Effect of Ultralow Doses of Antibodies to Histamine on the Development of Chronic Ulcerative Process in the Stomach of Experimental Animals

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Antiulcer activity of ultralow doses of antibodies to histamine was demonstrated on the model of chronic acetate-induced gastric ulcer in rats. Course therapy with the preparation accelerated healing of chronic experimental ulcer by correcting hemodynamic disturbances and stimulating mucus formation in the gastric wall.

Key Words: *antiulcer activity; ultralow doses of antibodies to histamine*

Pharmacological activity of low and ultralow doses (ULD) of drugs attracts much recent attention [1,10]. The effects of ULD of many substances are as strong as the effects of these substances in therapeutic doses. ULD of antibodies do not suppress activity of the corresponding endogenous regulator, but modify its effects. A new class of drugs created on the basis of ULD of antibodies is characterized by the efficiency of therapeutic application and the absence of toxic effects. In gastroenterology, regulatory molecules can be actual targets for creation of these drugs. Since histamine is a key molecule in the pathogenesis of ulcer disease of the stomach and duodenum, ULD of antibodies to histamine (ULD of anti-H) presumably modulating histamine-dependent activation of histamine receptors were selected as a potential antiulcer preparation.

We previously reported pronounced antiulcer properties of ULD of anti-H and demonstrated their antacid, antiinflammatory, and analgesic effects on the models of acute gastric ulcers of different etiologies. However, the main drawback of acute ulcers is rapid disappearance of ulcer defects; therefore, the wound-healing effect of the preparation cannot be detected in this model. Protracted course of the pathological process in experimental chronic acetate-induced ulcer makes it possible to evaluate the therapeutic effect of the test drug.

Here we studied the effect of ULD of anti-H on the development of chronic ulcerative process in the stomach of rats.

MATERIALS AND METHODS

Experiments were carried out on 42 outbred male rats weighing 220-240 g (Laboratory of Experimental Biomodeling, Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences).

The preparation used in the study contained affinity-purified ULD of anti-H and was administered intragastrically in a single daily dose of 0.5 ml for 21 day starting from the day of ulcer induction. The control group received potentiated water in the same volume and according to the same scheme.

Chronic acetate-induced ulcer was modeled as follows [9]: laparotomy along the abdominal midline was performed under light ether narcosis and 0.05 ml 5% acetic acid was injected under the serosa of the anterior wall of the stomach. The operation wound was sutured layer-by-layer. The rats were sacrificed by

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ether narcosis on days 7, 14, and 21 of the experiment. After autopsy, the stomachs were washed and under a magnifying glass the area of ulcers was determined by the formula: $S=p\times[(R+r)/2]^2$, where R and r are halves of the greater and lesser diameters, respectively. The antiulcer effect of the test preparations was evaluated by the area of ulcers in the above specified terms.

For morphological study, the fragments of the stomach wall with ulcer were fixed in formalin and embedded into paraffin. Deparaffinized sections (5 μ) were stained with hematoxylin and eosin after van Gieson (for connective tissue), with Schiff reagent (for acid glycosaminoglycans, GAG), and with methyl green—pyronine after Brachet (for RNA) [8]. On sections stained after Brachet, tissue basophils in the ulcer edge per 1 mm^2 section area were counted and differentiated by the amount of granules into maximally granulated and partially and maximally degranulated [11].

The data were processed statistically using nonparametric Mann—Whitney test.

RESULTS

Macroscopic examination of the stomach from untreated rats on day 7 of the experiment revealed ulcers in the gastric mucosa (GM) with a depth of 1-2 mm and bottom area of 23.52±3.38 mm^2, the ulcers were surrounded by a wall due to inflammatory infiltration and swelling of the mucosa. The mean size of the ulcer with the wall in the control group was 98.06±15.04 mm^2. On day 14 of the experiment, the size of ulcers in the group receiving potentiated water remained unchanged: ulcer area and bottom area decreased by 21.8 and 19.9%, respectively (Table 1). Appreciable dynamics of ulcer healing in this group was noted only by day 21: ulcer area and bottom area decreased by 55.6 and 75.7%, respectively, compared to day 7.

Ulcer defects in rats receiving ULD of anti-H were smaller than in controls at all terms of observation (Table 1). On day 7, ulcer area decreased by 14.4% and the bottom area decreased by 19.9% compared to the corresponding values in the control group. Appreciable dynamics of ulcer healing by day 14 in the group receiving ULD of anti-H should be noted: ulcer area decreased by 61.2% and the bottom area decreased by 75.6% compared to the corresponding values in the control group. On day 21, ulcer area remained unchanged, but bottom area significantly decreased by 37.8% compared to that in untreated animals (Table 1).

The positive effect of ULD of anti-H on healing of chronic acetate-induced gastric ulcer is confirmed by morphological study. After 7 days, deep ulcer defect of GM, submucosa, and muscular layers filled with necrotic mass and granulation tissue was seen on histological preparations from rats of the control group. The serosa was thickened, infiltrated with leukocytes, and often adhered to the liver and pancreas. GM surrounding the ulcer defect was characterized by hyperemia, edema, and leukocytic infiltration (primarily with neutrophils and eosinophils). Degenerative changes in the surface epithelium culminating by desquamation and formation of surface erosions were noted. The content of GAG and RNA in the cytoplasm of pit epitheliocytes was not high. In rats with experimental gastric ulcer receiving ULD of anti-H, edema and hyperemia of GM and submucosa on day 7 were less pronounced. The content of RNA in the cytoplasm of chief cells of fundal glands was higher than in the control, which was confirmed by more intensive pyronine staining of these cells compared to the control. The number of partially degranulated mast cells in the ulcer edge increased (Table 2).

On day 14 of the experiment, an extensive ulcer defect filled with granulation tissue and residual cell

TABLE 1. Effect of Course Treatment with ULD of Anti-H on Healing of Chronic Acetate-Induced Gastric Ulcer in Male Rats (*M*±*m*)

Group	Area of ulcer defects, mm^2					
	day 7		day 14		day 21	
Control	98.06±15.04 / 23.52±3.38	(*n*=6)	76.65±20.82 / 18.83±5.77	(*n*=7)	43.53±9.77 / 5.71±0.83	(*n*=8)
Experimental	83.91±14.53 / 18.84±4.19	(*n*=7)	29.75±5.58* / 4.60±1.08**	(*n*=7)	30.53±7.33 / 3.55±1.11*	(*n*=7)

Note. Numerator: area of the ulcer with the wall; denominator: area of ulcer bottom. Here and in Table 2: *$p<0.05$, **$p<0.01$ compared to the control group.

TABLE 2. Characteristics of Mast Cells in GM of Rats with Experimental Chronic Ulcer Treated with ULD of Anti-H (*M*±*m*)

Terms of observation, days	Group	Number of mast cells per 1 mm^2	Degranulation degree, %		
			maximally granulated	partially granulated	maximally degranulated
Day 7	Control	41.2±2.7	59.6±3.3	31.8±3.6	9.3±2.9
	Experimental	56.6±6.4	28.5±1.6*	57.3±2.3*	14.1±1.6
Day 14	Control	45.8±2.5	49.2±6.8	40.0±6.7	10.7±1.7
	Experimental	69.0±3.1*	27.1±2.7*	57.9±2.1	15.0±1.6
Day 21	Control	54.0±51	51.2±2.4	38.8±7.0	10.0±4.7
	Experimental	60.8±5.5	42.1±2.2*	43.5±3.8	14.4±3.3

detritus was still seen in the gastric wall. The tissues surrounding the ulcer were characterized by hyperemia, edema, and degenerative changes of surface and glandular epithelium. In animals receiving ULD of anti-H, the pit epithelium was better preserved, the content of GAG in the cytoplasm of these cells and on the surface of GM around the ulcer defect was higher. Moreover, the absolute number of mast cells in the ulcer edge was also higher (Table 2).

On day 21, the ulcer defect in the control was completely cleansed from nectrotic mass and was partially filled with mucous glands, muscular layer was partially restored, but edema and plethora were still observed. Some fundal glands underwent cyst dilatation, chief cells were flattened. The number of cells with high GAG content in the cytoplasm increased (mucoid transformation of fundal glands), while the content of RNA in chief cells did not differ from normal. In rats receiving ULD of anti-H, no edema and hyperemia of the gastric wall were seen. The degree of ulcer healing was higher than in the control, but in none cases complete epithelization of the ulcer was observed. The granulation tissue filling the ulcer defect looked more mature and contained less cells and thicker collagen fibers. The content of RNA and GAG in the cytoplasm of epithelial cells was higher than in the control. Chief cells of fundal glands had usual structure and higher cytoplasmic content of RNA compared to the control.

Thus, against the background of chronic acetate-induced indolent ulcer and atrophic gastritis with reorganization of the function of fundal glands by the pyloric type in GM surrounding the ulcer, administration of ULD of anti-H accelerated ulcer healing compared to the control group.

A possible mechanism of the observed gastroprotective effect of ULD of anti-H is improvement of the defense barrier function of supraepithelial mucous layer of the gastric wall. Moreover, the increase in the number of mast cells involved into maintenance of the trophic processes in GM also plays a role in the mechanism of more active healing of chronic experimental gastric ulcer in rats treated with ULD of anti-H [3]. It was shown antihistamine preparations stimulate synthesis and secretion of antiinflammatory and protective substances improving circulation, increasing resistance to oxygen deficiency, and normalizing tissue homeostasis by mast cells. Mast cells are known to actively participate in reparative process during cicatrisation of gastric ulcer [3,12].

Our experiments demonstrated accelerated healing of experimental gastric ulcer due to alleviation of hemodynamic disturbances and stimulation of mucus formation in the gastric wall.

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