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The influence of the short peptide of arginyl-alfa-aspartyl-lysyl-valyl-tyrosyl-arginine on the activity of NO-synthase system and processes of lipoperoxidation in experimental gastric lesions in rats

Wpływ krótkiego peptydu arginyl-alfa-aspartyl-lizyl-walyl-tyrozyl-argininy na aktywność systemu syntazy NO i procesy lipoperoksydacji w doświadczalnym uszkodzeniu żołądka u szczurów

INTRODUCTION

It is known that short-chained peptides participate in the regulation of a variety of physiological functions in humans, particularly in cytoprotection of gastric mucosa (GM). The gastroprotective effects were evaluated in such short-chained peptide substances as glyprolins and pentadecacapeptide BDC 157 [5, 10, 13]. The positive influence of the hexapeptide arginyl-alfa-aspartyl-lysyl-valyl-tyrosyl-arginine ("Imunofan") was also reported on the course of the peptic ulcer but the mechanisms of its cytoprotective influence on GM is not clear [9]. That is why the aim of the present investigation was to study the role of Imunofan in the processes of lipoperoxidation and the status of L-arginine/NOS/NO system in experimental gastric lesions (GL) in rats.

MATERIAL AND METHODS

Investigations were conducted on 122 rats. The structure of this study and animal experimental procedures were approved by the Ethical Committee of Lviv National Medical University. Stress GL was induced by intraperitoneal injection of epinephrine (2 mg/kg) [2]. We conducted 4 series of the experiments: 1) the study of epinephrine action on GM; 2) the study of Imunofan action (1 μg/100g) on GM on the background of stress; 3) the study of the combined action of Imunofan (1 μg/100g) and L-arginine on the background of stress; 4) the study of the combined action of Imunofan (1 μg/100g) and selective iNOS blocker aminoguanidine (20 mg/kg) on GM on the background of stress.

With the purpose of investigating the NO-synthase system status in GM homogenates, the following indices were determined: activity of NO-synthase [11] and the content of NO [4] in GM and the level of L-arginine in plasma [1]. Processes of lipoperoxidation were analized by the contents of tiobarbituric acid products (TBAP) [12], antioxidant system status – by superoxidedismuthase

activity (SOD) [3] and catalase [7]. The area and severity of GL were investigated using the method of planiometry and 12-grade scale. The results were processed using the method of variation statistics.

RESULTS AND DISCUSSION

The rats introduced to epinephrine developed severe GL in the form of erosions, ulcers and hemorhages. The mean damaged area made up 38.5±5 mm^2 and 10.9±2 grades. In rats who were administered Imunofan under conditions of stress the damaged area of GL was 42% smaller and made up 22±4 mm^2, the severity of GL decreased by 39.44% and made up 6.6±2.8 grades. The group of animals introduced to the combined action of Imunofan and L-arginine on the background of stress GM remained almost intact and the damaged area made up 2.75±0.5 mm^2 and 2.5±0.4 grades respectively. Under conditions of NOS blockage by amine guanidine and Imunofan administration the damaged area of GM made up 6.6±1.7 mm^2 and 3.6±1.2 grades. Thus, the combined administration of Imunofan and L-arginine induced significant gastroprotective effect.

In healthy conditions, the activity of eNOS in GM is dominating and iNOS activity is low [6]. In our experiments, the proportion between eNOS та iNOS in intact animals made up 3.8 and the level of NO in GM made up 16.1±2.55 μmol/l, the concentration of L-arginine in plasma was 39.2±2.09 μmol/ml.

In stress, the NOS activity was significantly increased: total NOS activity increased by 133% from 0.876 ± 0.42 to 2.65 ± 0.827 nmol/min·g ($p<0.01$), eNOS activity did not change significantly and iNOS activity was 6 times higher ($p<0.001$). The content of NO increased from 16.1±2.55 to 24.1±2.6 μmol/l (by 50 %, ($p<0.05$), and the concetration of L-arginine in plasma decreased by 37% ($p<0.05$). The proportion between eNOS and iNOS made up 0.68, and between NO in GM and L-arginine in plasma – 1.22, respectively.

In stress-induced GL, the activity of lipoperoxidation processed also increased – the content of the products of TBAP increased from 224.5±24.4 to 312.4±10.7 μmol/g ($p<0.05$); SOD activity increased by 57%, ($p<0.05$); catalase activity did not change significantly. Thus, in experimental epinephrine-induced GL, massive structural and hemorrhagic damages of the GM were supervised, accompanied by the increase of iNOS activity and content of NO in GM. The level of L-arginine in plasma decreased appropriately. The activity of lipoperoxidation and SOD activity increased.

Imunofan administration in dose of 1 μg/100 g of body weight under the influence of epinephrine induced significant decrease of the total NOS activity by 56% ($p<0.05$), eNOS activity decreased by 41%, and iNOS – by 62%. The content of NO decreased by ($p<0.05$) in comparison to animals who were introduced to epinephrine. The tendency to a decrease of the activity of lopoperoxidation processes was also noted, meanwhile SOD activity decreased by 54%. Thus, Imunofan administration induces the decrease of the damaged area, NOS activity and the content of NO while L-arginine concentration increased.

Imunofan action on the background of iNOS blockage by amine guanidine induced tendency to enhance the inhibition of iNOS activity and the content of NO in GM decreased; respectively. The content of TBAP diminished by 11%, SOD activity had a tendency to decrease in comparison to Imunofan effect. The concentration of L-arginine in blood did not change significantly. Thus, iNOS

blockage under the influence of Imunofan enhanced iNOS inhibition, lipoperoxidation processes, a decrease of NO and SOD activity. Meanwhile L-arginine concentration in plasma increased.

A combined action of L-arginine and Imunofan induced a significant decrease of the activity of total NOS and a decrease of iNOS and the proportion between the activity of total NOS and iNOS made up about 1. NO content did not change significantly and L-arginine concentration was higher, TBA products decreased by 15%, meanwhile SOD and catalase activity did not change significantly in comparison to monotherapy with Imunofan.

Analyzing the obtained data, we conclude that the ulcerogenic effect of epinephrine induces the development of oxidative stress, activation of proinflammatory enzymes - iNOS and COX-2, increase of GM infiltration by polymorphonuclear leukocytes, increase of proinflammatory cytokins production – IL-1β, IL-6 and TNF-α in GM [14]. In oxidative stress, the generation of active forms of oxygen and fatty acid radicals is significantly increased, leading to the damage of the blood vessels membranes, secretory cells and epiteliocytes, which induces the formation of GL [9].

Epinephrine enduces GL formation with a typical increase of TBAP, activation of iNOS, increase of NO and peroxinitrite production. Increased NO-synthase activation leads to the decrease of L-arginine level in blood, which indicates the activation of L-arginine transportation from blood into the cells of the damaged area and its utilization by iNOS. Imunofan inhibits NO-synthases activity in GM [4, 5]. It was proved that Imunofan shows a significant immunomodulatory effect, stimulating differentiation and proliferation of T-lymphocytes, increasing antibodies production, phagocytic activity of macrophages and polymorphonuclear leukocytes and it inhibits the processes of lipoperoxidation [9].

In our experiments Imunofan induced the cytoprotective processes, which was determined on the basis of the area and severity of GL, accompanied by the decreased activity of eNOS and iNOS, NO, L-arginine and TBAP. The obtained indices confirm the cytoprotective action of Imunofan. This effect of Imunofan may be explained by its degradation into di- and tripeptides, containing arginine, which may act as NO-synthase blockers, and its stimulating influence on polymorphonuclear leukocytes.

CONCLUSIONS

Administration of Imunofan in stress decreases the severity of GL, the activity of eNOS, iNOS, NO, SOD in GM and it increases the level of L-arginine in plasma. As the most significant gastroprotection was evaluated under the conditions of the combined action of Imunofan and L-arginine, we suggest this combination to be recommended for the treatment of inflammatory diseases of the stomach.

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SUMMARY

In the experiments on rats, under conditions of modeled ulcer of the stomach, the role of arginyl-alfa-aspartyl-lysyl-valyl-tyrosyl-arginine – Imunofan was investigated on the processes of lipoperoxidation and the status of the system of L-arginine/NOS/NO. It was shown that Imunofan action at the background of ulcerative lesions of the stomach caused a steep decline of the activity of NO-synthases, decrease of NO content in GM and increase of L-arginine concentration in the plasma of blood. The action of Imunofan at the background of blockage of iNOS with amine guanidine induced the inhibition of iNOS activity and a decrease of NO content in GM, whereas the concentration of L-arginine in the plasma of blood increased. Under conditions of combined action of Imunofan with L-arginine, the activity of NO-synthases reduced, the content of nitrogen oxide slightly increased, and the concentration of L-arginine in the plasma of blood was determined higher than in the control animals. The action of Imunofan also caused a decrease of the processes of lipoperoxidation and SOD activity.

Keywords: arginyl-alfa-aspartyl-lysyl-valyl-tyrosyl-arginine, gastroprotection, lipoperoxidation, NO-synthase system.

STRESZCZENIE

W badaniu na szczurach w warunkach modelowanych wrzodów żołądka badano wpływ arginyl-alfa-aspartyl-lizyl-walyl-tyrozyl-argininy – Imunofanu na procesy lipoperoksydacji i stan systemu L-arginina/NOS/NO. Wykazano, że Imunofan w przebiegu wrzodziejącego uszkodzenia żołądka powodował stopniowy spadek aktywności syntaz NO, spadek zawartości NO w śluzówce żołądka i wzrost stężenia L-argininy w osoczu krwi. Imunofan w przypadku blokady iNOS za pomocą guanidyny indukował inhibicję aktywności iNOS i spadek zawartości NO w śluzówce żołądka, podczas gdy stężenie L-argininy w osoczu krwi było podwyższone. W warunkach jednoczesnego oddziaływania Imunofanu i L-argininy aktywność syntaz NO była zmniejszona, zawartość tlenku azotu lekko podwyższona, a stężenie L-argininy w osoczu krwi wyższe niż u zwierząt kontrolnych. Imunofan powodował także spadek aktywności procesów lipoperoksydacji i SOD.

Słowa kluczowe: arginyl-alfa-aspartyl-lizyl-walyl-tyrozyl-argininy, gastroprotekcja, lipoperoksydacja, układ syntazy NO