https://doi.org/10.31925/farmacia.2021.2.9 **ORIGINAL ARTICLE**

BIOCHEMICAL MECHANISMS OF OXIDATIVE STRESS IN ANIMALS EXPOSED TO HEXAVALENT CHROMIUM COMPOUNDS IN THE CASE OF ISONIAZID–RIFAMPICIN HEPATITIS

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Abstract

We evaluated the effects of the combined administration of isoniazid, rifampicin and hexavalent chromium compounds for 7 and 14 days, in rats. Analysing the blood serum, it was observed a more significant accumulation of POL (lipid peroxidation products) at this level than in the liver, which may be a consequence of the damage of hepatocyte membranes and the incidence of toxic factors in blood. The maximum decrease of catalase activity in liver was recorded in the immature rats of the 3rd experimental group till the end of the experiment. The poisoning of rats by potassium dichromate on the background of induced hepatitis resulted in the most significant decrease of the content of reduced glutathione, on the 14th day of the study. The introduction of toxic substances in the animal organisms caused significant infringements and decreased levels of both enzymatic and non-enzymatic antioxidant defence systems, as evidenced by the strong correlation between catalase activity and the content of reduced glutathione. An indirect correlation was observed between the content of TBA-RP and catalase activity in the liver of animals, which were exposed to potassium dichromate (r = –0.86).

Rezumat

Au fost evaluate efectele administrării combinate a izoniazidei, rifampicinei și cromului hexavalent timp de 7, respectiv 14 zile, la șobolani. Analizând serul șobolanilor, s-a observat o acumulare semnificativă a produșilor de peroxidare lipidică la acest nivel comparativ cu ficatul, care poate fi o consecință a deteriorării membranelor hepatocitelor și a incidenței factorilor toxici în sânge. Scăderea maximă a activității catalazei în ficat a fost înregistrată la șobolanii imaturi din grupul 3, până la sfârșitul experimentului. Administrarea de dicromat de potasiu pe fondul hepatitei induse a dus la o scădere semnificativă a conținutului de glutation redus, în a 14-a zi a studiului. Administrarea substanțelor toxice în organismele animale a provocat dezechilibre semnificative și scăderea nivelurilor atât a sistemelor de apărare antioxidantă enzimatică, cât și a celor neenzimatice, dovadă fiind corelația puternică dintre activitatea catalazei și conținutul de glutation redus. S-a observat o corelație inversă între conținutul de TBA-RP și activitatea catalazei în ficatul animalelor care au fost expuse la dicromat de potasiu (r = –0,86).

Keywords: isoniazid, rifampicin, hexavalent chromium, oxidative stress, hepatitis, rats

Introduction

Compounds containing heavy metals are one of the most common chemical factors of professional risk under conditions of modern industry. According to some predictions, heavy metal compounds as a threat to the ecological state of the environment may come to the fore in the future, outpacing nuclear waste and organic anthropogenic pollution in this respect [4, 12]. Some of the toxic compounds of heavy metals include hexavalent chromium (Cr(VI)) compounds [21].

It was established, that the heavy metals' concentrations in wastewater effluents affect the water quality of the aquifer. The water quality and resistance of the aquifer to heavy metals pollution increased with the presence of a renewable recharging of freshwater along with the contamination source [2]. Background levels of Cr in surface water and groundwater aquifers are a direct function of regional geology, mineral weathering processes, sediment loading rates and precipitation patterns [9]. High concentrations of Cr may occur naturally in groundwater in areas with mafic or ultramafic volcanic or metamorphic rocks, being particularly prevalent in ophiolite complexes and serpentine-rich units [7].

Humans can be exposed to Cr, primarily to trivalent (Cr(III)) and hexavalent Cr(VI) forms through its wide distribution in air, soil, ground and drinking water, originating from natural and anthropogenic sources. Once

absorbed, Cr(VI) readily penetrates cell membranes, while Cr(III) does not [4, 7].

Based on the new, high quality data from chronic drinking water carcinogenicity and mode of action studied for Cr(III) and Cr(VI), the risk assessment of Cr(VI) in drinking water considers both cancer (in the case of Cr(VI) and non-cancer (in the case of Cr(III) and Cr(VI)) endpoints. The most serious cases of anthropogenic contamination of drinking water in the U.S. are due to discharges of toxic Cr(VI) by cooling towers [17].

Carcinogenicity of Cr(VI) compounds was established at the end of the XIX century by a survey of the workers of chrome pigments producing plants in Scotland, which revealed a high frequency of the appearance of nasal tumours [14]. In the 30's of XX century in the literature there began to appear reports of cases of lung cancer in workers of the chrome industry in Germany, and in 1936 this disease was recognized as a professional one [18]. Today Cr(VI) is recognized by the International Agency of the Cancer Researches of the European Union as one of six chemical elements (arsenic, beryllium, cadmium, cobalt, nickel and chromium) that reveal a carcinogenic effect on human body [20].

It is known from literature, that the supply of Cr (VI) compounds to the organism of animals and humans, as well as cells cultivated *in vitro*, is accompanied by the reduction of Cr(VI) to Cr(III) [6, 11]. This process leads to the formation of active forms of oxygen (AFO), which are characterized by high reactivity [6, 11, 20]. As a result, the content of antioxidants decreases, which is accompanied by the activation of chain reactions of lipid oxidation and the development of oxidative stress [5].

It is known that increasing of the intensity of AFO formation in cells in certain conditions can lead to the development of oxidative stress [5, 15], which underlies many diseases. Due to the potential threat posed by reactive oxygen metabolites, the protection system from the effect of excess AFO operates in living systems. It includes low molecular weight non-enzymatic antioxidants and antioxidant enzymes [23].

In modern ecological conditions, the combined effect of several xenobiotics is associated with the human body that is connected with the chemicalization of industrial production and life, uncontrolled use of hepatotoxic drugs. About one thousand drugs with a high or negligible potential for hepatotoxicity are known. In the context of aetiology, anti-TB and anti-bacterial agents come to the fore [27], then non-steroidal anti-inflammatory drugs, drugs regulating the functions of nervous system, hormonal, cytostatic, antihypertensive, antiarrhythmic pharmaceutical preparations, etc.

It was established that hepatotoxic effects in cases of combined effect of isoniazid and rifampicin are due to the fact that the drug metabolism occurs predominantly in the liver and is accompanied by significant disruptions of the oxidation reactions [16, 19]. The analysis of literary sources in recent years has shown that the intensification of lipid peroxidation (POL) processes [25] is one of the mechanisms of damage and degradation of liver cells due to the development of systemic oxidative stress, which arises as a result of the imbalance between hyper production of AFO and the lack of antioxidant system (AOS) [22]. It can clearly be argued that for the objective assessment of the damage degree of the hepatobiliary system, the absolute quantity of POL products is not as important as it is the comparison with different links of the AOS defence and other disorders of metabolic homeostasis.

The objective of the study was to examine the development of free radical oxidation processes of biopolymers and to estimate oxidative stress markers in the organism of animals of different age in cases of Cr (VI) compounds affection on the background of isoniazid-rifampicin hepatitis.

Materials and Methods

Laboratory animals and procedures

The experiments were conducted on outbred white male rats of three age groups: the 1st group – immature (3-month-old animals, 90 - 110 g); 2nd group – mature (6-month-old animals, 150 - 170 g) and the 3rd group – senile (18-months-old animals, 280 - 300 g). They were kept on a standard diet at the *vivarium* of Ternopil National Medical University, Ukraine.

The housing and manipulations of animals were carried out according to ethical guidelines [10]. The study was approved by the Institutional Ethics Committee.

Study design

All experimental animals of each age group were divided into four groups: the control group – intact animals (injected with normal saline solution); the 1st experimental group – animals that received potassium dichromate solution; the 2nd experimental group – animals that were administered isoniazid and rifampicin; the 3th experimental group – animals that simultaneously received potassium dichromate, isoniazid and rifampicin. Each study group comprised 6 animals.

Chemicals, reagents and drugs

The experimental toxic model was simulated by the combined effect of isoniazid, rifampicin and hexavalent chromium compounds. Isoniazid and rifampicin were administered intragastrically in aqueous solution to animals daily, 0.05 g/kg bw and 0.25 g/kg bw accordingly, for 7 and 14 days. Hexavalent chromium compounds were administered to animals in a similar manner using a solution of potassium dichromate, 3 mg/kg bw. Euthanasia was performed by means of thiopental sodium (25 mg/kg bw) on the 7th and 14th day from the first day of xenobiotics administration.

Biochemical laboratory analyses

The study of liver homogenate and blood serum was performed. The blood was taken from the heart of animals and subjected to centrifugation at 3000 rpm for 30 min. The obtained blood serum (a sedimentary liquid) was used for further investigations. Selected liver (250 mg) was used to obtain the homogenate by the method of differential homogenization; it was used after previous perfusion in physiological solution.

The state of the antioxidant system was evaluated by change of oxidative processes in the organism of animals – by determining the content of TBA (thio-barbituric acid)-reactive products (TBA-RP) [24] and the antioxidant system indexes: by determining the catalase activity [13] and the content of reduced glutathione (G-SH) [3].

The content of TBA-RP was calculated based on the molar extinction coefficient of the coloured complex (malonic dialdehyde forms a red colour complex with thiobarbituric acid), which is equal to 1.56×10^5 $cm^{-1} \bullet mol^{-1}$. The catalase activity was determined by the ability of hydrogen peroxide to form a stable coloured yellow complex with ammonium molybdate. The content of reduced glutathione was determined by the interaction of 5,5-dithiobis-(2-nitrobenzoic) acid (Ellman's reagent) with free SH - groups of the reduced glutathione with formation of a yellow colour of thionitrophenyl anion, the amount of which is directly proportional to the content of SH - groups. The content of G-SH was calculated based on the molar extinction coefficient for the thionitrophenyl anion, which is 1400 $mol^{-1} \bullet cm^{-1}$.

Statistical analysis

The processing of statistical data was carried out using the SPSS-22 software package. The distribution of data was analysed according to Kolmogorov-Smirnov's criterion of normality. The obtained values had a nonparametric distribution, so the difference between the groups was analysed according to the Student's t-criterion and the non-parametric Wilcoxon's criterion for the connected samples. The criterion $\chi 2$ was used to evaluate the difference between categorical data. Differences were considered statistical significant at $p < 0.05$.

Results and Discussion

It is known that the emergence of pathological processes in an organism, that are caused by different endogenous and exogenous factors, leads to the development of oxidative stress as a result of excessive formation of the AFO, that is accompanied by disturbance of the balance in the system of "oxidants-antioxidants" and even functional changes in the system of antioxidant protection [5]. The content of AFO in cells increases in expressed prolonged stress, especially with the development of pathological processes, and its excess inhibits the ability of protective systems of cells, which can lead to their death. One of the manifestations of a toxic effect of oxygen metabolites is the intensification of free radical oxidation reactions. The intensification of free radical oxidation processes under the action of AFO leads to increased POL, oxidative modification of proteins, denaturation of nucleic acids, carbo-hydrates, that causes structural and metabolic disorders in cells [8].

TBA-RPs are formed in the process of lipid per-oxidation and reflect the intensity of this process in the affected organism. Results considering their concentration are given in Table I.

Table I

The content of TBA-RP in blood serum (μmol/L) and liver homogenate (μmol/kg) of rats exposed to isoniazid, rifampicin and Cr (VI) compounds (M ± S.D.)

Research material	Group of animals	Age group of animals					
		immature		mature		senior	
		Study duration, days					
		7th	14th	7th	14th	7th	14th
Blood serum	Control group	0.89 ± 0.10		0.74 ± 0.05		0.62 ± 0.02	
	1st group	2.03 ± 0.19*	2.28 ± 0.15*	2.85 ± 0.14*	2.03 ± 0.19*	2.28 ± 0.15*	2.85 ± 0.14*
	2nd group	3.27 ± 0.19*	3.41 ± 0.18*	2.76 ± 0.15*	3.27 ± 0.19*	3.41 ± 0.18*	2.76 ± 0.15*
	3rd group	5.31 ± 0.32*	5.48 ± 0.48*	3.44 ± 0.09*	5.31 ± 0.32*	5.48 ± 0.48*	3.44 ± 0.09*
Liver	Control group	16.66 ± 2.25		5.88 ± 0.45		6.94 ± 0.20	
	1st group	27.46 ± 1.44*	29.06 ± 1.35*	12.61 ± 0.57*	27.46 ± 1.44*	29.06 ± 1.35*	12.61 ± 0.57*
	2nd group	37.50 ± 1.77*	41.88 ± 2.14*	12.39 ± 0.46*	37.50 ± 1.77*	41.88 ± 2.14*	12.39 ± 0.46*
	3rd group	53.20 ± 2.82*	58.08 ± 2.78*	15.71 ± 0.72*	53.20 ± 2.82*	58.08 ± 2.78*	15.71 ± 0.72*

* – significant differences between the control group and the animals exposed to toxic substances, $p \leq 0.05$; S.D. – standard deviation

The content of TBA-RP in blood serum increased by 156% in the immature animals of the 1st experimental group, by 311% – in the mature animals and by 490% – in the senior animals in comparison with the control ($p < 0.05$). Such a tendency in increase of the content of lipid peroxidation products can be explained by the fact that potassium dichromate solution causes the formation of the AFO in the organism. The latter interact with cells biopolymers, taking part in the reactions of POL, and stimulate the processes of free radical oxidation of biomolecules.

The similar increase of the content of TBA-RP in blood serum of all age groups was established in the organisms of rats of the 2nd experimental group. The

most susceptible to these toxic substances were immature rats; the content of TBA-RP in blood serum exceeded the level of the intact control ($p < 0.05$) by 283% till the end of the experiment. The combined action of the above-mentioned xenobiotics (the 3rd experimental group) had led to an even greater increase of the TBA-RP content already on the 7th day of the experiment: 597% in animals of immature age, in animals of mature age – 465%, and in animals of senior age – 581% (Table I).

In the liver of intoxicated animals there was observed a similar pattern, registering an increase of the content of lipid peroxidation products (Table I). On the 7th day of the experiment in the liver of the immature animals of the 1st experimental group the content of TBA-RP increased by 65%; in animals of the 2nd experimental group – by 114% and in animals of the 3rd experimental group – by 143% in comparison with the rats of the control group ($p < 0.05$). Obviously, the seventh day is a toxicogenic phase of the action of xenobiotics, since it is known that in the influence of the salts of heavy metals, the activation of POL processes occurs most precisely in the first days after the affection.

We recorded the highest content of TBA-RP on the 14th day in all experimental age groups of animals: 345% in the immature rats, in the mature rats – 298% and in the senior rats – 364% (Table I). This, in our opinion, is related to the combined effect of toxic substances on the organism, which activates the processes of free radical oxidation, lipid peroxidation, in particular, and creates an additive effect on the organism.

In stress and pathological conditions, there is a shifting in the antioxidant defence processes, in particular the change of the activity of enzymes and the amount of non-enzymatic natural compounds, which decompose hydrogen peroxide and block the formation of an aggressive hydroxyl radical. The hydrogen peroxide that is formed by the dissonation of the superoxide anion is decomposed by catalase. The active centre of the enzyme includes trivalent iron ion and proto-porphyrin, which interacts with hydrogen peroxide according to catalase or peroxidase mechanism, depending on the concentration of substrate [28].

Table II

Catalase activity in blood serum (μcat/L) and liver homogenate (μcat/kg) in rats exposed to isoniazid, rifampicin and Cr (VI) administration (M ± S.D.)

Research material	Group of animals	Age group of animals					
		immature		mature		senior	
		Study duration, days					
		7th	14th	7th	14th	7th	14th
Blood serum	Control group	11.70 ± 0.67		21.16 ± 0.28		27.06 ± 1.19	
	1st group	9.12 ± 0.51*	8.72 ± 0.55*	15.70 ± 0.26*	15.27 ± 0.21*	23.71 ± 0.33*	20.07 ± 0.23*
	2nd group	9.21 ± 0.83*	8.30 ± 0.83*	14.27 ± 0.44*	12.70 ± 0.28*	23.78 ± 0.29*	20.14 ± 0.41*
	3rd group	8.86 ± 0.72*	7.77 ± 0.48*	11.50 ± 0.28*	10.12 ± 0.34*	17.69 ± 0.39*	15.21 ± 0.56*
Liver homogenate	Control group	10.55 ± 0.88		14.23 ± 0.33		15.38 ± 0.60	
	1st group	3.44 ± 0.44*	3.20 ± 0.27*	9.39 ± 0.36*	5.93 ± 0.53*	10.57 ± 0.55*	7.35 ± 0.22*
	2nd group	5.19 ± 0.29*	4.86 ± 0.35*	8.99 ± 0.53*	6.70 ± 0.34*	10.15 ± 0.38*	7.30 ± 0.25*
	3rd group	3.20 ± 0.32*	2.66 ± 0.49*	8.66 ± 0.28*	5.77 ± 0.46*	8.08 ± 0.31*	6.15 ± 0.24*

* – significant differences between the control group and the animals exposed to toxic substances, $p \leq 0.05$; S.D. – standard deviation

It was assessed the catalase activity in blood serum and liver homogenate of animals after their affection by hexavalent chromium compounds, isoniazid and rifampicin. From the data presented in Table II, it results that the catalase activity in the blood serum of the 2nd experimental group (7th day) was decreased by 21% in the immature animals, by 33% – in the mature animals and by 12% – in the senior animals compared with the animals of the intact control group ($p < 0.05$). Reducing the activity of the enzyme in the blood serum of animals in this experimental group leads to the accumulation of H_2O_2, destruction or modification of biological molecules, and, as a consequence, the collapse of cell structures that causes the death of cells.

In the 3rd experimental group of animals it was registered a decrease of the catalase activity in both types of samples, in all age groups (Table II).

The maximum decrease of the catalase activity in blood serum was registered in mature rats (the 3rd experimental group) on the 14th day of the research by 52% compared with animals of the control ($p < 0.05$), which testifies the low level of neutralization of toxic products by the antioxidant system of the mature organism. Along with the catalase activity assessment, we determined the content of reduced glutathione in the organism of animals, which is a structural component of the glutathione antioxidant system.

The main function of glutathione is its participation in the detoxification of xenobiotics. The concentration of free SH-groups and SH-groups of protein and non-protein origin decreases by the affection of toxic substances.

The content of G-SH decreased by 25% in the blood serum in the 1st experimental group of immature animals (Table III) after 7 days, it was lower by 29% in comparison with its indicator at the end of the experiment in the animals of the control group ($p < 0.05$). A similar tendency was observed in the case of

rats exposed to anti-TB drugs and by the combined action of toxic substances. The critical decrease of G-SH content in blood serum may be due to its exhaustion, in the first days of toxic occurrence in the organism.

Table III

The content of G-SH in blood serum (mmol/L) and liver homogenate (mmol/g) in rats exposed to isoniazid, rifampicin and Cr (VI) administration (M ± S.D.)

Research material	Group of animals	Age group of animals					
		immature		mature		senior	
		Study duration, days					
		7th	14th	7th	14th	7th	14th
Blood serum	Control group	3.67 ± 0.17		3.40 ± 0.06		6.60 ± 0.15	
	1st group	2.74 ± 0.12*	2.60 ± 0.14*	2.53 ± 0.11*	2.39 ± 0.11*	4.88 ± 0.16*	4.64 ± 0.17*
	2nd group	2.70 ± 0.05*	2.55 ± 0.22*	2.26 ± 0.11*	1.88 ± 0.09*	4.39 ± 0.09*	4.26 ± 0.12*
	3rd group	2.43 ± 0.13*	2.36 ± 0.10*	2.17 ± 0.10*	1.56 ± 0.09*	4.35 ± 0.11*	3.91 ± 0.09*
Liver homogenate	Control group	2.78 ± 0.12		3.27 ± 0.10		5.51 ± 0.11	
	1st group	2.39 ± 0.11*	2.09 ± 0.10*	2.22 ± 0.09*	1.67 ± 0.10*	4.33 ± 0.12*	3.95 ± 0.16*
	2nd group	1.75 ± 0.10*	1.48 ± 0.11*	1.52 ± 0.10*	1.24 ± 0.12*	3.88 ± 0.13*	3.67 ± 0.16*
	3rd group	1.50 ± 0.10*	1.37 ± 0.11*	1.33 ± 0.09*	1.14 ± 0.10*	3.80 ± 0.14*	3.42 ± 0.07*

* – significant differences between the control group and the animals exposed to toxic substances, $p \leq 0.05$; S.D. – standard deviation

The content of G-SH in animals of the 3rd experimental group decreased by 34% on the 7th day of the experiment in the immature and senior animals, and 36% – in the mature animals from the level of the control animals ($p < 0.05$). The lowest content of G-SH in blood serum was observed on the 14th day of the research in the mature age animals of the 3rd experimental group (Table III).

In the liver of the 2nd experimental group, this indicator decreased in the immature rats by 37% on the 7th day of the experiment and by 47% on the 14th day in comparison with the control group ($p < 0.05$), in the mature – by 54% and 62 %, in the seniors – by 30% and 33%, respectively (Table III).

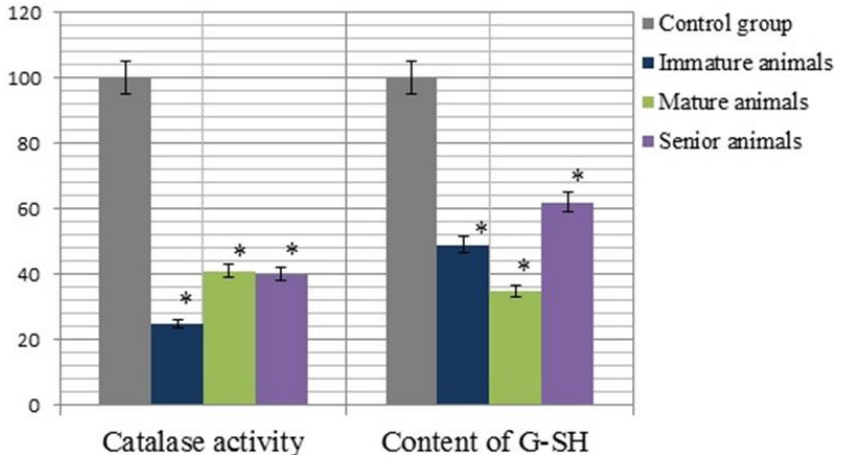

Figure 1.

G-SH content and catalase activity in the liver of rats exposed to isoniazid, rifampicin and Cr (VI) compounds (14th days), %;

* – significant differences between the control group and the animals exposed to toxicants, $p \leq 0.05$

The toxic effect of the tested compounds (isoniazid, rifampicin and Cr (VI) compounds) is associated with their hepatotoxicity that led to a decrease in the content of G-SH and catalase activity in the liver of all experimental age groups of animals (Figure 1). The maximum enzyme activity decreased by 75% in liver of the immature animals in comparison with the control group ($p < 0.05$).The maximum decrease of the content of G-SH was recorded in the senior animals on the 14th day of the research: 62% in comparison with the control group ($p < 0.05$) (Figure 1).

From the obtained results we can assume that the glutathione system is much faster incorporated in protecting the organism from free radicals. It becomes clearer since its functioning leads to a breakdown of the chain of free radicals' reactions at an earlier stage and its components are synthesized in the liver.

Toxic products that were formed when xenobiotics reached the organism of the rats caused the activation

of free radical oxidation with the participation of AFO, and, as a result, the increase of the POL and the decrease of the protective and compensatory forces of the organism. Based on the above, we made the correlation analysis between the content of TBA-RP and the AOS indicators (Table IV).

It was established that the strongest correlation connection (inverse) exists between the content of TBA-RP and the catalase activity in the liver of animals in the first experimental group (r = -0.86). This suggests that lipid peroxidation products are accumulated in the liver and decrease the synthesis of the catalase. It is obvious that AFO, which are formed by enzymatic pathways in the affected animal organism, lead to the initiation of free radical oxidation processes. A direct correlation between the catalase activity and the content of reduced glutathione was observed (Table IV).

Table IV

Correlation analysis between the content of TBA-RP, catalase activity and the content of G-SH in the serum and at the hepatic level of rats exposed to isoniazid, rifampicin and Cr (VI) administration

Research material	Experimental groups	Correlation coefficient, r_{xy}		
		Pairs of correlation relationships		
		Content of TBA-RP/ Catalase activity	Content of TBA-RP/ Content of G-SH	Catalase activity/ Content of G-SH
Blood serum	1	+0.40	+0.30	+0.81
	2	-0.23	-0.1	+0.82
	3	-0.27	-0.13	+0.82
Liver homogenate	1	-0.86	-0.18	+0.59
	2	-0.30	-0.16	+0.56
	3	-0.65	-0.13	+0.41

In the blood serum of animals of the 3rd experimental group of mature age, the content of TBA-RP on the 14th day of the study was the smallest in comparison with the immature and senior rats (Table I). It testifies the least pronounced development of the oxidative processes in the organism of the mature animals and less permeability of the cell membranes, the integrity disruption may lead to activation of phospholipases and oxygenases. The increase of their activity leads to the formation of a significant amount of free radicals [26] that provoke disturbances in the system of POL and affect the nature of flow of membrane-destructive processes in cells.

It should be noted that in the blood serum of the affected animals there is a more significant accumulation of lipoperoxidation products than in the liver that may be a consequence of the damaging the hepatocyte membranes by potassium bichromate and tuberculostatics, and as a consequence – the incidence of toxic factors in the blood. Probably, the reduction of hexavalent chromium in the rats' organisms caused a significant increase in the content of AFO, which have a destructive effect on the hepatocyte membranes and start up the cascade of oxidative processes in the organism.

The results of our study have shown that the affection of animals by isoniazid and rifampicin is accompanied by a profound modification of the enzymatic level of AOS. The activity of one of the main antioxidant enzymes, the catalase, decreases on the 7th day of the research in the blood serum of the 2nd experimental group of rats. The maximum decrease of the catalase activity in blood serum was recorded in the mature rats of the 3rd experimental group till the end of the experiment (Table II). The toxic effects of our pollutants are related to their hepatotoxicity that led to the decrease of the catalase activity in the animals' liver in all age groups (Table II). By chemical toxicity, the protein synthesis functions are dramatically affected.

We investigated the activity of one of the components of the non-enzymatic part of the antioxidant protection system – glutathione. The concentration of G-SH decreased in animals of the 1st experimental group, both in the blood serum and in the liver (Table III). Obviously, under the action of the oxidizer Cr (III) (the reduced form of Cr (VI)) the G-SH oxidizes with the formation of a disulphide bond, thereby fulfilling the function of antioxidant. This is confirmed by literary data [1, 2], as the cytotoxicity of Cr is due to three interrelated mechanisms: the intensification of POL as a result of the decrease of antioxidant protection of the cell, and as a result of direct prooxidant activity of some metals; the inhibition of mitochondrial respiration because of the changing of the membrane potential of mitochondria and contravention of the activity of the respiratory chain enzymes and the Krebs cycle; the imbalance of calcium homeostasis of the cell due to the change in the intracellular flow of calcium, the replacement of calcium on specific receptors with subsequent activation of calcium-dependent enzymes. The tuberculostatics administration and the subsequent combined exposure to potassium dichromate led to the most significant decrease of the content of G-SH in the test material of the 3rd experimental group of animals till the end of the experiment (Figure 1). The decrease of the content of G-SH is associated with the decrease of the activity of the glutathione reductase.

The administration of toxic compounds caused an imbalance between the AFO and the indicators of the antioxidant protection system in the direction of increasing the reactive oxygen level and decreasing

the content of antioxidants. It is confirmed by the strong inverse correlation interaction between the catalase activity and the content of G-SH in the blood serum and the liver of rats of all age groups (Table IV).

Conclusions

The novelty of the study is the exposure of animals to potassium dichromate and anti-tuberculosis drugs, followed by the activation of lipids radical oxidation in blood serum and liver of animals, evidenced by the increase of the TBA-RP content. The most pronounced metabolic disorders in the cases of chrome-isoniazid-rifampicin exposure were evidenced in the immature and senile group of animals in comparison with the mature animals. This indicates the alteration of the liver protein synthesis function and the degradation of proteins. The imbalance in the AOS functioning and the oxidative stress development is confirmed by the correlation between the TBA-RP content, catalase activity and the G-SH content. Considering this model as fit for humans with anti-TB hepatitis, in case of environmental pollution, it might be possible to assess the severity of chemical toxicity.

Conflict of interest

The authors declare no conflict of interest.

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