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THE INFLUENCE OF EXOGENOUS XENOBIOTICS ON ADSORPTION OF IRON IONS AND HEMATOPOIESIS

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Abstract

An acute hepatotoxic liver injury by administering a single peritoneal bolus injection of thioacetamide causes an imbalance between the synthesis and degradation of proteins in the extracellular matrix and is a trigger of fibrogenesis. In the scientific publications, the intoxication-triggered changes are described using the changes with time in proinflammatory and profibrotic cytokines, without considering hematological indices.

The aim of the study was to establish the nature of morphometric changes in hematological parameters caused by a prolonged toxicity of thioacetamide and ethanol.

The results of the study. It has been noted that changes in RBC indices in the group of test animals consisted in an insignificant reduction in absolute counts of red blood cells and mean corpuscular volume while maintaining the homogeneity of the population, as suggested by slight fluctuations of such indices as RDW-SV, which in the test group increased significantly ($p<0.05$) by 4.38%, and RDV-CV, which decreased by 0.71%. Activation of megakaryocytic cells was detected, as evidenced by a significant ($p<0.05$) 28.21% increase in immature platelet counts (P-LCC) in the group of test animals and a 22.54% increase in large platelets P-LCR fraction compared with the finding in the group of intact animals. The hepatotoxic effect of thioacetamide, a hydrophilic xenobiotic, was shown to cause changes in iron adsorption, which consisted in a significant ($p<0.05$) 28.07% ferritin increase in the test group against the background of a 17.68% reduction in plasma levels of Fe^{+3} ions in the animals compared to controls. The reduction in Fe^{+3} stores in plasma as a result of its deposition as ferritin is leading to a reduction in volume fraction of RBCs in whole blood, as suggested by a 12.23% hematocrit reduction in the group of test animals. Prolonged intoxication by exogenous xenobiotics for 21 days caused the development of inflammation, as suggested by the increased concentration of C-reactive protein (up to 1.67 mg/dl*10^{-2}) and corresponding to its significant increase by 23.35% compared to controls. The development of the inflammation involved the population of neutrophils and lymphocytes, the absolute counts of which increased significantly in the test group ($p<0.05$): by 58.73% and by 46.28%, respectively.

Keywords: *thioacetamide, xenobiotics, hepatotoxicity, RBC indices.*

Introduction

The liver is the main detoxifying organ to perform biological transformation of exogenous xenobiotics, which underlies the development of a number of models of hepatotoxic injury [1, 2]. The scientific literature provides a sufficient evidence base for the hepatotoxicity of thioacetamide as increases in proinflammatory, profibrotic and hepatic cytokines [9, 10, 11, 19]. The negative effects of ethanol in the body consist not only in adverse remodeling of hepatic parenchyma (the liver being the principal organ of ethanol metabolism), but also in development of extrahepatic syntropies and complications, due to the lack of tissue specificity of the ethanol molecule and its relatively straightforward penetration into the cells by simple diffusion along the concentration gradient [3, 4].

Scientists have found thioacetamide to be a trigger of fibrogenesis in hepatic parenchyma, as suggested by an increase in levels of transcription of transforming growth factor β, connective tissue growth factor, platelet-derived growth factor receptor β, tumor necrosis factor, interleukin-6 and by intensive deposition of type I collagen due to activation of fibroblast-type hepatic stellate cells [12, 15].

However, in the case of both thioacetamide and ethanol, the research into morphofunctional changes, as well as into the compensation and adaptive responses with involvement of the blood system are little investigated areas, which at the same time are essential for understanding the mechanisms behind the development of disorders. Since polyfunctional blood cells interact closely with the majority of tissues and organs, assessment of their statuses and levels of resistance to such toxic substances as thioacetamide and exogenous ethanol is an important source of information [5].

The most adverse effect of alcohol intoxication includes structural and functional changes in erythrocytic membranes, which consist in reduced fluidity of erythrocytic membranes due to increased cholesterol content and formation of protein-protein cross-links, which reduce the plasticity and increase the rigidity of membranes [16, 20]. The previously mentioned changes in the membranes trigger hemolysis of red blood cells; as a result, hemoglobin breaks down into the protein component and the iron-containing porphyrin component, i.e. the heme. Since the heme, owing to its chemical properties and its structure, may participate in oxidative processes and act as a prooxidant and an antioxidant at the same time, this phenomenon sparks active interest in the biochemical research community.

The scientific novelty and the relevance of this study is attributable to the fact that from a biochemical point of view, acetaldehyde (the principal toxic intermediate of ethanol) is a highly active alkylating agent, which causes inhibition of hematopoiesis. However, exact knowledge of the mechanism behind such inhibition is currently lacking [21].

Alcohol abuse leads to impaired hematological indices already at the bone marrow level. In a setting of chronic alcohol toxicity, proerythroblasts show evidence of vacuolization, which results in reduced production of fully functional cells [13]. Recent data suggest that acetaldehyde may also be metabolized in hematopoietic stem cells, and the loss of one of the aldehyde dehydrogenase-1 isoforms by these cells is leading to disruption of the cellular processes. Thus, Smith C. emphasized that different cytopenias (leukopenia, anemia and thrombocytopenia) and the potentially increased risk for myelodysplasia and acute leukemia in persons who abuse alcohol are caused by polymorphism in the genes responsible for the metabolism of reaction-capable aldehydes resulting from ethanol metabolism and for the reduction of their DNA adducts in hematopoietic stem cells and in other hematopoietic cells [17].

As an exogenous xenobiotic, ethanol has a negative impact on carbohydrate metabolism in red blood cells. Despite their lack of certain metabolic systems, red blood cells are not just intact protoplasmic elements, since an active glucose metabolism is taking place in those blood cells [6]. A noteworthy detail is that prolonged exogenous ethanol toxicity causes disruptions of carbohydrate and energy metabolism in red blood cells and consists in inhibition of activity of the enzymes responsible for glycolysis, pentose phosphate shunt and Krebs cycle [8, 10, 18].

Therefore, the analysis of literature sources suggests that the syntropic effects of thioacetamide and ethanol on the mechanism behind inhibition of

hematopoiesis is a little-explored area, which is why it has become our area of interest.

The aim of the study was to establish the nature of morphometric changes in hematological parameters and the changes of iron ions metabolism in a setting of prolonged toxicity of thioacetamide and ethanol.

Methods

The hepatotoxic liver injury caused by a double effect of two exogenous xenobiotics (thioacetamide and ethanol) over 21 days, which included the following administration schedule:

- 4 consecutive intraperitoneal injections of thioacetamide at the dose of 250 mg/kg of live weight of animals at four days intervals;
- intra-gastric administration of ethanol at the dose of 3 g/kg at one day intervals.

The study was performed in 20 Wistar rats, which have been divided into two groups: the intact group and the test group. In order to assess the changes in hematological indices, whole blood was taken from the cardiac cavity via cardiotomy with subsequent testing on a Horiba Yumizen H_{500} analyzer. Plasma concentrations of C-reactive protein were determined on an automatic biochemical analyzer Accent 200 using an antigen-antibody agglutination reaction determined by the absorption level, which is directly proportional to the amount of C-reactive protein in the sample. The concentration obtained was determined by interpolation using a calibration curve determined using a calibrator with known concentration. The Fe^{+3} ions were determined in the serum free from traces of hemolysis using a colorimetric method with ferrozine without deproteinization. The Fe^{+3} ions were released in an acidic medium in the presence of detergents, after which they were reduced to Fe^{+2} when interacting with ascorbate. The Fe^{+2} ions react with 3-(2-pyridyl)-5,6-bis(2-[4-phenylsulfonic acid])-1,2,4-triazine (ferrozine) to form a colored complex compound, whose coloration intensity is directly proportional to the iron content. The ferritin content was investigated using the method of enzyme linked immunosorbent assay by determination of serum concentrations of ferritin-class immunoglobulins using a "Ferritin Vector-Best" test system. Statistical analysis of the results obtained was carried out using the "Microsoft Excel" software package. The results obtained were summarized as M±m. The significance of the difference between the indices was assessed using the student's t-test. The differences were considered significant at $p<0.05$.

Results

It has been noted that changes in RBC indices in the group of test animals consisted in an insignificant reduction in absolute counts of red blood cells and mean corpuscular volume while maintaining the homogeneity of the population, as suggested by slight fluctuations of such indices as RDW-SV, which in the test group increased significantly ($p<0.05$) by 4.38%, and RDV-CV, which decreased by 0.71%. Activation of megakaryocytic cells was detected, as evidenced by a significant ($p<0.05$) 28.21% increase in immature platelet counts (P-LCC) in the group of test animals and a 22.54% increase in large platelets P-LCR fraction compared with the finding in the group of intact animals. The hepatotoxic effect of thioacetamide, a hydrophilic xenobiotic, was shown to cause changes in iron adsorption, which consisted in a significant ($p<0.05$) 28.07% ferritin increase in the test group against the background of a 17.68% reduction in plasma levels of Fe^{+3} ions in the animals compared to controls. The reduction in Fe^{+3} stores in plasma as a result of its deposition as ferritin is leading to a reduction in volume fraction of RBCs in whole blood, as suggested by a 12.23% hematocrit reduction in the group of test animals. Prolonged intoxication by exogenous xenobiotics for 21 days caused the development of inflammation, as suggested by the increased concentration of C-reactive protein (up to 1.67 mg/dl*10^{-2}) and corresponding to its significant increase by 23.35% compared to controls. The development of the inflammation involved the population of neutrophils and lymphocytes, the absolute counts of which increased significantly in the test group ($p<0.05$): by 58.73% and by 46.28%, respectively.

Discussion

Over 21 days, thioacetamide and ethanol toxicities have caused insignificant changes in RBC indices. Based on analysis of the diagram (Fig. 1), we could assert a 6.29% reduction in mean corpuscular volume (MCV) and a 4.62% reduction in absolute red blood cell count (RBC), as well as an insignificant 5.53% reduction in hemoglobin (HBG) in the group of

test animals compared to the findings in intact animals.

The analysis of the distribution width of red blood cells based on such parameters as RDW-SD and RDW-CV suggested homogeneity of the RBC population. Thus, RDW-CV in the test group significantly increased by 4.39%, while RDW-SD reduced by 0.71%.

As can be seen from the results of changes with time in the platelet indices caused by the hepatotoxic effects of thioacetamide and ethanol (Fig. 2), at Day 21 the animals of the test group were found to have a 6.96% increase in the absolute platelet count (RLT) and a 10.53% increase in plateletcrit. Mean platelet volume has significantly increased by 3.84% in rats of the test group.

It is worth noting that as the absolute platelet count grew, there was an insignificant increase of heterogeneity of the population, as confirmed by a significant 4.92% increase in platelet distribution width (PDW) in the group of test animals. Complete blood count also detected activation of megakaryocytic cells, since the P-LCC parameter, which included immature platelets, was significantly increased in the group of test animals by 28.21%. In the test group, an increase in the fraction of large platelets (P-LCR) was noted (by 22.54% compared to the group of intact animals).

In order to perform a comprehensive assessment of hepatic metabolism of iron in a setting of hepatotoxic injury by exogenous xenobiotics, the authors have compared the changes with time in the iron-containing protein complex (ferritin), the concentration of Fe^{3+} ions and hematocrit (Fig. 3). The analysis of the histogram suggests a mutually inverse relationship between the concentration of Fe^{+3} ions and the content of ferritin in the plasma of test group animals. At Day 21 during the experiment, the findings in the test group of animals included a significant ($p<0.05$) increase of plasma ferritin by 28.07% and a significant ($p<0.05$) reduction in plasma Fe^{+3} ions by 15.02%, as well as a 10.89% hematocrit reduction in the animals compared to controls.

Based on analysis of Pearson correlation coefficient r = - 0.93 (Fig. 4), a significant negative linear interdependence between the content of Fe^{3+} ions and plasma ferritin concentrations was established for the control group. The approximation significance $R^2 = 0.73$. This means that the change in Fe^{3+} content was mainly caused by the changes of ferritin concentration (73%), while 26% of this change depended on other factors. Therefore, the correlation analysis has confirmed the inverse interdependence between Fe^{3+} ions and ferritin concentration in the control group of animals. The data obtained also allow us to ascertain a significant negative linear interdependence between plasma ferritin concentrations and whole blood hematocrit, since the value of Pearson correlation coefficient approximates 1 with a "-" sign and equals r = - 0.84, with the approximation significance $R^2 = 0.71$.

The data in Fig. 4. are also suggesting that a prolonged influence of exogenous xenobiotics did not discriminate the presence of a strong negative linear interdependence between ferritin concentration and Fe^{3+} ions, which has been established in the control group and confirmed in the test group. Thus, Pearson correlation coefficient r = - 0.98, and the approximation significance $R^2 = 0.98$. Therefore, the hepatotoxic effect of thioacetamide and ethanol is leading to reduction in Fe^{+3} stores due to the active deposition of iron into ferritin, a complex iron-protein compound. Such a change in the metabolism of iron ions in the plasma has negative erythropoietic implications, which is suggested by reduced volume fraction of RBCs in whole blood (Fig. 1).

Statistical analysis had demonstrated that the correlation between plasma ferritin concentration and hematocrit in the test group had a weak negative non-linear nature as evidenced by the r-value of -0.49. Therefore, the increase in ferritin concentration was not associated with hematocrit reductions in the test group, as confirmed by the significant approximation value $R^2 = 0.24$.

When modeling toxic liver injury, it is important to assess the level of proinflammatory cytokines and to conduct a quantitative analysis of changes in immune defense cells. Given the fact that the synthesis of C-reactive protein is occurring specifically in the hepatocytes under the influence of proinflammatory cytokines IL-1 and IL-6, as well as the fact that our chosen experimental model was based on liver disease modeling, the determination of this acute phase protein is essential for objective

assessment of changes in the immune system and identification of the nature and the degree of inflammation development [5]. Increases in neutrophil counts (by 142.3%) and lymphocyte counts (by 86.15%) were found in the test group (Fig. 5) compared to controls. The increase in absolute counts of neutrophils and lymphocytes (as the principal immune system cells involved in inflammation development) correlated positively with a 30.49% increase in C-reactive protein concentration in the plasma of test animals.

The performed statistical analysis has shown the absence of correlation between plasma concentrations of C-reactive protein and absolute neutrophil counts (Fig. 6). This was suggested by the value of Pearson correlation coefficient r =0.62 with an approximation coefficient $R^{2=}$0.39. However, there was a significant positive linear interdependence between acute phase protein and neutrophils in the group of test animals. Thus, Pearson correlation coefficient r = 0.74 with the approximation significance R^2 = 0.55 suggests a non-absolute interdependence of changes in test parameters. In our opinion, such correlation of acute phase protein with neutrophils and lymphocytes is associated with an increased production of cytokines and free radicals through stimulation of these cells by C-reactive protein on condition of complement activation.

The syntropic influence of thioacetamide and exogenous ethanol on erythroid cells consisted in reducing the absolute counts and mean corpuscular volumes of red blood cells while maintaining the homogeneity of the population, activation of megakaryocytic cells due to an increase in the fraction of small and large platelets and an insignificant increment in population heterogeneity. In course of prolonged intoxication with thioacetamide and ethanol, changes have been detected in adsorption of iron ions in the test group of animals, which consisted in reduction of plasma Fe^{+3} stores due to active deposition of iron into ferritin, a complex iron-protein compound. The test group was identified with increased neutrophil and lymphocyte counts and increased concentrations of C-reactive protein, suggesting the development of an inflammatory response of the hepatic parenchyma.

In addition to thioacetamide and ethanol, a trigger factor to be included in development of an effective model of liver cirrhosis is retinol. The latter contributes to intensification of fibrogenesis in hepatic parenchyma and to biological activation of thioacetamide. Therefore, inclusion of vitamin A into the studies of the pathogenesis of cirrhosis in addition to hepatotoxic substances is a promising and unstudied area.

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Figure 1. The changes in morphometric parameters of red blood cells caused by the hepatotoxic effects of thioacetamide and ethanol

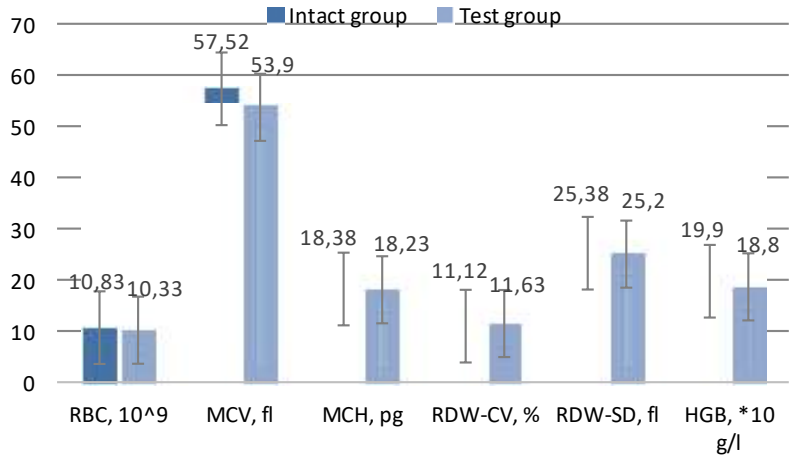

Figure 2. Changes with time in platelet indices caused by the hepatotoxic effects of thioacetamide and ethanol

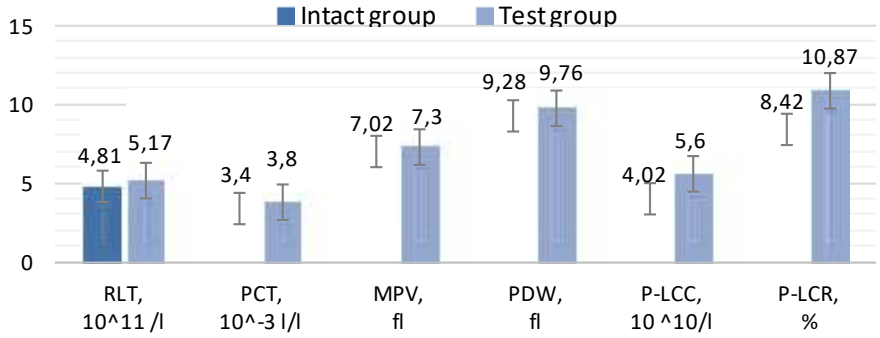

Figure 3. The changes in serum ferritin concentration, serum Fe^{3+} ions concentration and hematocrit caused by hepatotoxic effects of thioacetamide and ethanol in Wistar rats

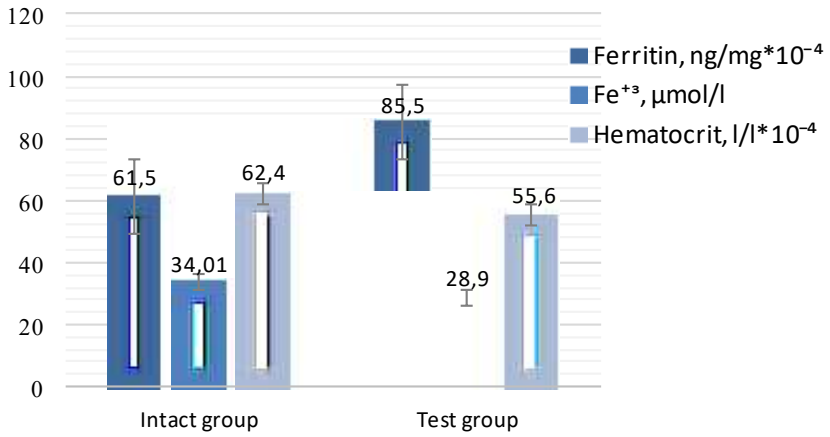

Figure 4. The correlation between the plasma levels of ferritin and Fe^{3+} ions and HCT in control animals (a) and test animals (b)

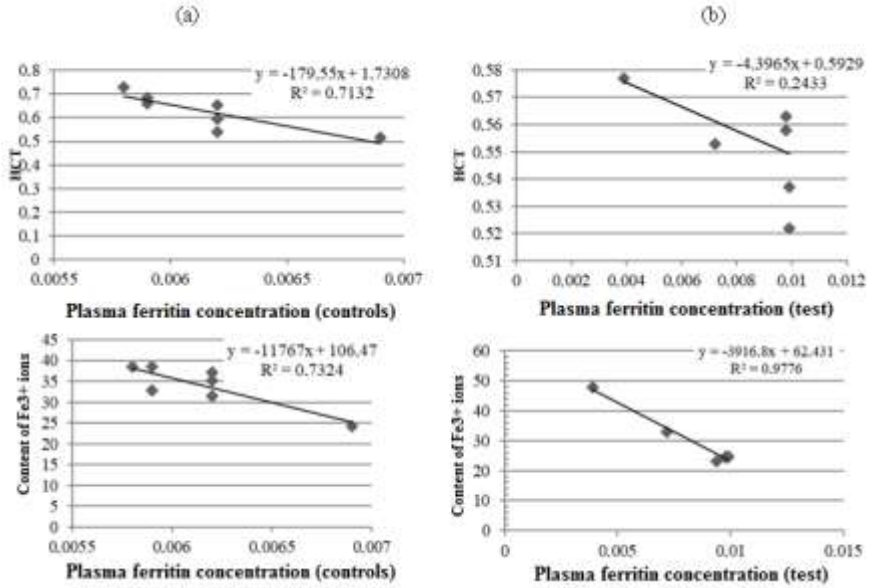

Figure 5. Changes with time in C-reactive protein, neutrophils and lymphocytes in a setting of hepatotoxic injury by thioacetamide and ethanol

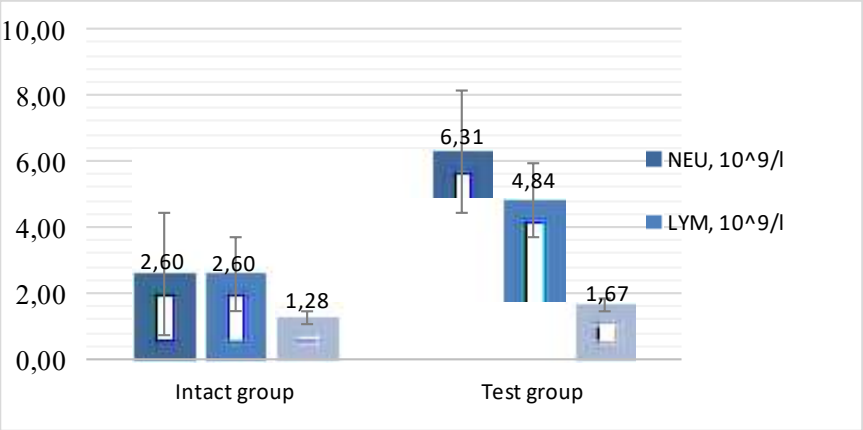

Figure 6. The correlation between plasma levels of C-reactive protein and NEU/LYM counts in the whole blood of control animals (a) and test animals (b)