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Pharmacologically Active Tripeptide Leu-Ile-Lys in Indomethacin-Induced Gastric Ulcer

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Abstract

The aim of the study was to examine the effect of a new tripeptide, Leu-Ile-Lys, on an experimental indometacin-induced gastric ulcer in rats.

Materials and Methods: The experiment was performed with 24 male Wistar rats (average weight of 150 g). Rats were randomly divided into 3 groups: Group 1 (n=8) – the ulcer control group (IIGU), Group 2 (n=8) – the experimental group (IIGU+pre-treating with the tripeptide Leu-Ile-Lys), and Group 3 (n=8) – the comparison group (IIGU+pre-treating with omeprazole). The model of IIGU in rats was performed by a single intragastric administration of indomethacin (60 mg/kg in 1ml of physiological saline). In Group 1, indomethacin caused the appearance of severe injuries of the mucosa with the presence of extensive edema and leukocyte infiltration in the submucosal layer. In animals of Group 2, which were pre-treated with the tripeptide Leu-Ile-Lys, macroscopically gastric mucosa also looked smooth and atrophic changes were not found. Destructive changes were not severe; they appeared only in the form of small spot erosions. The number of spot erosions was 2.6 times less than in Group 1. The average erosion depth was 6.8 times less than in Group 1, and 2.0 times less than in Group 3.

Conclusion: Results of this study demonstrated the high, comparable to the action of omeprazole, gastroprotective activity of the new tripeptide Leu-Ile-Lys. (**International Journal of Biomedicine. 2018;8(4):351-354.**)

Key Words: peptic ulcer • Indometacine • oligopeptides • gastroprotective activity

Introduction

Peptic ulcer disease is responsible for substantial premature mortality worldwide.[1,2] The major causative factors of peptic ulcer disease are Helicobacter pylori infection and the use of nonsteroidal anti-inflammatory drugs and anti-thrombotic agents.[3] Despite the fact that the modern arsenal of antiulcerogenic drugs is quite extensive, a problem of finding new methods for the pharmacological treatment of gastric ulcer remains highly relevant, largely because existing drugs have limited efficacy and several serious side effects.

Previously, we discovered a gastroprotective effect of a peptide complex of pork kidney tissue that was manifested in a significant reduction of gastric mucosal damage in indomethacin-induced gastropathy in rats.[4] In the study of the amino acid composition of this peptide complex, it was found that it includes 15 amino acids, with the greatest mass percentage of leucine, isoleucine and lysine.[5] In this context, we suggest that these amino acids, and/or the peptides containing them, can be responsible for the gastroprotective effect. To test this hypothesis, we decided to synthesize the tripeptide Leu-Ile-Lys and examine its impact on the course of the experimental, indomethacin-induced gastric ulcer (IIGU), which was the purpose of the present study.

Materials and Methods

The experiment was performed with 24 male Wistar rats (average weight of 150 g). Rats were randomly divided into 3 groups:

Group 1 (n=8) – the ulcer control group (IIGU)

Group 2 (n=8) – the experimental group (IIGU+pre-treating with the tripeptide Leu-Ile-Lys)

Group 3 (n=8) – the comparison group (IIGU+pre-treating with omeprazole).

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Animals were housed in keeping with the rules for good laboratory practice. The experiments were performed in accordance with the norms for the humane treatment of animals, which are regulated by the International Guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care in accordance with the protocol approved by the Institutional Animal Care and Use Committee of Altai State Medical University.

The tripeptide Leu-Ile-Lys was synthesized at Shanghai Apeptide Co., Ltd. (Shanghai, China), supported by "Evalar" ZAO (Biysk, Russia). The purity of the sample was at least 98%.

In animals of Group 2, the tripeptide Leu-Ile-Lys was intragastrically administered in a dose of 11.5 mg/kg per day via gavage for 7 days prior to simulation of an indomethacin-induced gastric mucosal injury. The last tripeptide administration was performed 1 hour before the indometacine administration. The animals of Group 1 and Group 3 received physiological saline in equivolume amounts and omeprazole (37 mg/kg), respectively, by the same mode of administration. The model of IIGU in rats was performed by a single intragastric administration of indomethacin (60 mg/kg in 1ml of physiological saline).

The animals were euthanized 4 hours after the indomethacin administration. The stomachs were removed, cut along the lesser curvature, washed with a 0.9% solution of NaCl, and fixed in 10% neutral formalin. Gastric mucosal damage was scored by counting the total number of erosions, the number of linear deep erosions, the number of spot erosions, and the percentage distribution of various types of erosions. Paul's index was calculated for each type of injury by the formula: (N×K)/100, where N - average number of erosions on one animal, K - percentage of injured animals in the group.

For microscopic examination, specimens were stained with H&E to evaluate the tissue structure. Histochemical neutral mucopolysaccharides were detected using the Schick reaction, and acid mucopolysaccharides were detected by intensity of staining with Alcian blue (pH=2.5). The density of the inflammatory infiltrate in 1mm^2 was estimated using an Avtandilov`s ocular grid. Morphometric gastric mucosa were measured using the software Image Tool 3.0.

Statistical analysis was performed using StatSoft Statistica v6.0. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SD for continuous variables. Multiple comparisons were performed with one-way ANOVA. The Mann-Whitney U Test was used to compare the differences between the two independent groups. A probability value of $P<0.05$ was considered statistically significant.

Results

The macroscopic examination of gastric mucosa surface of the Group 1 rats showed clearly visible changes in form of the linear and spot-shaped erosions (Fig.1). The results of microscopic examination in this group demonstrated that indomethacin caused the appearance of severe injuries of the mucosa with the presence of extensive edema and leukocyte infiltration in the submucosal layer. Gastric mucosa appeared atrophic, coating-patching epithelium was dystrophic. Necrotic changes in ulcerative defects reached the muscle layer. Massive deposits of hydrochloric acid hematin occurred in many erosions. Conditions of moderate edema and inflammation were fixed in the muscle layer.

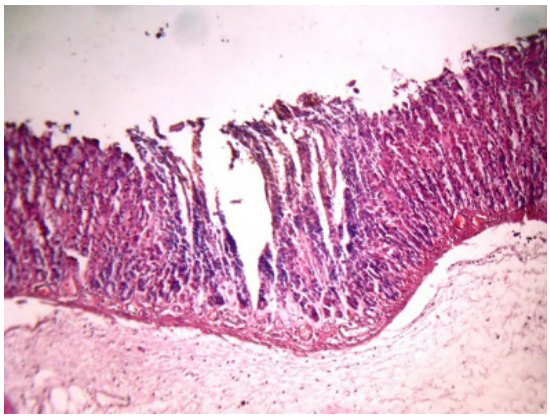

Fig. 1. *Deep linear erosion of gastric mucosa in a rat of Group 1. H&E staining, magnification ×100.*

In rats of Group 3, pre-treated with omeprazole, gastric mucosa macroscopically looked smooth and atrophic changes were not found. Weakly expressed destructive changes in the form of small spot erosions containing acid hematin were determined only in certain areas. All injuries were represented by spot erosions (Fig.2). Visible effects of mild inflammation occurred in the submucosal layer. The number of mucosal injuries was 6.9 times less than in Group 1 and the thickness of the mucous membrane was 1.8 times greater. The depth of spot erosions was 3.3 times less than in Group 1 (Table 1).

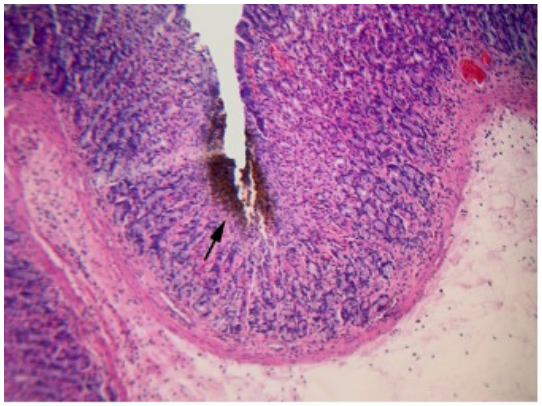

Fig. 2. *Spot erosion of gastric mucosa in a rat of Group 3. H&E staining, magnification ×100.*

Against this background, in animals of Group 2, which were pre-treated with the tripeptide Leu-Ile-Lys, macroscopically gastric mucosa also looked smooth and atrophic changes were not found. Destructive changes were not severe; they appeared only in the form of small spot erosions (Fig.3). Such destructive changes were observed in all animals.

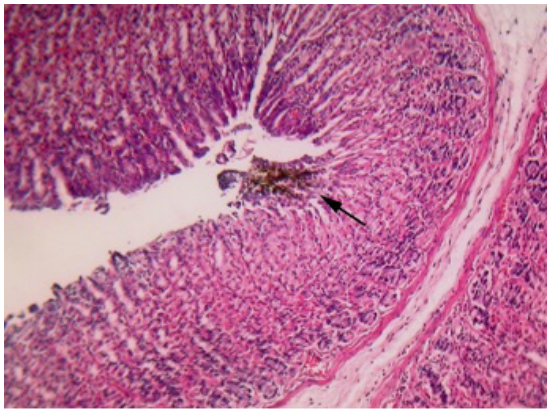

Fig. 3. *Spot erosion of gastric mucosa in a rat of Group 2. H&E staining, magnification ×100.*

Table 1.

Morphological indicators of the state of the gastric mucosa of rats in the studied groups

Index	Group 1	Group 2	Group 3	Statistics
The total number of injuries	9.6±1.7	1.75±0.25	1.4±0.4	F=165.764 P=0.000 P_{1-2}=0.000 P_{1-3}=0.0000
The number of linear erosions	4.8±0.9	0	0	
The number of linear erosion,%	51.1	0	0	
The number of spot erosions	4.6±1.4	1.75±0.35	1.4±0.4	F=32.972 P=0.000 P_{1-2}=0.000 P_{1-3}=0.000
The number of spot erosion,%	48.9	100	100	
Paul's index for the linear erosions	24	0	0	
Paul's index for spot erosions	16.8	1.75	1.4	
The thickness of the mucosa, microns	335.6±12.4	529.5±25.4	609.7±13.7	F=483.109 P=0.000 P_{1-2}=0.000 P_{1-3}=0.000 P_{2-3}=0.000
The depth of erosion, mm	385.75±23.7	56.7±6.4	115.8±5.2	F=1172.893 P=0.000 P_{1-2}=0.000 P_{1-3}=0.000 P_{2-3}=0.000
The density of the inflammatory infiltrate in 1 mm^2	1333.3±70.5	673.3±33.3	1040±211.7	F=51.563 P=0.000 P_{1-2}=0.000 P_{1-3}=0.001 P_{2-3}=0.04

The number of spot erosions was 2.6 times less than in Group 1 (Table 1). The average erosion depth was 6.8 times less than in Group 1, and 2.0 times less than in Group 3. Paul's index for spot erosions was 5.5 times less than in Group 1. The thickness of gastric mucosa on the periphery of erosions was 1.6 times greater than in Group 1. The density of the inflammatory infiltrate was 2.0 times less than in Group 1 and 1.5 times less than in Group 3. Parts of the columnar epithelium surface of mucosa, which were stained with Schick reagent, indicated a well-defined response to neutral mucopolysaccharides. In Stidmen staining of acidic mucopolysaccharides, an intensive staining was detected in cells of deep parts of the gastric pits. Weakly pronounced inflammation was observed in the submucosal layer.

Discussion

Thus, this study demonstrated a significant gastroprotective effect of the new tripeptide Leu-Ile-Lys on the model of indomethacin-induced gastric mucosal injury in rats. The effectiveness of the tripeptide Leu-Ile-Lys was comparable to the traditional drug, omeprazole, which is considered to be one of the most effective anti-ulcer agents.

It is commonly known that omeprazole suppresses basal acid secretion by inhibiting the proton pump in gastric parietal cells. At the same time, there were no data in the available literature showing that short-chain peptides may exhibit anti-secretory activity. It is possible that the mechanism of gastroprotective activity of tripeptide Leu-Ile-Lys, at least in part, can be determined by its ability to weaken the activity of the process of oxidation stress in gastric mucosa tissue, whose pathological role in the development of stomach ulcers is now recognized.[6,7] The reason to consider that suggestion is that the results of a previously conducted study showed us the pronounced antioxidant activity of the peptide complex from porcine kidney tissue in experimental urolithiasis and experimental gastropathy.[8,9] In addition, after the application of the tripeptide Leu-Ile-Lys, depth and density of the resulting erosion of the inflammatory infiltrate was significantly lower than after administration of omeprazole. Perhaps the tripeptide Leu-Ile-Lys has the ability to decrease an inflammatory reaction in the ulceration. Indirect confirmation of this ability can be regarded as recorded in our previous experiments that showed significant reduction of COX-2 expression in the gastric mucosa under the influence of the peptide complex from porcine kidney tissue in experimental gastropathy.[9]

In any case, the results of the present study clearly demonstrate the high gastroprotective activity, comparable to the action of omeprazole, of the new tripeptide Leu-Ile-Lys, patented by Altai State Medical University (priority date of 24.07.2018).

Conflict of interest

The authors declare that they have no competing interests.

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