BIOGERONTOLOGY

Peptidergic Regulation of Expression of Genes Encoding Antioxidant and Anti-Inflammatory Proteins

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Geroprotective peptide T-34 regulates the expression of mRNA for various genes. The development of gastric ulcer is associated with morphological and molecular changes resulting from modulation of the synthesis of antioxidant and anti-inflammatory proteins. Peptide T-34 normalizes the synthesis of these proteins by regulating the expression of the corresponding genes.

Key Words: *geroprotective peptide; gene expression; antioxidant proteins; anti-inflammatory proteins*

Geroprotective peptides are characterized by stimulatory effects manifesting both at the level of organs and systems and at the molecular genetic level [1,3,5]. It is demonstrated that peptide preparations of the thymus exhibit immunomodulatory and anti-inflammatory effects, pineal peptides exhibit a pronounced antioxidant effect and are used in the treatment of not only age-associated diseases, but also in stress exposure of different kind [4,6,10,11]. However, the mechanisms of antioxidant and anti-inflammatory effects of peptide bioregulators at the genome level remain not quite clear.

We studied peptide regulation of the genome on the model of gastric ulcer. This model was chosen because gastric ulcer is paralleled by disorders in the functioning of antioxidant and anti-inflammatory system proteins [9].

MATERIALS AND METHODS

The study was carried out on male Sprague-Dawley rats (n=32; 180-220 g) divided into 4 groups, 8 per group: 1) intact rats; 2) ulcer+saline; 3) ulcer+T-34 peptide (Glu-Asp-Gly); and 4) ulcer+clarithromycin (antibiotic).

Ulcer was induced by three (at 4-h intervals) intragastric doses (25 mg/100 g) of cystamine-HCl (Aldrich, Milwannee, WI). The ulcer (28.0±3.5 mm^2) emerged at the interface between the antral and fundal portions of the stomach 12 h after the last dose. Simultaneously with the first cystamine-HCl dose the animals received intragastrically *Helicobacter pylori* culture (Curtin Matheson Scientific Inc.), strain Cag J117, 100 bacterial cells.

Peptide T-34 was injected subcutaneously (0.5 μg in 0.5 ml saline) over 5 days after ulcer appearance. Clarithromycin (Abbott) was injected intramuscularly (10 mg in 1 ml saline) for 5 days starting from the moment of ulcer development.

The material for analysis was collected from the ulcer edge on day 7 (for Western blotting and RT-PCR) and on days 7 and 21 for morphological studies.

Constitutive and inducible NO synthases (cNOS and iNOS), HSP70 heat shock protein, and NF-κBp65 transcription factor were selected as the signal molecules, whose expression reflected biochemical disorders in the gastric wall and reparative changes during

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the development of ulcer destruction [5,9]. The expression of these factors was studied by Western blotting. The specimens were electrophoresed in monomeric gel plates on nitrocellulose with 0.45-μ pores (Millipore) using sponges with abrasive coating (Scotch-Brite).

The electrophoretic chamber with a nitrocellulose sheet contained 0.7% acetic acid, the voltage gradient of 6 V/cm was maintained for 1 h. The sheets were treated with 3% BSA in saline (0.9% NaCl/10 mM Tris-HCl, pH 7.4) for 1 h at 40°C, washed in saline, incubated with antibodies (BioRad), washed 5 times for 30 min, and incubated with the second antibodies. Labeled spots were visualized using fluorescein isothiocyanate conjugated with horseradish peroxidase (1:100, Fluka) for 30 min at ambient temperature, after which the spots were photographed in far UV light through a yellow filter.

According to some reports, the effects of peptide geroprotectors manifest at the level of gene expression, and therefore we used RT-PCR [2,7,8]. The expression of matrix RNA (mRNA) was evaluated for antioxidant enzyme superoxide dismutase (SOD), TNF-α, and cycloxygenase (Cox-2). RT-PCR was carried out in 25 μl mixture containing buffer (40 mM Tris-HCl, pH 8.0, 2.5 mM $MgCl_2$, 25 mM KCl), 20 fmol DNA, 1 U activated Taq-DNA polymerase, 0.5 pmol of each primer labeled with the corresponding donor and acceptor fluorochrome. RT-PCR was carried out in an iCycler iQ DNA amplifier (BioRad): denaturation of double-stranded DNA at 94°C for 30 sec (1 cycle), primer annealing and elongation at 45°C for 10 sec with fluorescence registration, and denaturation of the target product at 80°C for 10 sec (a total of 25 cycles). Electrophoretic control of RT-PCR products was carried out in 8% PAAG in Tris-acetate buffer (pH 7.8) under non-denaturing conditions at voltage gradient of 4 V per 1 cm gel length in a vertical chamber for 4 h. The gel was stained with ethidium bromide and photographed on a Gel Camera System (UVP, Inc.).

Fragments of the gastric wall collected from the ulcer edge for morphological studies were fixed in 10% neutral formalin (pH 7.2), dehydrated in a Leica TP1020 automated station, and embedded in paraffin. Paraffin sections (5 μ) were mounted on slides coated with poly-L-lysine film (Sigma). For visual examination, the sections were stained with hematoxylin and eosin and with picrofuchsin after van Gieson.

RESULTS

Morphological and molecular studies showed that pathological changes characterizing the development of gastric ulcer were directly associated with expression of antioxidant and anti-inflammatory proteins.

In group 2, the ulcer on day 7 remained as large as on day 1 after induction. Pronounced perifocal edema was seen; the ulcer walls were coated with fibrinous necrotic deposit with signs of chronic inflammation and small erosions. A wide leukocytic necrotic layer was found on the ulcer bottom. Granulation tissue with numerous thin-walled blood vessels and predominating fibroblasts were found under it. Collagen fibrils with partially orderly orientation and few vessels were found in deeper layers of the granulation tissue. Pronounced diffuse inflammatory infiltration with predominating neutrophils was seen in granulation tissue adjacent to the necrotic zone and in deeper layers.

In group 2, ulcer defects in the gastric mucosa persisted on day 21 (Fig. 1, *a*). The ulcer bottom was covered with a thin necrotic layer and was diffusely

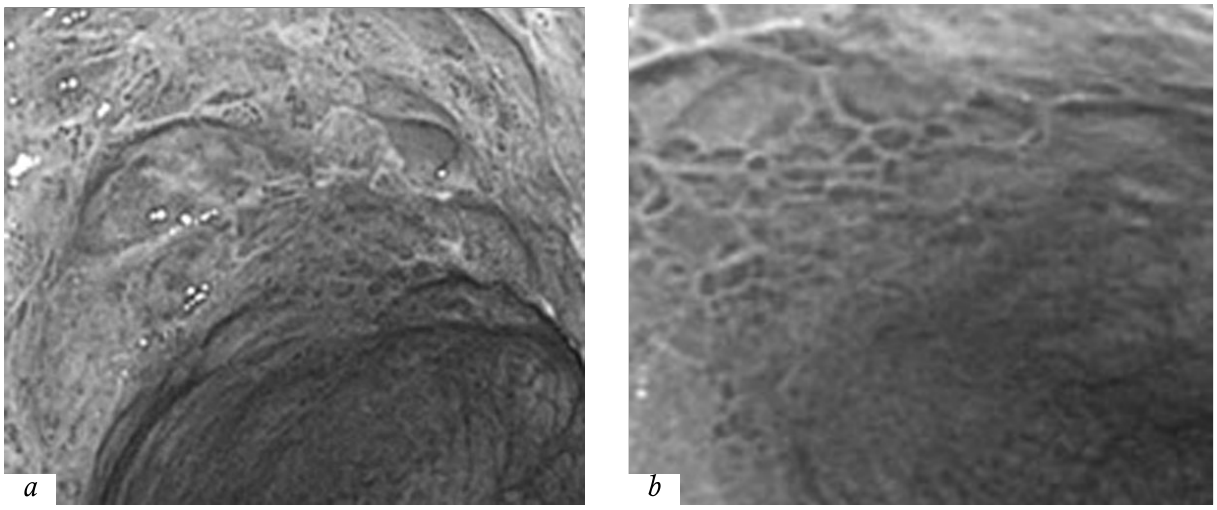

Fig. 1. Pathomorphosis of induced gastric ulcer. *a*) ulcer on day 21 without peptide treatment; *b*) complete epithelialization of ulcer on day 21 after T-34 peptide treatment.

infiltrated with leukocytes. Maturing granulation tissue in the sublying layer contained partially orderly oriented collagen fibrils and was moderately infiltrated with histiocytes, lymphocytes, and neutrophils.

In group 3 (injections of T-34 peptide), the area and depth of ulcer defect decreased on day 7; the ulcer bottom was free from detritus and inflammation round the ulcer reduced. Histological studies of the gastric ulcer showed a thinner leukocytic necrotic layer. The sublying layer consisted of maturing granulation tissue with numerous vessels and moderately pronounced diffuse leukocytic infiltration (mainly histiocytes, lymphocytes, neutrophils). Cyst-like dilated glands with epitheliocyte proliferation were detected in the ulcer edges.

No ulcer defects were detected on day 21 after T-34 peptide treatment (Fig. 1, *b*). The gastric mucosa was evenly epithelialized and was pink. The ulcer defect epithelialized. No ulcerative defects were found in the mucosa. There were just zones with glandular cavities lined with proliferating epithelium. Fibrous tissue with orderly oriented collagen fibrils and slight inflammatory lymphoid histiocytic infiltration were found in the sublying stroma. Injections of the antibiotic (clarithromycin; group 4) led to the same effects on days 7 and 21 as treatment with T-34 peptide.

Western blotting showed a significant (3-5-fold) increase of the expression of all the studied signal molecules in specimens of the mucosa from the gastric ulcer edges on day 7 after its induction in comparison with normal gastric mucosa from intact animals (Fig. 2). Enhanced expression of both NOS forms, HSP70, and p65 in the gastric ulcer indicated induction of proinflammatory cytokine synthesis, more intensive apoptosis, and excessive free radical reactions attesting to a pathological process.

Peptide T-34 repaired the gastric mucosa, which was seen from significant reduction of the expression of both NOS forms, HSP-70, and p65 to the control level (Fig. 2). The antibiotic (clarithromycin) exhibited a similar effect.

Hence, morphological and molecular studies showed that the reparation effect of T-34 peptide can be explained by normalization of the above proteins synthesis.

RT-PCR studies showed that the expression of mRNA for SOD, TNF-α, and Cox-2 increased significantly in the mucosa specimens from the edge of ulcers on day 7 after their induction (Fig. 3). This fact indicated changed expression of the genes producing proteins involved in antioxidant and antiapoptotic defense.

Treatment with T-34 peptide led to reduction of SOD, TNF-α, and Cox-2 mRNA synthesis to the control level. This indicated that the reparation effect of this peptide was due to regulation of the expression of

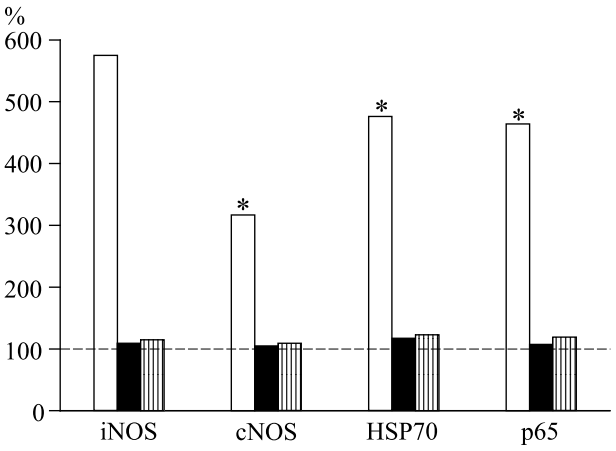

Fig. 2. Expression of signal molecules in the gastric mucosa. The level of expression is shown in % of control (100%; dotted line). Light bars: gastric ulcer (group 2), dark bars: ulcer+peptide T-34 (group 3), vertically hatched bars: ulcer+clarithromycin (group 4). Here and in Fig. 3: **p*<0.05 in comparison with the corresponding values in control.

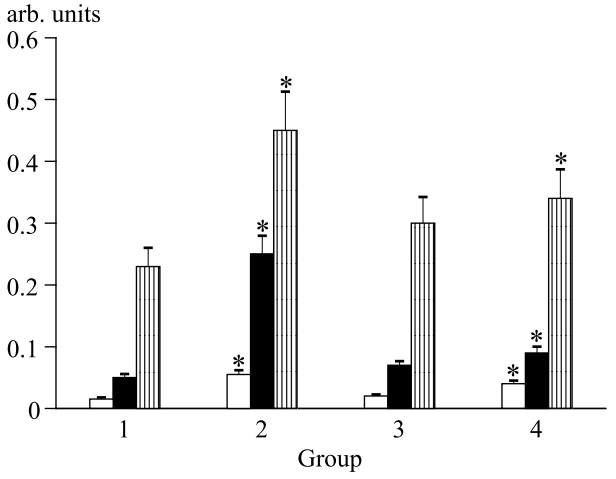

Fig. 3. Expression of signal molecules mRNA in the gastric mucosa. The expression is shown in arb. units reflecting the intensity of gel staining. Light bars: TNF-α; dark bars: SOD; vertically hatched bars: Cox-2.

genes encoding proteins of the antioxidant system and inflammatory reaction.

Clarithromycin also reduced the expression of the studied mRNA in comparison with group 2, but none of the values reached the control level (Fig. 3). The reparation effect of T-34 peptide used for the treatment of gastric ulcer was stronger at the gene level.

Hence, T-34 peptide regulation of the expression of genes encoding antioxidant system and inflammatory reaction proteins underlies the mechanism of its reparative effect.

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