of the hepatic disease with an increase up to 0.2 at late stage.

Use of MC and Medicas E adsorbent for the duration of one week causes a significant reduction of catalase activity in the liver, however it returns to control levels after 14 days of treatment. Medicinal charcoal used in the treatment of rats with experimental hepatitis for 1 and 2 weeks did not significantly influence GR activity in the liver compared with the control group, but it diminished the effect of hepatotoxic substance at an earlier stage of the hepatic affliction. In such conditions the treatment with Medicas E adsorbent for 7 days contributed to an increase of GR in the liver from 3.56 ± 0.15 up to 4.39 ± 0.17 (23%, $p < 0.001$) as compared with the control group. Use of this enterosorbent for 14 days in the experimental group was associated with an increase in antioxidant enzyme levels comparable to the levels in the control group. There was a significant reduction of CT/GR proportion on the 8th day of treatment with MC and Medicas E to 0.12 and 0.1 respectively. Subsequently this proportion was restored to control levels after 2 weeks of treatment.

At the early stage of the hepatic lesion an increase in lipid peroxidation exhausts the detoxification pathway of glutathione-peroxidase with the ultimate production of oxidized glutathione(GSSG). In such conditions, GR activity increases with the restoration of glutathione (GSH) quantity. In later stages of hepatotoxicity activated charcoals, through elimination of toxic products of CCl_4, likely contribute to the removal of tissue hypoxia with restoration of catalase and GR activity, expressed through normalization of CT/GR proportion.

Exposure of rats with hepatic diseases to hyperbaric oxygen for curative reasons (7 and 10 sessions) reduced catalase activity in the liver with an increase of GR activity from 3.56 ± 0.15 to 7.64 ± 0.77 (114%, $p < 0.001$) after 7 sessions and from 3.56 ± 0.15 to 9.75 ± 0.77 (173%, $p < 0.001$) after 10 sessions of HBOT. In such conditions an important reduction

Table 1

Activity of antioxidant system in the liver of rats with acute toxic hepatitis under the influence of enterosorption, oxygen-baric therapy and their association

Nr. gr.	Conditions of experiment	SOD, M ± m u/g. prot.	Gama - GTP, M ± m u/s. g. prot.	GR, M ± m μmol/s. g. prot.	CT, M ± m μmol/s. g. prot.
1.	Witness group	9.06 ± 0.53	0.243 ± 0.07	3.56 ± 0.15	0.81 ± 0.05
2.	CCl_4 – 7 days (control)	8.41 ± 3.78	0.197 ± 0.003	4.59 ± 0.34*	0.71 ± 0.1
3.	CCl_4 – 14 days	13.15 ± 1.02*	0.217 ± 0.01	3.8 ± 0.22	0.79 ± 0.05
4.	CCl_4 + CM – 7 days	8.46 ± 1.1	0.298 ± 0.025**	3.74 ± 0.23	0.45 ± 0.02*
5.	CCl_4 + CM – 14 days	9.0 ± 0.94**	0.123 ± 0.02***	3.9 ± 0.26	0.96 ± 0.08***
6.	CCl_4 + Medicas E – 7 days	7.06 ± 1.02	0.321 ± 0.006**	4.39 ± 0.17*	0.46 ± 0.03* **
7.	CCl_4 + Medicas E – 14 days	12.68 ± 0.96****	0.132 ± 0.013***	3.69 ± 0.27***	0.98 ± 0.05****
8.	CCl_4 + OBT – 7 days	32.49 ± 2.55* **	0.048 ± 0.009***	7.64 ± 0.77* **	0.41 ± 0.03* **
9.	CCl_4 + OBT – 14 days	47.39 ± 3.29* **	0.059 ± 0.005***	9.75 ± 0.77* **	0.57 ± 0.05* **
10.	CCl_4 + CM + OBT – 7 days	35.67 ± 1.24******	0.047 ± 0.002******	7.56 ± 0.47******	0.41 ± 0.03* **
11.	CCl_4 + CM + OBT– 14 days	49.23 ± 2.68******	0.061 ± 0.002******	8.63 ± 0.3******	0.97 ± 0.17
12.	CCl_4 + Medicas E + OBT – 7 days	42.16 ± 3.76******	0.051 ± 0.004******	7.14 ± 0.55******	0.53 ± 0.05*
13.	CCl_4 + Medicas E + OBT – 14 days	41.09 ± 2.77******	0.108 ± 0.055***	9.55 ± 1.23******	0.57 ± 0.07******

Note: * - statistically significant with the control group (1); ** - statistically significant with CCl4 + respective preparation (4 and 6).

of CT/GR proportion was seen after 7 and 14 days to 0.053 and 0.054, respectively, compared with the control group (0.23) and experimental groups 2 and 3 (0.15 and 0.2).

In case of co-administration of HBOT and MC for 7 days, the content of catalase in the liver diminished compared with both the control group and the CCL4 experimental group (CCl_4 – 7 days). Combined treatment with MC and HBOT (10 sessions) contributed to an increase of catalase activity compared with the group that received only hyperbaric oxygen. During the concomitant use of Medical E with hyperbaric oxygen for 7 and 14 days catalase activity was at the level of the group treated with HBOT.

Associated treatment of acute toxic hepatitis with MC and Medical E during 1 and 2 weeks contributed to a marked increase of GR in the liver (table 1). In these conditions we determined an essential reduction of CT/GR proportion, which constituted 0.055 in the group subjected to MC + HBOT for 7 sessions, 0.11 – MC + HBOT for 10 sessions, 0.074 – Medicas E + HBOT for 7 sessions and 0.059 – Medicas E + HBOT 10 sessions.

During modeling of acute toxic hepatitis there was a reduction of y – GTP activity in the liver from 0.243 ± 0.07 to 0.197 ± 0.003 (19%, p > 0.05) on the 8th day and to 0.217 ± 0.01 (10.7%, p > 0.05) on the 15th day. Having administered MC for one week, a significant increase in the antioxidant enzymes in the liver was observed, as compared with the CCl4-only experimental group , whereas no significant difference was observed with the control group. At the same time, while using activated charcoal for 14 days, a decrease of gamma-GTP activity was seen as compared with the control animals, from 0.217 ± 0.01 to 0.123 ± 0.02 (43.4%, p < 0.05). Similar effects have been revealed when using Medicas E for 7 and 14 days.

Hyperbaric oxygen therapy (7 and 10 sessions) in rats with acute toxic hepatitis was associated with marked reduction of gamma-GTP activity in the liver. While using concomitantly hyperbaric oxygen and activated charcoal (MC and Medicas E) the activity of the respective enzyme was twice or five times less than in the CCl4 only group and control group. Thus, activated charcoals and oxygen under pressure manifested an effect which is opposite to the hepatotoxic substance at early and late stages, but during the association of the therapeutic agents the effect of the hyperbaric oxygen predominated.

Thus, changes of enzymes of the antioxidant system are dependent on the early or late evolution of the hepatic disease. At early stage activation of processes of lipids peroxidation takes place (increase of DAM and HPL), involving the enzymes of antioxidant system. Later, in untreated animals, there was an initiation of the processes of correction of the oxidative stress marked by a reduction of ROS products and an increase of antioxidant enzyme activity. Carbon tetrachloride (CCl_4), being a lipophilic substance, a relatively non-reactive molecule, can cross the membrane of hepatocytes becoming more toxic after enzymatic activation on the level of the smooth endoplasmatic reticulum. CCl_4 is metabolized in the microsomes where it is reduced by the P450 cytochrome resulting in the formation of free radicals: trichlormethyl (CCL_3) and peroxichlormethyl ($OOCCl_3$), which subsequently attack the lipid structures of the cellular membranes, leading to their peroxidation [4].

Metabolization of CCl_4 depends on the presence or absence of oxygen. During the reduction of concentration or absence of oxygen (hypoxia, including that induced by the hepatoxic substance), the enzymes of P450 cytochrome metabolize the whole quantity of CCl_4 producing CCl_3, which overcomes the antioxidant capacity of the liver, ultimately harming lipid membrane structures.. In anaerobic conditions CCl_4 can also metabolize in an alternative way, forming products of carbyne type, which attach to proteins and nucleic acids. When there is a sufficient supply of molecular oxygen, it

inhibits cytochrome P450 and respectively inhibits formation of CCl_3. Based on other literature, we suppose that CCl_4 exerts a hepatotoxic effect through several mechanisms. One of them is lipid peroxidation. Supporting this is the correlation that exists between the increase of transaminases in the serum and the products of lipid peroxidation in the liver, primarily the increase of malonic dialdehyde. Hepatotoxicity of CCl_4 manifests through formation of free radicals CCl_3, $OOCCl_4$ and Cl that act upon membranous lipids by triggering lipid peroxidation (POL) responsible for physiologic functions of the membranes, bioenergetic processes and finally for the cellular functions.

The therapeutic effect of enterosorption, including that of activated charcoal (MC and Medicas E), in acute toxic hepatitis induced with CCl_4, can be explained by interference with absorption of unsaturated fatty acids (a source of lipoperoxidation), biliary acids and toxins, as well as the intermediate products eliminated through bile in the intestine (CCl_4, CCl_3 etc.), as well as other POL products.

Hyperbaric oxygen, by increasing partial pressure of oxygen and inhibition of P450 cytochrome activity (especially isomorphs 3A4 and 2E1), contributes to diminishing the formation of CCl_3 and its transformation into a less reactive $OOCCl_3$. HBOT, through reduction of tissue hypoxia induced by the hepatotoxic substance, probably diminishes the alternative pathway of CCl_4 metabolization with carbyne formation, reducing subsequent consequences. Simultaneously, oxygen under pressure in curative ways intensifies significantly SOD activity in the liver, thus intensifying enzymatic inactivation of SRO (superoxide anion etc.). This effect of HBOT is synergistic with enterosorption, a phenomenon of enhancement of beneficial effects of the therapeutic agents, leading to accelerated hepatocytes restructuring and functional remodeling.

Conclusions

1. Some insignificant changes of the antioxidant system activity in the liver occur in acute toxic hepatitis induced by CCl_4; the changes manifest through reduction of the level of SOD, catalase, gamma-GTP and an increase of GR.

2. Medicinal charcoal and enterosorbent Medicas E do not influence significantly the enzymes of the antioxidant system while being used for 7 days, but they can contribute to the restoration of the revealed disturbances, especially while being used for 14 days.

3. Enterosorption in association with oxygen-baric therapy reduce y-GTP activity accompanied by reverse changes in activity of SOD, catalase and GR.

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