Histomorphological Characteristics of Accelerated Healing of Acetate Ulcers under the Effect of Glyprolines

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Glyprolines (PGP, GPG, GPGP, PGPGP, and GPGPGP) modulated histomorphological characteristics of acetate ulcers. They accelerated healing of acetate ulcers, promote complete differentiation of the surface epithelium and glands in the gastric mucosa, contributed to the appearance of a considerable number of fibroblasts at the site of the regenerating mucosa, and significantly decreased the count of macrophages.

Key Words: *glyprolines; acetate ulcer; histomorphological characteristics*

Proline-containing oligopeptides PGP, PG, and GP belong to a family of glyprolines [2]. They not only increase the resistance of the gastric mucosa (GM) to ulcerogenic factors, but also decelerate the development and accelerate healing of acetate ulcers [1,3,5,6]. Histomorphological and temporal characteristics of these ulcers are similar to those of peptic ulcers in humans.

Here we studied the effect of 5 glyproline-containing peptides PGP, GPG, GPGP, PGPGP, and GPGPGP on histomorphological characteristics and healing of acetate ulcers in rats.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 190-250 g. The animals were anesthetized with ether and abdominal cavity was opened to get access to the stomach. Glacial acetic acid was applied to the serous layer of the gastric body for 15 sec. The stomach was put in the abdominal cavity. The cut was sutured. One of the test peptides (PGP, GPG, GPGP, PGPGP, and GPGPGP) in a dose of 3.7 μmol/kg (0.5 ml per 200 g) was administered intragastrically on day 4 after surgery (for 3 days). This period corresponds to most severe ulceration [3]. Control rats received an equivalent volume of physiological saline (0.5 ml per 200 g). The animals were euthanized on day 7 after surgery. The area of ulcers was measured using a binocular magnifier equipped with an ocular micrometer. In 3-4 animals of each group with moderate gastric ulceration, the ulcer was excised, fixed with freshly prepared Carnoy fluid (70% ethanol-chloroform-glacial acetic acid mixture, 6:3:1), and after 2 h transferred into 70% ethyl alcohol.

Sections (5 μ, cut along the ulcer) were prepared on a microtome and stained with hematoxylin and eosin by the standard method. This study was performed at the Institute of Human Morphology. Each section was examined for qualitative and quantitative characteristics, including cell composition (monocytes, macrophages, and fibroblasts), stage of inflammation, and architectonics of GM. Monocytes, macrophages, and fibroblasts were counted under a Mikmed-1 immersion microscope (LOMO, ×1000). The total number of examined cells was 100. The results were analyzed by Mann—Whitney

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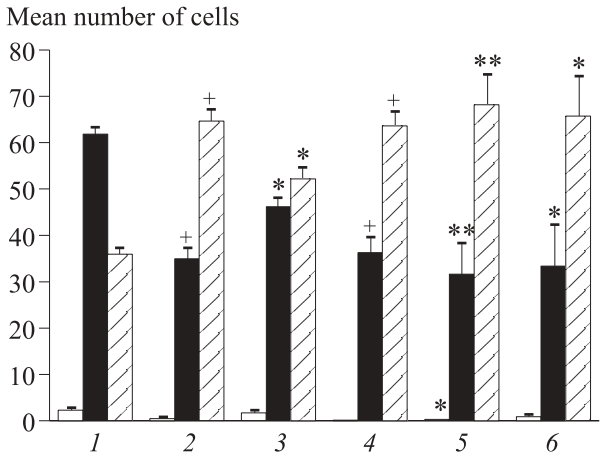

Fig. 1. Mean number of monocytes (light bars), macrophages (dark bars), and fibroblasts (shaded bars) in the site of ulceration after administration of peptides. Control (7/29, *1*); PGP (2/7, *2*); GPG (3/12, *3*); GPGP (2/6, *4*); PGPGP (6/12, *5*); and GPGPGP (3/13, *6*). The number of animals/sections is shown in brackets. **p*<0.05 and ***p*<0.005 compared to the control; +insufficient number of animals.

test (Statistica software). The differences were significant at $p<0.05$.

Microphotographs of histological preparations were made using an OLYMPUS photo device (×300, ×600).

RESULTS

Severe damage to GM was found in control animal. The area of damage was 30.3 mm^2. Examination of histological preparations revealed destruction of the epithelium and underlying tissues, which extended to the smooth muscle layer. The ulcer mainly included mature cells of the macrophage system (up to 62 macrophages). The number of fibroblasts corresponding to the stage of proliferation was 2-fold lower (up to 36 fibroblasts per 100 cells). Low number of monocytes was found (2 monocytes per 100 cells, Fig. 1). It should be emphasized that the number of monocytes (macrophage precursors) is high at the early stage of inflammation. Hence, day 7 of ulceration coincides with the end of inflammation and early stage of proliferation in control animals. These changes correspond to the initial

TABLE 1. Effect of Glyprolines on Healing of Acetate Ulcer in Rats

Group	Mean area of ulcer, mm^2	LST test (relative to the control)
Control (*n*=30)	30.30±1.82	
PGP (*n*=5)	15.30±2.14	0.000013
GPG (*n*=4)	17.63±3.41	0.000053
GPGP (*n*=8)	13.30±3.41	0.000000
PGPGP (*n*=7)	20.93±3.31	0.000002
GPGPGP (*n*=10)	16.49±2.36	0.000000

Note. *n*, number of animals.

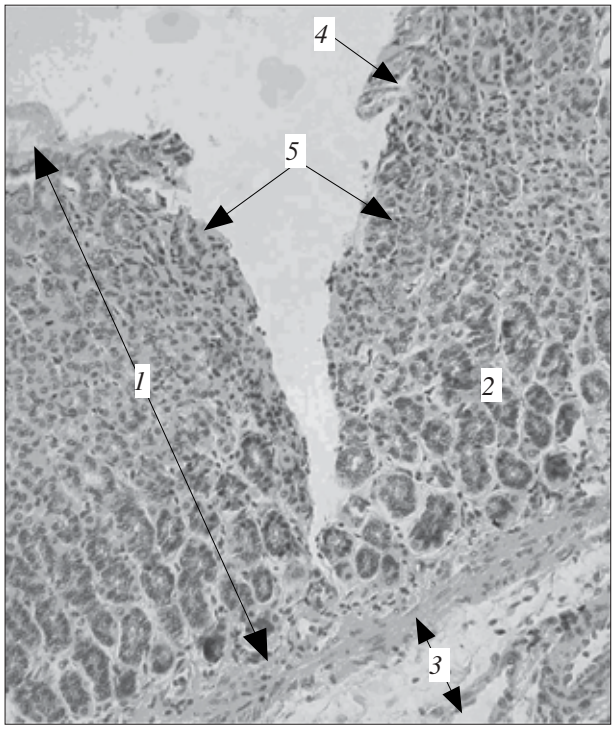

Fig. 2. GM in a control animal on day 7 after application of acetic acid. Hematoxylin and eosin staining (×600). GM (*1*); GM glands (*2*); smooth muscle layers (*3*); destructed epithelium (*4*); and cluster of cells of the macrophage system at the site of ulcerative lesion.

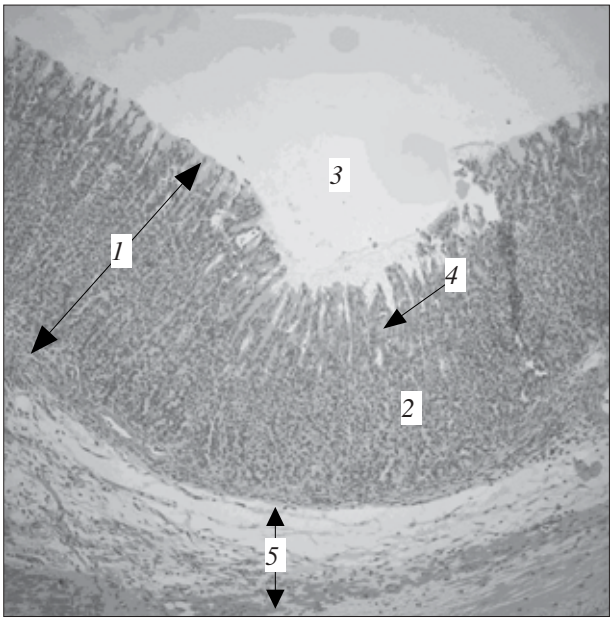

Fig. 3. GM after intragastric administration of PGPGP in a dose of 3.7 μmol/kg for 3 days. Hematoxylin and eosin staining (×300). GM (*1*); undifferentiated glands of GM (*2*); zone of ulceration (*3*); regenerated epithelium (*4*); and smooth muscle layer (*5*).

period of healing, but not to the stage of ulcer formation. Our results are consistent with published data [3].

All peptides (PGP, GPG, GPGP, PGPGP, and GPGPGP) significantly decreased the area of damage, which reflected acceleration of ulcer healing (Table 1). No significant differences were found in the effect of different glyprolines.

Study of histomorphological characteristics revealed complete regeneration of the epithelium and glands in GM (Figs. 2 and 3). The number of fibroblasts was highest in the regenerating mucosa of treated animals (52-68 fibroblasts per 100 cells). These cells produce collagen and glycosaminoglycans that are involved in reparation of damaged tissues. As differentiated from macrophages and fibroblasts, monocytes were practically absent in treated rats (similarly to control animals, 1 monocyte per 100 cells, Fig. 1). Intergroup differences were insignificant. The only exception was the PGPGP group. The number of monocytes in PGPGP-treated rats was far below the control level. The number of macrophages characterizing the inflammatory process decreased in animals of both groups (compared to the control).

Histological study showed that intragastric administration of PGP, GPG, GPGP, PGPGP, and GPGPGP accelerates ulcer healing and cell differentiation. This conclusion was derived from the decrease in the area of damage.

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