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**РЕАКЦИЯ НЕЙТРОФИЛОВ В УСЛОВИЯХ КОМБИНИРОВАННОГО**  
**ИСПОЛЬЗОВАНИЯ L-АРГИНИНА И АМИНОГУАНИДИНА**  
**У КРЫС С ПЕРИТОНИТОМ**

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**RESPONSE OF NEUTROPHILS WITH COMBINED ADMINISTRATION**  
**OF L-ARGININE AND AMINOGLUANIDINE IN RATS WITH PERITONITIS**  
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**Резюме.** Исследование показало, что комбинированное введение L-аргинина и аминогуанидина крысам с перитонитом снижает уровень незрелых нейтрофилов (метамиелоцитов и палочкоядерных) в крови и перitoneальной жидкости, а также повышает фагоцитарную активность нейтрофилов. Это свидетельствует о противовоспалительном и иммуномодулирующем эффекте, вероятно, за счёт коррекции микроциркуляции, снижения цитотоксического действия активных форм азота и увеличения биодоступности L-аргинина.

**Ключевые слова:** перитонит, нейтрофилы, оксид азота, L-аргинин, аминогуанидин.

**Resume.** The study showed that combined administration of L-arginine and aminoguanidine to rats with peritonitis reduced the levels of immature neutrophils (metamyelocytes and band cells) in the blood and peritoneal fluid while increasing neutrophil phagocytic activity. This indicates an anti-inflammatory and immunomodulatory effect, likely due to improved microcirculation, reduced cytotoxicity of reactive nitrogen species, and enhanced bioavailability of L-arginine for immune cells.

**Keywords:** peritonitis, neutrophils, nitrogen monoxide, L-arginine, aminoguanidine.

**Relevance.** The relevance of this research in this direction are, the morphological target in peritonitis is the peritoneum with its cavity, it is important to determine the response of peritoneal leukocytes, especially neutrophils, in the dynamics of the inflammatory process. Additionally, the effects of nitric oxide (NO) in inflammation are dual: pro- and antioxidant, pro- and anti-inflammatory, pro- and anti-adhesive [1-5]. The contribution of NO generated by different isoforms of NO synthase (NOS) - constitutive (cNOS) and inducible (iNOS), to the development of peritonitis has not been disclosed; there are no approaches to its pathogenetic therapy that involve influencing the L-arginine-NO system.

**Purpose:** to assess the composition and phagocytic activity of peritoneal neutrophils in rats with experimental peritonitis and combined administration of L-arginine and aminoguanidine.

**Materials and methods.** Rats were divided into 2 equal series, which were injected intraperitoneally: 1st series (control) – 0.9% sodium chloride, 2nd series (experimental peritonitis, EP) – 15% fecal suspension in a volume of 0.6 ml/100 g of body weight, according to the method of Blinkov Yu.Yu. et al. [6], 3rd series (EP + L-arg + AG) – the

rats with peritonitis and combined administration of NOS substrate – L-arginine, L-arg, and iNOS inhibitor – aminoguanidine, AG. Standardization of fecal suspension was achieved by its two-stage filtration through a filter of a larger and then a smaller diameter then by subsequent standardization of the suspension by performing spectrophotometric and densitometric analysis. At the same time, 15 % filtered fecal suspension spectrophotometrically corresponds to 2.8 units of optical density and densitometrically – to 11.3 McFarland units,  $3396 \times 10^6$  bacteria/ml ( $\lambda=550$  nm) [7]. The study of the quantitative composition of neutrophils was determined in blood smears and peritoneal fluid stained according to Romanovsky-Giemsa. Determination of the phagocytic activity of neutrophils of the peritoneal exudate was carried out after half a day, 1 day and 3 days of EP using the adapted method of Patsula Yu.I., Vlasenko V.S. [8]. For this, peritoneal exudate (20  $\mu$ l) was incubated with an equivolumed 0.1% solution of nitrosine tetrazolium in the wells of the plate for immunological studies, followed by the addition of 160  $\mu$ l of 3 % acetic acid after thermostating at 37 °C for 30 minutes. Phagocytic activity was assessed based on the number of formazan-positive neutrophils (FPN) which contains dark purple formazan granules in the hemocytometer, followed by calculation of their percentage. Statistical data processing was performed using the Statistica 10.0 program for Windows (StatSoft Inc., USA) using the nonparametric Kruskal-Wallis test and post hoc comparisons; data are presented: Me (LQ; UQ) – median (lower quartile; upper quartile).

**Results and their discussion.** When assessing the composition of neutrophils in the peritoneal fluid in all the studied periods, an increase in the number of segmented and band neutrophils was noted along with the appearance of metamyelocytes. In addition, after 1 day and 3 days, myelocytes were detected, which characterizes the transformation of the regenerative shift of the leukocyte differential count to the left into hyperregenerative one. When studying the relative leukocyte formula in rats with the combined administration of L-arg and AG, it was revealed that the content of morphologically and functionally immature neutrophils – metamyelocytes and band neutrophils in the peritoneal fluid was the lowest, compared with the results in animals with EP without their administration or with the isolated use of the studied NOS modulators, along with the absence of myelocytes. In the studied periods in rats with the combined use of the studied NOS modulators, a decrease in the absolute number of metamyelocytes and band neutrophils in the absence of myelocytes was observed, indicating a significant decrease in the severity of the nuclear shift of the leukocyte formula to the left, which is characterized as regenerative. In comparison, with EP, including with the isolated administration of L-arg and AG, changes in the leukocyte formula were expressed in the development of a hyperregenerative nuclear shift to the left.

The obtained data reflect the anti-inflammatory effect of the combined use of L-arg and AG, which may be due to the correction of microcirculation and metabolic disorders under the conditions of L-arg use, as well as a decrease in the activity of oxidative stress due to the inhibition of iNOS activity by AG. This, in turn, reduces the severity of secondary alteration and the exudation it initiates with the emigration of leukocytes to the site of inflammation.

Along with a change in the quantitative composition of neutrophils, a decrease in their ability to phagocytosis was noted. This was evidenced by a decrease in the percentage of formazan-positive neutrophils in the peritoneal fluid after half a day – by 13% ( $p<0.05$ ), after 1 day – by 22% ( $p<0.05$ ), and after 3 days – by 13% ( $p<0.05$ ), compared with the value in the control. It is assumed that the decrease in the phagocytic activity of neutrophils may be due to the cytotoxic effect of reactive oxygen and nitrogen species on peritoneal microphages. Along with a decrease in the severity of leukocytosis, under conditions of combined administration of L-arg and AG, an increase in the phagocytic activity of neutrophils was established, as evidenced by a greater number of peritoneal FPN than in animals with EP without the administration of NOS modulators. Moreover, the increase in the studied parameter was: after half a day, one day and three days – 67 (63; 69)%, 60 (58; 62)% and 64 (62; 67)%, respectively, which was more than with EP – by 23% ( $p<0.05$ ), by 25% ( $p<0.05$ ) and by 20% ( $p<0.05$ ), than with the use of L-arg – by 16% ( $p<0.05$ ), by 16% ( $p<0.05$ ) and by 14% ( $p<0.05$ ) and than with the administration of AG – by 8% ( $p<0.05$ ), by 7% ( $p<0.05$ ) and by 7% ( $p>0.05$ ), respectively. The absence of obvious changes in the content of FPN in the peritoneum in the dynamics of the studied periods when using a combination of the NOS modulators indicates a stable level of functional activity of peritoneal microphages. Compared with the value of the indicator in the control group, in rats with the combined administration of L-arg and AG, the amount of FPN was higher: after half a day and 3 days - by 10% ( $p < 0.05$ ) and by 7% ( $p < 0.05$ ), respectively, in the absence of differences after one day of EP ( $p > 0.05$ ). Thus, the combined administration of L-arg and AG to rats with EP led to the most pronounced increase in the ability of peritoneal neutrophils to phagocytosis among the studied NOS modulators. This may be due to a decrease in the formation of reactive nitrogen species, which have a cytotoxic effect on peritoneal phagocytes, as well as an increase in the bioavailability of L-arg to correct metabolic disorders in immunocompetent cells.

**Conclusions:** the response of neutrophils in rats with acute experimental peritonitis in the form of a hyperregenerative nuclear shift of the leukocyte formula to the left, along with a decrease in their phagocytic activity indicates the inhibition of nonspecific immune defense and a significant intensity of the infectious and inflammatory process. Combined administration of the NOS substrate – L-arg and the iNOS inhibitor – AG to rats with EP had the most significant corrective effect in maintaining the activity of constitutive NOS isoforms and eliminating the negative effects of excess NO concentrations. At the same time, a significant decrease in the quantitative changes in peritoneal leukocytes and their functional activity was noted. The severity of the inflammatory process in EP under the conditions of AG administration can be reduced due to a decrease in the activity of oxidative stress, since NO is a free-radical molecule and suppression of its formation due to inhibition of iNOS activity prevents excessive production of active nitrogen forms. In addition, under the conditions of administration of the NOS substrate – L-arg, maintenance of the activity of constitutive NOS isoforms is realized, as well as important metabolic processes not associated with the functioning of the NO synthase mechanism.

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