Effect of Glyprolines PGP, GP, and PG on Homeostasis of Gastric Mucosa in Rats with Experimental Ethanol-Induced Gastric Ulcers

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The decrease in the severity of erosions and ulcer lesions after preventive treatment with PGP or PG correlated with a decrease in the content of lipid peroxidation products to a control level. Activities of SOD and catalase also returned to control values. GP produced the weakest effect on pro- and antioxidant state of the gastric mucosa. We concluded that the pronounced preventive effect of PGP and PG on the development of ethanol-induced erosions and ulcer lesions is largely determined by their antioxidant properties. Glyprolines can be considered as a promising means for prevention and treatment of stomach and duodenal ulcers.

Key Words: *glyprolines; ethanol; gastric mucosa; lipid peroxidation; antioxidant enzymes*

Glyprolines are endogenous regulatory peptides, because they are produced in the organism during synthesis and degradation of collagen, elastin, and other connective tissue proteins [1]. The glyproline family presented by short proline- and glycine-containing peptides PGP, GP, PG, *etc.* is now intensively studied because these peptides can be used as the basis for creation of natural gastroprotectors.

Experiments on various models of ulcer formation in the gastric mucosa (GM) showed PGP, GP, and PG reduced the area of lesions induced by stress, ethanol, indomethacin, and pylorus ligation [2,3,5,6,8,10]. However, each of PGP metabolites in relatively low concentrations was shown to be effective in a certain model of ulcer formation: PG in ethanol-induced and GP in stress-induced ulcers [2,5]. This effect was associated with stimulation of blood flow in GM and inhibition of hydrochloric acid secretion [4,7]. *In vitro* cell culture experiments also showed that PGP considerably reduced cell death under conditions of oxidative stress [9], which also attested to possible antioxidant properties of glyprolines and their possible use in ulcer disease. However, the effect of PGP, GP, and PG on LPO, the major mechanism of damage to GM, is poorly studied.

Here we studied the effects of PGP, GP, and PG on the formation of LPO products and activity of antioxidant defense enzymes in GM of rats with experimental ethanol-induced stomach ulcer.

MATERIALS AND METHODS

Acute experiments were carried out on albino male Wistar rats weighing 180-220 g (n=45). One day before the experiment the animals were deprived of food, but had free access to water. GM lesions were induced by intragastric administration of 96° ethanol in a dose of 1 ml/200 g body weight [2,3]. The animals were divided into 5 groups (9 rats per group). Group 1 animals were intact controls (intraperitoneally received 0.4 ml physiological saline). Group 2 animals (ethanol control) received 0.4 ml physiological saline (placebo)

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15 min before intraperitoneal administration of ethanol. Rats of groups 3, 4, and 5 received PGP, GP, PG, respectively, in a dose of 3.7 μmol/kg in 0.4 ml physiological saline 15 min before intraperitoneal administration of ethanol. One hour after administration of ethanol, the animals were sacrificed by a lethal dose of urethane (3 g/kg intraperitoneally). The stomach was cut along the lesser curvature and the mucosa was exposed, thoroughly washed, and examined using a transillumination gastroscope and a magnifying glass (×4). In each stomach, the area of ulcers and length of erosions were determined. Then the mean area of ulcers and mean length of erosions per one stomach for each group were calculated. In GM homogenate, the content of the main LPO products was determined: conjugated dienes (spectrophotometrically), TBA-reactive products (by the reaction with TBA), and Schiff bases (fluorometrically). The state of antioxidant defense system was evaluated by activity of SOD and catalase. The correspondence of the experimental results to normal distribution was verified using Shapiro–Wilcoxon test. The samples were compared using Student *t* test for independent samples.

RESULTS

The main area of ethanol-induced ulcers was 9.09±1.35 mm^2. The mean length of erosions per stomach was 6.89±1.44 mm. Ethanol also considerably intensified LPO processes leading to accumulation of conjugated dienes, TBA-reactive substances, and Schiff bases in GM by 85% ($p<0.001$), 52% ($p<0.001$), and 77% ($p<0.001$), respectively. Activity of the key enzymes of antioxidant defense system SOD and catalase also decreased by 35.3% ($p<0.01$) and 34% ($p<0.01$), respectively (Table. 1).

Preventive treatment with PGP and PG reduced the area of ethanol-induced ulcers in GM by 55% ($p<0.01$) and 85% ($p<0.001$), respectively. PGP and PG also reduced the length of erosions by 61% ($p<0.05$) and 77% ($p<0.01$), respectively. Dipeptide GP had no effect on the area of ethanol-induced ulcers, but reduced the length of erosions by 56% ($p<0.01$).

The decrease in the severity of erosions and ulcer lesions after preventive treatment with PGP correlated with a decrease in the content of LPO products to the control level. SOD activity in these animals did not significantly differ from the corresponding pa-

TABLE 1. Effect of PGP on Content of LPO Products and Activity of Antioxidant System Enzymes in GM Homogenate (*M*±*m*)

Parameter	Group		
	control	ethanol	PGP+ethanol
Conjugated dienes, nmol/mg protein	358.44±17.95	663.51±31.70***	372.15±27.55^{+++}
TBA-reactive substances, nmol/mg protein	86.35±5.26	131.17±10.02***	79.42±5.39^{+}
Schiff bases, arb. units/mg protein	74.35±5.94	131.59±10.52***	81.48±5.66^{+}
SOD, arb. units/min/mg protein	0.17±0.01	0.11±0.01**	0.15±0.01^{+++}
Catalase, μmol H_2O_2/min/mg protein	4.86±0.20	3.21±0.25**	5.15±0.27^{+++}

Note. Here and in Table 2: **$p<0.01$, ***$p<0.001$ compared to the control; $^{+}p<0.05$, $^{++}p<0.01^{+++}$, $p<0.001$ compared to ethanol.

TABLE 2. Effect of GP and PG on Content of LPO Products and Activity of Antioxidant System Enzymes in GM Homogenate (*M*±*m*)

Parameter	Group			
	control	ethanol	GP+stress	PG+stress
Conjugated dienes, nmol/mg protein	358.44±17.95	663.51±31.70***	501.06±32.17***$^{++}$	419.68±25.98^{+++}
TBA-reactive substances, nmol/mg protein	86.35±5.26	131.17±10.02***	119.37±10.39**	91.81±4.61^{++}
Schiff bases, arb. units/mg protein	74.35±5.94	131.59±10.52***	125.32±10.02**	92.40±7.87^{+}
SOD, arb. units/min/mg protein	0.17±0.01	0.11±0.01**	0.13±0.01	0.19±0.01^{+}
Catalase, μmol H_2O_2/min/mg protein	4.86±0.20	3.21±0.25**	2.73±0.20***	4.18±0.22^{+++}

rameter in the control, while catalase activity tended to normal.

PG produced similar effect on the pro- and antioxidant status of GM under conditions of ethanol-induced ulcer development. The effect of PG on catalase activity surpassed that of PGP (Table 2). GP produced the weakest effect on pro- and antioxidant status of GM.

Thus, the pronounced preventive effect of PGP and PG on the development of ethanol-induced erosions and ulcers is largely determined by their antioxidant properties. The gastroprotective effect of GP is probably determined by other important properties of glyprolines: suppression of basal and stimulated secretion of hydrochloric acid, stimulation of basal and stimulated secretion of bicarbonates, activation of blood flow in GM, *etc.* [4,7,8,11].

Our findings suggest that glyprolines can be considered as a promising means for prevention and treatment of stomach and duodenal ulcers.

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