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Participation of No-Synthase in the Mechanisms of Oxidative Stress and Endothelial Injury in Rodent Peritonitis

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ABSTRACT

Background: The slow tendency of decrease in mortality from peritonitis may be due to an incomplete understanding of its pathogenesis. The participation of NO-synthase (NOS) in the mechanisms of development of oxidative stress and endothelial lesion during rodent peritonitis has not been fully studied, which may be of importance for researchers and for elaboration of adequate pathogenetic treatment of the pathology through the influence on the activity of this enzyme.

Objective: To study participation of NO-synthase in the mechanisms of oxidative stress and endothelial injury in rodent peritonitis.

Material and Methods: Experiments were carried out on white male rats (n=30), divided into 5 equal series, which were injected intraperitoneally, 0,6 ml/100 g: 1st (control) – 0.85% NaCl, 2nd (peritonitis, P)–5th– 15% fecal suspension, with subsequent intramuscular injection of: 3rd serie – substrate of NOS – L-arginine, L-Ar (300 mg/kg); 4th serie – inhibitor of inducible NOS – aminoguanidine, Ag (15 mg/kg); 5th series – analogous doses of L-Ar and Ag. The activity of oxidative stress by concentration of malonic dialdehyde (MDA) and content of reduced glutathione (GSH) and endothelial injury by number of circulating endotheliocytes (EC) were studied.

Results: The research of the rodent peritonitis course under combined administration of L-Ar and Ag revealed more significant corrective effect, in comparison with the results in peritonitis with their separate administration, in relation to oxidative stress and vascular injury, showing decrease in the concentration of lipid peroxidation product – MDA, increase in level of GSH and decrease in number of EC in blood plasma.

Conclusion: The most significant corrective effect of combined use of the NOS-substrate, Ar, and the inhibitor of inducible isoform of enzyme, Ag, in rodent peritonitis may be the result of inhibition of the excessive NO production by Ag, as well as the maintenance of the endothelial NOS activity and different metabolic pathways under Ar administration.

Keywords: Rodent Peritonitis; NO-Synthase; Oxidative Stress; Endothelium

Abbreviations: NOS: NO-Synthase; MDA: Malonic Dialdehyde; GSH: Glutathione; EC: Endotheliocytes; Ar: Arginine; Ag: Aminoguanidine

Introduction

The problem of treating peritonitis remains relevant due to the high mortality rate, reaching 85-90% when complications develop [1]. One of the reasons for the high mortality rate in peritonitis may be the insufficiency of its pathogenetic therapy, which is based on an incomplete understanding of the pathogenesis of this pathology. In particular, the effects of activation or inhibition of various NO synthase isoforms in peritonitis remain insufficiently studied. It is important to note that this enzyme takes part in the implementation of oxidative reactions, regulation of blood flow and bactericidal properties of leukocytes [2]. The substrate of NO synthase is the amino acid L-arginine (Ar), and the enzyme itself is represented by neuronal, inducible and endothelial isoforms. A special role in inflammation belongs to the inducible (macrophage) isoform of NO synthase. Thus, studying the effects of activation or inhibition of various synthase isoforms during peritonitis can make it possible to detail its pathogenesis and develop adequate approaches to the pathogenetic therapy of peritonitis.

Materials and Methods

The animals were divided into 5 series:

1. Rats with intraperitoneal injection of 0.85% sodium chloride solution (control);
2. Rats with peritonitis modeled by intraperitoneal injection of 15% fecal suspension (peritonitis, P) according to the modified method of Lazarenko V.A, et al. [3,4], followed by intramuscular injection of 0.85% sodium chloride solution;
3. Rats of group "P" with intramuscular injection of NOS substrate – amino acid L-arginine (P+Ar) at a dose of 300 mg/kg (Sigma, USA);
4. Rats of group "P" with intramuscular injection of an inhibitor of the inducible isoform of NOS – aminoguanidine (Ag) at a dose of 15 mg/kg (Sigma, USA);
5. Rats of group "P" with intramuscular injection of L-Ar (Sigma, USA) and Ag (Sigma, USA) at doses of 300 mg/kg and 15 mg/kg, respectively.

Blood from rats was taken from the abdominal aorta after 3 days of peritonitis. Surgical interventions were performed under conditions of adequate analgesia, in accordance with the ethical standards for the treatment of animals set out in the documents of the world community "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" Strasbourg [5]. The activity of oxidative stress was studied by determining the content of the secondary product of lipid peroxidation, malondialdehyde (MDA), and the antioxidant defense factor, reduced glutathione (GSH), in the blood plasma of rats [6]. The severity of morphological damage to the endothelium of blood vessels was determined by the number of circulating endothelial cells (EC) using a Goryaev chamber [7]. Statistical processing of data was carried out using nonparametric methods (Kruskal-Wallis test and post hoc comparisons using Dunn's test) using the Statistica 10.0 program for Windows (StatSoft Inc., USA); data are presented in the form Me (LQ; UQ), where Me is the median, LQ and UQ are the values of the lower and upper quartiles, respectively; differences are statistically significant at $p<0.05$ [8].

Results and Discussion

In rats with peritonitis, an increase in the processes of lipid peroxidation and a decrease in the activity of the antioxidant system was noted, as evidenced by an increase in the content of the secondary product of lipid peroxidation – malondialdehyde (MDA) and a decrease in the level of the antioxidant protection factor – reduced glutathione (GSH) in the blood plasma of rats after 3 days of inflammatory process [9]. In turn, under conditions of administration of arginine (Ar) and aminoguanidine (Ag), opposite changes were noted – a decrease in the activity of oxidative stress and an increase in antioxidant protection based on the studied indicators, which was more pronounced with the combined use of these substances. In particular, in the group of rats with combined administration of Ar and Ag, a decrease in the MDA content in the blood plasma was noted – 3.4 times ($p<0.01$), compared with the values with peritonitis without their administration. In addition, the concentration of MDA in the blood plasma of rats with peritonitis and the introduction of a combination of the studied modulators of NOS activity decreased, compared with the results with the introduction of only Ar or only Ag, by 61 (56; 67)% ($p<0.01$) and 43 (35; 47)% ($p<0.01$), respectively, which indicates the most pronounced corrective effect of the combined use of modulators, compared with their isolated use, in relation to the activity of oxidative processes (Figure 1).

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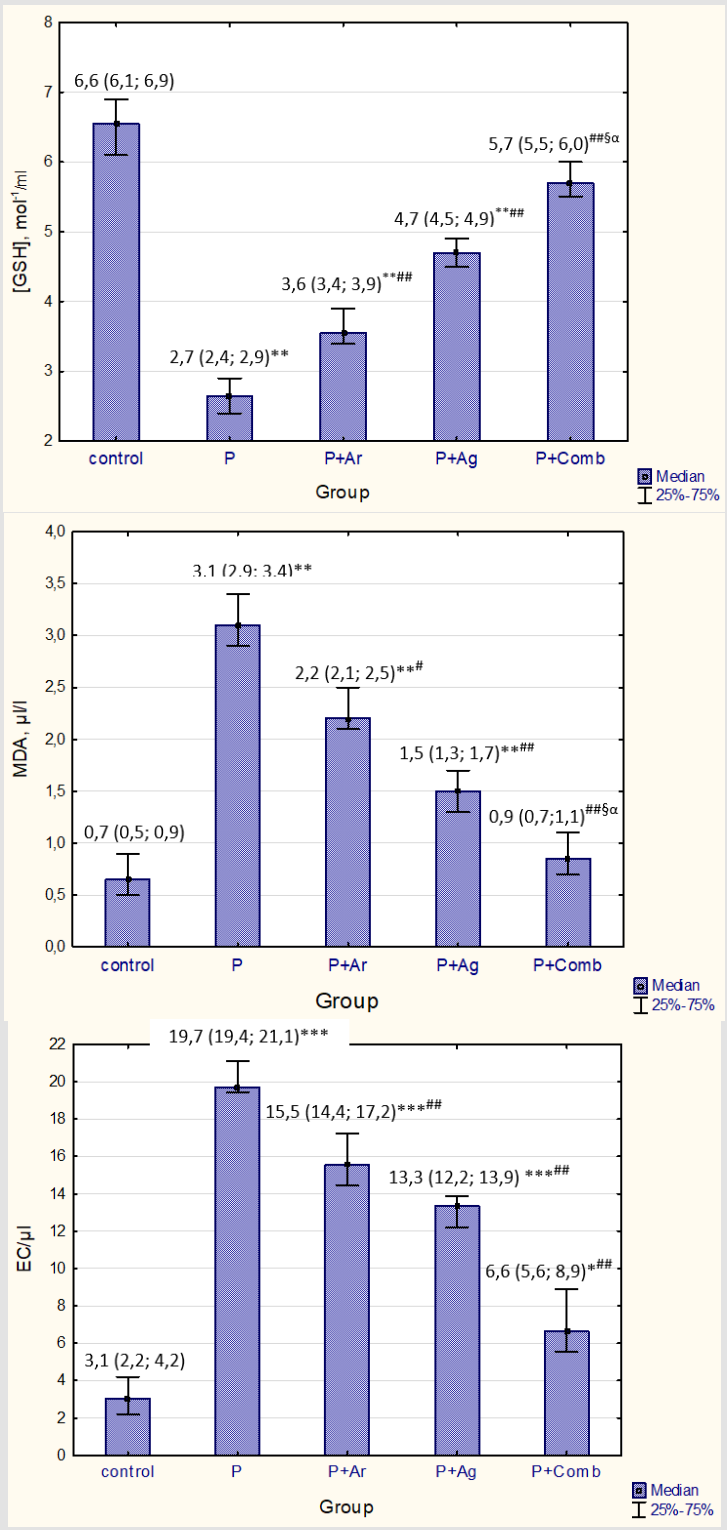

Note: Significant differences regarding: * – p<0.05, ** – p<001, *** – p<0.001 – control group;
– p<0.05, ## – p<0.01, ### – p<0.001 – "P" group; § – "P+Ar" group; α – "P+Ag" group;
MDA – malondialdehyde, GSH – reduced glutathione, EC – endothelial cells in blood plasma

Figure 1: The level of malonic dialdehyde, MDA, and reduced glutathione, GSH, endothelial cells, the in blood plasma of rats with peritonitis, P, and separate or combined (Comb) administration of arginine, Ar, and aminoguanidine, Ag, in 3 days of peritonitis.

Simultaneously with the decrease in MDA content in the group of rats with peritonitis and the combined administration of Ar and Ag, there was an increase in the concentration of GSH in the blood plasma after 3 days of inflammation – 2.1 times ($p < 0.01$), compared with the values in animals with peritonitis without their introduction. Comparison of the studied parameters in groups of rats with isolated and combined administration of these modulators of NOS activity revealed the most pronounced increase in [GSH] in the blood plasma with the introduction of a combination of Ar and Ag, as evidenced by an increase in the indicator by 60 (54; 61)% ($p < 0.01$) and by 22 (22; 22)% ($p<0.01$), compared with the separate use of Ar or Ag, respectively.

Thus, the decrease in the prooxidant-antioxidant imbalance, which was expressed in a decrease in the content of the lipid peroxidation product – MDA and an increase in the level of antioxidant protection indicator – GSH in the blood plasma of rats with peritonitis, was expressed to the greatest extent with the combined administration of Ar and Ag, indicating the corrective effect of these modulators regarding oxidative stress activity. The results obtained can be explained by the inhibition of the formation of cytotoxic concentrations of nitrogen monoxide and peroxynitrite formed with its participation – $ONOO^-$ under the conditions of using Ag [10], as well as the properties of Ar, which is a precursor in the formation of the antioxidant defense factor – glutathione.

In addition, in rats with peritonitis and combined administration of Ar and Ag, there was a decrease in the number of EC in the blood plasma after 3 days of the inflammatory process by 3.0 times ($p<0.01$), compared with the values of the indicator for peritonitis without their administration. The detected values were less than for peritonitis using only Ar or Ag, 2.3 times ($p<0.01$) and 2.0 times ($p<0.01$), respectively, indicating the most significant corrective effect the effect of these modulators on the integrity of the endothelial layer of blood vessels during peritonitis in rats. Moreover, compared with the number of EC in the "control", under the conditions of combined administration of Ar and Ag, the number of EC in the blood plasma of rats after 3 days of peritonitis was 2.2 times greater ($p<0.05$), which may be due to the lack of time for their elimination by phagocytes [11].

Conclusion

The combined administration of the NOS substrate – L-arginine and the inhibitor of the inducible isoform of the enzyme – aminoguanidine had the most significant corrective effect on the course of peritonitis in rats, which was expressed in a significant decrease in the activity of oxidative stress and damage to the endothelial lining of blood vessels, compared with the results in peritonitis without their administration or with their isolated use. The identified corrective effects may be due to the competitive inhibition of the inducible NOS isoform by aminoguanidine with a decrease in the formation of perioxynitrite and cytokines with inflammatory properties [12], as well as an increase in the bioavailability of the amino acid L-arginine, which has antioxidant effects due to an increase in the formation of glutathione, maintaining the activity of the endothelial NOS isoform with improving microcirculation, suppressing the induction of Hsp60 and Hsp10 proteins [13]. In addition, less activity of oxidative processes and an increase in the degree of antioxidant protection prevents damage to the vascular endothelium, leading to a decrease in the severity of endothelial dysfunction [14].

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