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RELATIONSHIP BETWEEN LIPID PEROXIDATION AND ANTIOXIDANTS IN RATS WITH EXPERIMENTAL ACUTE GENERALIZED PERITONITIS AGAINST THE BACKGROUND OF STREPTOZOTOCIN-INDUCED DIABETES

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Abstract

Introduction. There are evidence that oxidative stress is involved in the pathogenesis of the acute generalized peritonitis and diabetes mellitus.

The aim of study was to examine levels of the products of lipid peroxidation and the antioxidants in rats with experimental acute generalized peritonitis against the background of streptozotocin-induced diabetes.

Material and methods. The experiment was performed on 66 adult white male rats weighing 220-300 g. Diabetes mellitus in experimental animals was simulated by a single intraperitoneal injection of streptozotocin (Sigma) at the dose of 60 mg/kg. Acute generalized peritonitis was induced with 10% faecal suspension in a dose of 0.5 ml per 100 g of animal weight in the abdominal cavity of laboratory animals by puncture method. The thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LOOHs), superoxide dismutase (SOD) activity, catalase (CAT) and ceruloplasmin (CP) were estimated and compared.

Results. Our results showed that during all stages of development of acute generalized peritonitis on the background of streptozotocin-induced diabetes there is the LOOHs decrease and the TBARS increase. We observed statistically significant inverse correlations between

the level of LOOHs and SOD in the animals of subgroup 1 (r = -0.88; p <0.05) and inverse correlations between the TBARS level and the SOD level in the subgroup 3 of animals (r = -0.74; p <0.05). We also found a statistically significant inverse correlation between the CAT level and the TBARS level (r = -0.86; p <0.05) and between the CP level and the TBARS level in the blood of animals in the subgroup 3 (r = -0.87; p <0.05).

Conclusion. Negative statistically significant correlations between the TBARS level and the antioxidants (SOD, CAT, CP) in the blood of animals with acute generalized peritonitis on the background of streptozotocin-induced diabetes indicate a predictor role of lipid peroxidation processes in the depletion of antioxidant resources.

Key words: acute generalized peritonitis; streptozotocin-induced diabetes; lipid peroxidation; antioxidant defense system; correlation analysis.

Global prevalence of diabetes is progressively increasing, and it is expected that 578 million people will have diabetes in 2030 and the number will increase to 700 million in 2045. [1]. The number of patients with acute peritonitis on the background of diabetes mellitus (DM) is constantly growing respectively [2]. Peritonitis due to a decrease in immunity and inhibition of the plastic properties of tissues in DM quickly becomes generalized with the development of sepsis with multiple organ failure [3] giving a mortality rate of 11 % [4]. Generalized peritonitis is one of the reasons that increase oxidative stress (imbalance between the prooxidant and antioxidant levels in favour of prooxidants) in the body. Increased lipid peroxidation (LPO), an indicator of oxidative stress, is mediated the harmful effects of oxidative stress in the body [5, 6, 7, 8]. Moreover, oxidative stress plays an important role in the development of DM by imbalance of reactive oxygen species production, and has close relationship with inflammation [9].

Establishing of the interaction between the products of LPO and the antioxidant defense system (ADS) provides an opportunity to clarify the metabolic pathways of the pathogenesis of acute peritonitis in conditions of concomitant DM.

The aim of this study was to examine levels of the products of LPO and the antioxidants in rats with experimental acute generalized peritonitis (AGP) against the background of streptozotocin (STZ) – induced diabetes.

Material and methods. Experimental studies were performed on 66 adult white male rats weighing 220-300 g. The rats were divided into main and control groups. The main group – animals with simulated AGP against the background of STZ-induced diabetes (n=56). Control group consisted of rats (n=8), which were injected with physiologic sline (0.9 %

NaCl) only. The animals of the main group were divided into 3 subgroups: subgroup 1 (diabetic rats on the 1st day of the AGP), subgroup 2 (diabetic rats on the 3rd day of the AGP), and subgroup 3 (diabetic rats on the 7th day of the AGP). These terms correspond to the reactive, toxic and terminal stages of peritonitis.

Rats had free access to food and water while living conditions included constant ambient temperature (23±1 °C and 50±5 % humidity) and a 12-h light-dark cycle. The animals were cared for in accordance the Law of Ukraine No3447-IV "On the protection of animals from cruel treatment" [10] and in accordance with the EU Directive of 10/10/2010 on the protection of vertebrates animals used for experimental and other scientific purposes [11].

DM induced by a single intraperitoneal injection of STZ (Sigma, USA) at a dose of 60 mg/kg body weight [12]. STZ is an antibiotic that causes pancreatic islet β-cell destruction and is widely used experimentally to produce a model of type 1 DM [13]. Two week following injection, blood glucose levels were monitored by Accu-check glucometer (Roche, USA). Blood glucose level of rats is higher than 300 mg/dL were considered diabetic.

On day 14 of development of the experimental model of diabetes, peritonitis was induced in 56 rats (96.6 %) using a 10% faecal suspension in a dose of 0.5 ml per 100 g of animal weight in the abdominal cavity of laboratory animals by puncture method [14]. The faecal suspension was prepared by mixing isotonic solution and the contents of the cecum of intact animals, then it was filtered through gauze in order to permit free passing through the interior of the needle in the direction of the cavity.

To assess the states of the LPO and ADS, the contents of the following parameters were determined: thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LOOHs), superoxide dismutase (SOD) activity, catalase (CAT) activity, as well as ceruloplasmin (CP) [15, 16].

Statistical analysis was performed using Statistica 6.0 and GraphPad Prism version 5.0 for Windows. The collected and calculated data are expressed as mean ± standard deviation (S.D.). Pearson's correlation coefficient was applied to find correlations (r) among continuous variables. Results were considered significant at $p<0.05$.

Results. We included 8 rats to subgroup 1 and 8 rats to subgroup 2 from the main group on the first and third days after the introduction of faecal suspension. Mortality in the main group of animals in these terms of the study, which were corresponded to the reactive and toxic stage of peritonitis on the background of diabetes, was 23.2 % and 31.4 %. On the seventh day of modelling of combined pathology (terminal stage of peritonitis), mortality was

37.5 %. Survival rats were randomly included into the subgroup 3 (n=8) to obtain statistically significant study results.

Levels of TBARS and LOOHs in the blood examined are presented in Figure 1. The concentration of TBARS was 2.1 times higher in subgroup 1 as compared to control group. Similarly, LPO was 3.4 times higher (concentration of TBARS) in the subgroup 2 and 7.8 times higher in the subgroup 3 when compared to control group ($p<0.05$). The maximum content of TBARS (almost 3.8 times higher than in subgroup 1 and almost 2.3 times higher than in subgroup 2) was observed on the 7th day after the introduction of faecal suspension in the animals of the subgroup 3.

The concentration of LOOHs was 5.7 times higher in subgroup 1 when compared to control group. However, the LOOHs level during all terms of development of AGP on the background STZ-induced diabetes decreased ($p < 0.05$). The lowest LOOHs level was observed in the subgroup 3. This product of the LPO was almost 1.5 times lower than in subgroup 1 and almost 1.4 times lower as compared to subgroup 2.

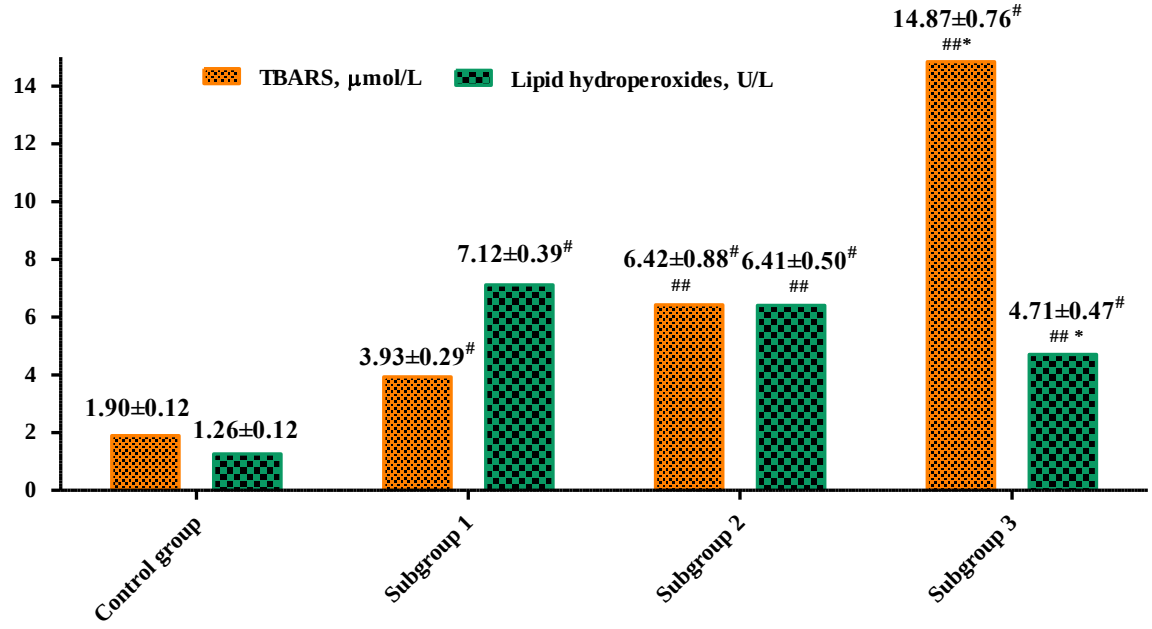

Figure 1. Lipid peroxidation content in the blood of rats with AGP on the background STZ-induced diabetes as compared to control group. Values are expressed as mean ± S.D. (n=8). # $p < 0.05$ to control group; ## $p < 0.05$ to subgroup 2; * $p < 0.05$ to subgroup 3.

The SOD activity in all samples examined subgroups was significantly decreased ($p<0.05$), versus the activity of the enzyme in control group (Fig. 2). Moreover, SOD activity

in blood was lower in rats of the subgroup 3 as compared to the subgroup 1 and as compared to the subgroup 2 ($p<0.05$).

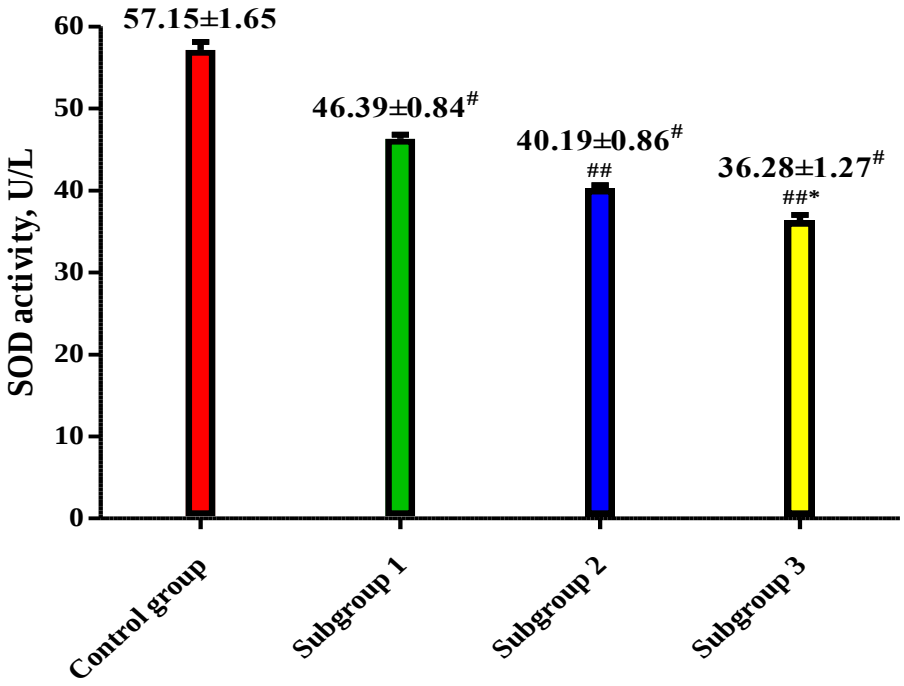

Figure 2. SOD levels in the blood of rats with AGP on the background STZ-induced diabetes as compared to control group. Values are expressed as mean ± S.D. (n=8). # $p < 0.05$ to control group; ## $p < 0.05$ to subgroup 2; * $p < 0.05$ to subgroup 3.

Levels of CAT activity and CP in all subgroups of animals with AGP on the background of STZ-induced diabetes were higher as compared to the corresponding indicators in the control group. During all terms of the study in combined pathology, we observed a decrease in CAT activity of blood and CP, but even on the 7th day of the development of AGP on the background of diabetes (subgroup 3), these markers were higher than controls (Fig. 3). The present study indicates that the increase in CAT activity in subgroup 1 was related with adaptive-compensatory reactions in response to the intensification of LPO.

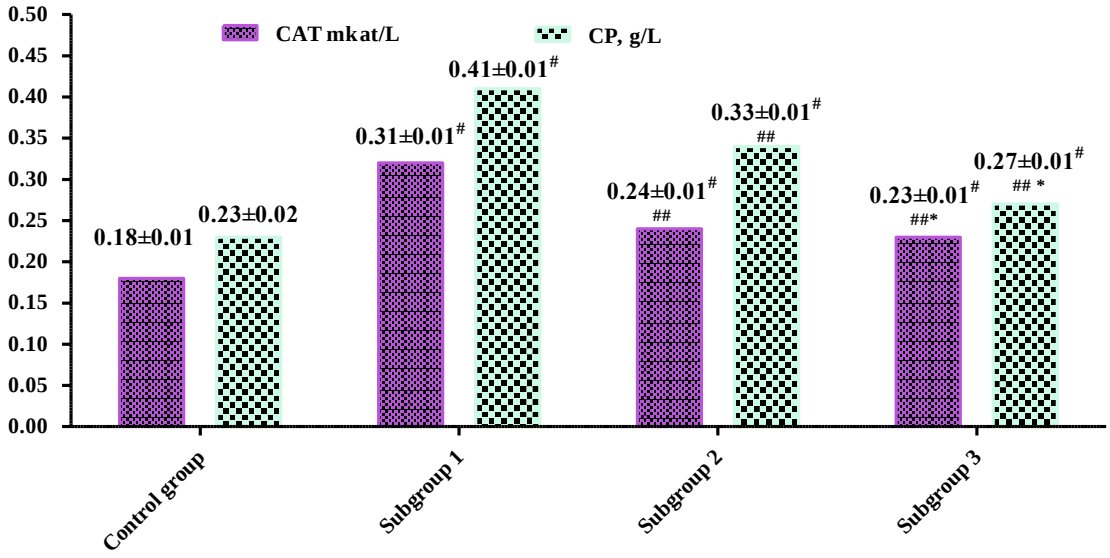

Figure 3. CAT level and CP concentration in the blood of rats with AGP on the background STZ-induced diabetes. Values are expressed as mean ± S.D. (n=8). # $p < 0.05$ to control group; ## $p < 0.05$ to subgroup 2; * $p < 0.05$ to subgroup 3.

After analysing the correlation between the products of LPO and antioxidants in animals with combined pathology, we found a statistically significant inverse correlation between the LOOHs level and the TBARS level in the blood of animals in the subgroup 2 ($r = -0.82$; $p < 0.05$) and in subgroup 3 ($r = -0.76$; $p < 0.05$) (Fig. 4).

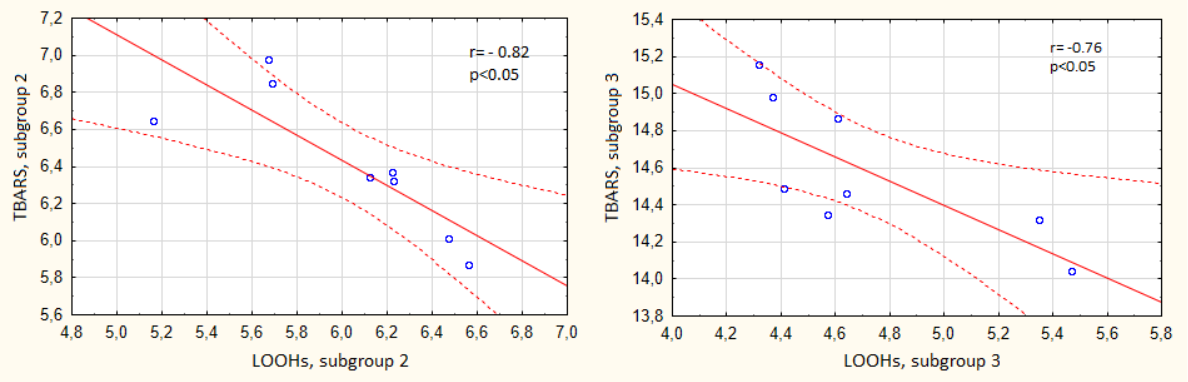

Figure 4. Correlation analysis between the TBARS and the LOOHs in the blood of rats with AGP on the background STZ-induced diabetes

We also found statistically significant inverse correlations between the level of LOOHs and SOD in the animals of subgroup 1 ($r = -0.88$; $p < 0.05$) and inverse correlations between the TBARS level and the SOD level in the subgroup 3 ($r = -0.74$; $p < 0.05$) (Fig. 5).

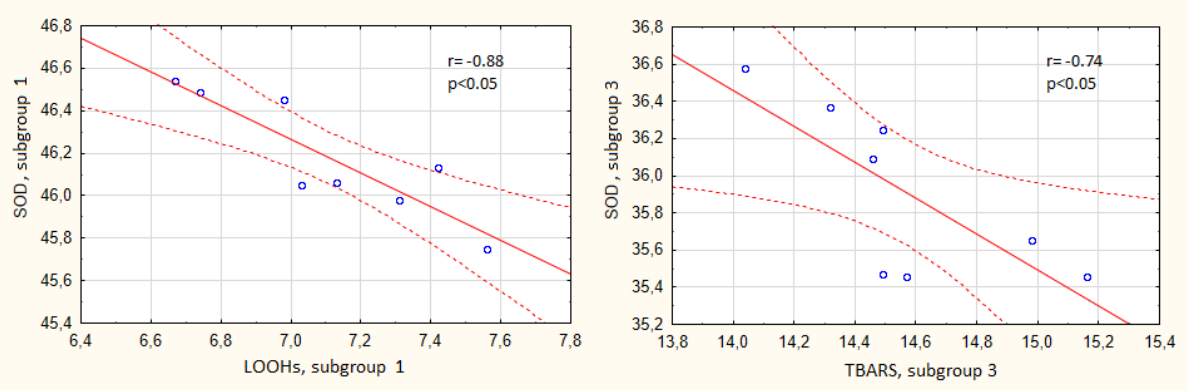

Figure 5. Correlation analysis between SOD activity and products of LPO in the blood of rats with AGP on the background STZ-induced diabetes.

After analyzing the correlation between the antioxidants and the TBARS level in the blood of animals with combined pathology, we found a statistically significant inverse correlation between the CAT level and the TBARS level ($r = -0.86$; $p < 0.05$) and between the CP level and the TBARS level in the blood of animals in the subgroup 3 ($r = -0.87$; $p < 0.05$). This only confirms the fact that with the increase of oxidative processes, antioxidant protection is depleted (Fig. 6).

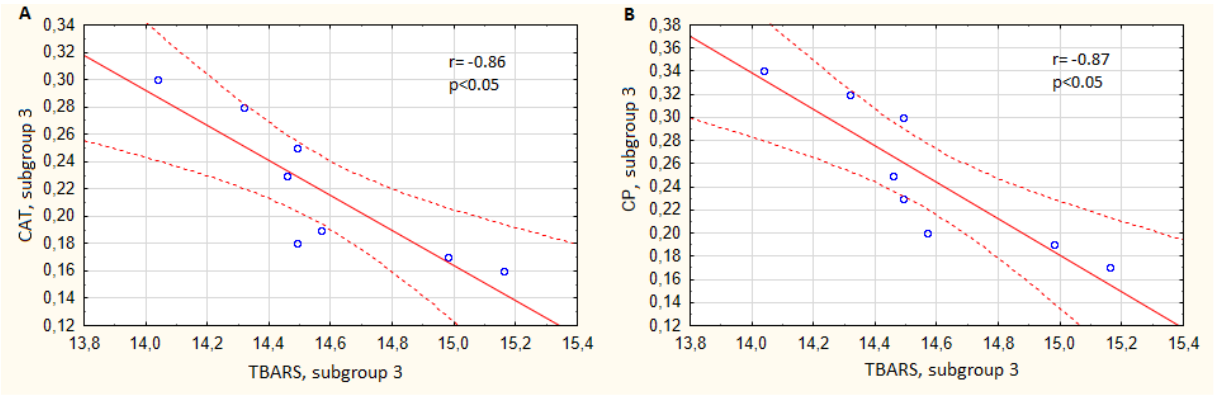

Figure 6. A. Correlation analysis between CAT activity and TBARS in the blood of rats with AGP on the background STZ-induced diabetes. B. Correlation analysis between CP and TBARS in the blood of rats with AGP on the background STZ-induced diabetes.

Discussion. Any imbalance between the reactive oxygen species and antioxidants leads to produce a condition known as “oxidative stress” that results in the development of wide range of diseases including diabetes and peritonitis. To date, the role played by interaction between the products of LPO and the ADS in the patient with AGP on the background of DM has not been fully investigated. Kashafeeva et al. found a decrease in LPO

of the peritoneum in inflammation [17]. Some study demonstrated increased LPO levels and oxidative stress in peritonitis [18, 19]. Our results showed that during all stages of development of AGP on the background of STZ-induced diabetes there is the LOOHs decrease and the TBARS increase. The highest TBARS concentration value in the subgroup 3, indicates an intensified process of LPO on the 7th day of combined pathology.

SOD and CAT respectively protect the cell against reactive oxygen species by scavenging superoxide radical and H2O2, which cause damage to the structure and function of membrane [20]. We observed reduced levels of SOD activity during development of combined pathology. The present study contrasts with the findings of Chen et al. [21] who found low SOD values and the continuous increase in animals with peritonitis. The increased mean CAT levels in the subgroup 1 may be explained on the basis that increased free radical production may enhance the ADS which counterbalances the prooxidant environment.

We also observed statistically significant inverse correlations between the level of LOOHs and SOD in the animals of subgroup 1. Furthermore, in the current study, we also found that TBARS were negatively correlated with the antioxidants (SOD, CAT, CP) in the animals of subgroup 3. That is, throughout the experimental modelling of AGP on the background of STZ-induced diabetes, the increase in LPO processes was accompanied by depletion of antioxidant resources.

Conclusion. Negative statistically significant correlations between the TBARS level and the antioxidants (SOD, CAT, CP) in the blood of animals with AGP on the background of STZ-induced diabetes indicate a predictor role of LPO processes in the depletion of antioxidant resources.

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