

Variability of Toxic Action of Carbon Tetrachloride in Rats of Different Ages

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Abstract – Age-specific changes define the difference in thresholds of sensitivity to the effect of poison and drug substances. The disclosure of features of mechanisms forming the basis of an observed phenomenon will allow increasing the efficiency of health risks management under the negative influence of chemical factors. The purpose of the study was to identify the hepatotoxic action of carbon tetrachloride in rats of different ages. The *Wistar* rats of two different age groups – 3 and 18 months divided into experiment and control were the objects of the study. The methods of study included clinicodiagnostic analysis of key biochemical indicators: bilirubin, cholesterol, alkaline phosphatase (ALP), ALAT, ASAT, hydroperoxides and malondialdehyde. The obtained results illustrate more expressed toxic xenobiotic impact on the organism of older rats. It was shown by relative (experiment/control) increase of hepatospecific enzymes activity in plasma: $ALP_{\text{young}} = 1.5$ times, $ALP_{\text{elderly}} = 2.4$ times, $ALAT_{\text{young}} = 5.9$ times, $ALAT_{\text{elderly}} = 10.5$; $ASAT_{\text{young}} = 3.5$, $ASAT_{\text{elderly}} = 4.9$ (the different in other parameters was not so obvious). The regularities revealed during the study contradict literary data on age decrease of activity of human and animal cytochromes ensuring metabolic activation of chemicals, including carbon tetrachloride. Thus, the strengthening of toxic impact of carbon tetrachloride on older animals may be caused by weak hepatic blood flow, reduced oxygenation of tissues and hence, longer contact of hepatocytes with high-reactive metabolites.

Keywords – age changes, mechanisms of hepatotoxic action, acute toxic hepatitis.

I. INTRODUCTION

In the conditions of global aging of the population the study of features of age reactivity of an organism to the effect of poison and drug substances is quite relevant. Scientific literature sources show various examples studying the

decrease of the activity of cytochromes with age [1–3]. This circumstance shall define the increase of thresholds of resistance to the toxic effect of chemicals, which contradicts clinical data confirming multiple cases of senile idiosyncrasy [4, 5].

Carbon tetrachloride – induced acute toxic hepatitis is a convenient model to study the hepatotoxic action of xenobiotics [6, 7]. The hygienic interest to this substance is caused by the fact that the modern literature describes clinical cases of poisoning with carbon tetrachloride when people deal with it in life and production [8]. The main links of pathogenesis at CCl_4 intoxication include the stage of molecule restoration on a microsomal device of hepatocytes thus forming high-reactive trichloromethyl radicals – initiators of lipid peroxidation [9, 10]. It is also known that free radical oxidation of hepatocyte biostructures is the universal mechanism of liver damage [11–13].

The **purpose of the study** is to analyze the hepatotrophic effect of carbon tetrachloride at rats of different age.

II. METHODS AND MATERIALS

The study covered 32 *Wistar* male rats of two age groups: 3 and 18 months, which, in turn, were divided into control and experiment. The animals of the control group were given solvents according to the scheme and volume similar to experiment animals. To form the model of acute toxic hepatitis the experiment rats were given *per os* 25 % oil solution of carbon tetrachloride within 4 days (once a day) in the amount of 0.2 ml/100 g of weight at 11:00. Water – *ad libitum*, feeding – at 13:00. In 24 hours after the last primer the animals were put to sleep (general anesthesia by intraperitoneal injection of Zoletil) and 3 ml of blood was

taken from their hearts. Using standard kits (LaChema, Vital Diagnostics) direct and total bilirubin was defined in blood plasma via Malloy-Evelyn method, cholesterol – via kinetic method with peroxidase, alkaline phosphatase – by the reaction of p-nitrophenol formation, alanin- and aspartate transaminase – by Reitman-Frankel method. Hydroperoxides were defined by Agat-Med kits, malondialdehyde in erythrocytes – by the reaction with thiobarbituric acid after preliminary sedimentation of proteins with trichloroacetic acid.

The animals were kept in the conditions of natural light mode and standard diet of a vivarium. The principles of work corresponded to the rules described in the National Research Council. The experimental study was approved at the meeting of the Committee on Ethics of the Institute of Biomedical Investigations (protocol No. 3 of 2019.01.17). The subsequent euthanasia of rats was performed in the exposure chamber via inhalation of 80 % carbon dioxide in oxygen.

The method of variation statistics of medicobiologic profile according to Student's t-test for paired observations was used to assess the statistical importance of average differences in cases of two samplings [14]. This approach was chosen due to even distribution of indicators (confirmed by Shapiro-Wilk test) in groups and the equality of general dispersions. Indicators of control and experimental tests were compared. The average arithmetic indicator (M) and standard error of the mean ($\pm m$) were calculated. The critical significance value when checking statistical hypotheses was

accepted smaller or equal 0.05. Statistically significant excess of the selective average indicator in the experiment in comparison with the selective average of the control sample served the evidence of pathogenetic effects created under the influence of carbon tetrachloride.

III. RESULTS

The introduction of hepatotropic poison to animals of different age is followed by reciprocal toxic reactions, which is shown in Table 1.

The analysis of key biochemical parameters reveals reliable pathobiocemical changes at rats of experimental groups in comparison with the control group. This is expressed by 75 % increase of bilirubin concentration in blood, which content still remained within the norm determined by the top level of 17 $\mu\text{mol/l}$ [15]. The indicators of liver enzymes tests were also authentically and considerably changed. The activity of alkaline phosphatase increased up to 2.4 times, which against the background of high bilirubin level detects the inflammatory process in hepatobiliary tract accompanied by bile outflow disorder. High ALAT and ASAT indicators in blood plasma of experimental animals demonstrate the disruption of permeability of cell membranes of hepatocytes caused by the action of hepatotoxicants. The acute pathological process may also be confirmed by low AST/ALT ratio, which is much lower in the groups of animals than the norm defined by the range of 1.0–1.3.

TABLE I. HEPATOTOXIC EFFECT OF CARBON TETRACHLORIDE ON RATS OF DIFFERENT AGE

No.	Parameter	Group of animals	Rats 3 months	Rats 18 months	<i>p</i> Sid.		
					<i>p</i> 1*	<i>p</i> 2**	<i>p</i> 3***
1	Direct bilirubin, $\mu\text{mol/L}$	Control	1.5 \pm 0.52	1.3 \pm 0.45	0.031	0.941	0.012
		Experiment	2.6 \pm 0.14	1.3 \pm 0.32			
2	Total bilirubin, $\mu\text{mol/L}$	Control	2.4 \pm 0.67	1.7 \pm 0.28	0.007	0.633	0.044
		Experiment	4.2 \pm 0.45	2.0 \pm 0.52			
3	Cholesterol, mmol/L	Control	1.8 \pm 0.04	1.5 \pm 0.06	0.821	0.007	0.000
		Experiment	1.8 \pm 0.09	1.1 \pm 0.07			
4	Alkaline phosphatase, IU/L	Control	661 \pm 86	278 \pm 20	0.010	0.008	0.093
		Experiment	976 \pm 107	678 \pm 109			
5	ALAT, $\mu\text{kat/L}$	Control	0.24 \pm 0.01	0.24 \pm 0.01	0.001	0.000	0.035
		Experiment	1.42 \pm 0.24	2.51 \pm 0.32			
6	ASAT, $\mu\text{kat/L}$	Control	0.26 \pm 0.01	0.25 \pm 0.02	0.001	0.002	0.212
		Experiment	0.90 \pm 0.13	1.22 \pm 0.22			
7	De Ritis ratio	Control	0.99 \pm 0.04	1.12 \pm 0.14	0.000	0.001	0.009
		Experiment	0.69 \pm 0.06	0.65 \pm 0.07			
8	Hydroperoxide, $\mu\text{mol/L}$	Control	5.14 \pm 0.31	3.39 \pm 0.06	0.118	0.113	0.000
		Experiment	4.59 \pm 0.12	3.16 \pm 0.12			
9	Malonic dialdehyde, $\mu\text{mol/L}$	Control	41.7 \pm 1.86	46.18 \pm 0.96	0.962	0.008	0.080
		Experiment	41.8 \pm 0.96	38.29 \pm 1.50			

* – Control/Experiment 3 months; ** – Control/Experiment 18 months; *** – Control/Experiment.

If to compare the indicators of two experimental groups, then it is obvious that the toxic effect of carbon tetrachloride at older animals is stronger. It was expressed by the relative increase of enzymatic activity of hepatospecific enzymes in blood plasma thus confirming the reduction of barrier functions of cell membranes. This is caused by the activation of lipid peroxidation under the influence of carbon tetrachloride metabolites. Thus, the activity of alkaline

phosphatase at young rats increased 1.5 times (experiment/control), and at old rats – 2.4 times. Similar changes are typical for aminotransferases: ALAT_{young} – 5.9 times, ALAT_{elderly} – 10.5 (the differences between the compared groups are reliable); ASAT_{young} – 3.5, ASAT_{elderly} – 4.9. The reduction of hydroperoxides and malondialdehyde content in blood revealing weak lipid peroxidation under the influence of xenobiotic was typical for old rats.

IV. DISCUSSION

Statistically significant differences of biochemical blood indicators between rats from two experimental groups allow revealing the age features of toxic damage of an organism with chemicals. The conversion of carbon tetrachloride into trichloromethyl radicals at young rats is more complete since, according to literary data, the activity of metabolic enzymes – cytochromes is higher than that at old rats [16, 17]. This is confirmed by high activity of hepatospecific enzymes in blood plasma – alkaline phosphatase, ALAT and ASAT (Table 1). The increase of bilirubin in blood at young rats can demonstrate compensatory reactions in the conditions of induced pathology since the pigments formed through hemoglobin disintegration have expressed antioxidant properties and are able to stop free-radical oxidation of membranes in lipids [18].

Nevertheless, the reduced activity of metabolic systems at old rats does not reduce the risk of intoxication. In comparison with the experimental group of young animals the indicators of liver enzymes test of old animals pathologically deviate by 40-78 %. The identified negative shifts at old rats proceed against the background of natural decrease in susceptibility of membrane lipids to the action of active forms of oxygen, which is confirmed by low markers of lipid peroxidation – hydroperoxides and malondialdehyde (Table 1) [19]. The revealed differences between two groups of young and old animals demonstrate the physiological features of a body changing the toxic effect on liver structures. First of all, they can be based on reduced clearance due to the general reduction of hepatic blood flow causing insufficient oxygenation of hepatocytes and longer contact of cells with high-reactive metabolites [20].

V. CONCLUSION

Experimentally studied features of hepatotoxic effect of the model poison demonstrate high risk of intoxication with age thus defining the need for individual selection of effective doses of medical drugs for elderly population and the need for the means of prevention of adverse health effects.

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