Antiulcer Activity of Ultralow Doses of Antibodies to Histamine under Experimental Conditions

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Ultralow doses of antibodies to histamine produced a considerable antiulcer effect in rats with gastric ulcers induced by various factors. Antibodies to histamine markedly decreased aggressiveness of the gastric juice.

Key Words: *antibodies to histamine; antiulcer effect; gastric juice*

Ulcer disease is a pathological condition with complex etiology and pathogenesis. More than 500 drugs are used for conservative therapy of this disease [2,5]. The incidence of recurrences and complications of ulcer disease progressively increases [2,6]. Effective antiulcer preparations with antisecretory activity (blockers of H_2 receptors and H^+/K^+-ATPase) produce various undesirable effects. For example, long-term suppression of hydrochloric acid secretion impairs barrier function of the stomach and promotes dissemination of *Helicobacter pylori* in the gastric mucosa (GM) [5,6]. Antibody production after long-term treatment with selective blockers contributes to the development of drug resistance [2,5]. The search for new highly efficient preparations affecting the mechanisms of ulceration and producing no side effects is an actual problem. Ultralow doses of antibodies to histamine obtained by homeopathic potentiation hold much promise in this respect.

MATERIALS AND METHODS

Experiments were performed on 71 female outbred rats. The animals weighing 220-240 g were obtained from the Laboratory of Biological Models (Institute of Pharmacology) and kept according to the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

For preventive treatment potentiated antibodies to histamine (PAB-H) in a daily dose of 0.5 ml were administered through a gastric tube for 5 (indomethacin-induced ulceration), 7 (pyloric ligation), and 8 days (ethanol-induced ulceration). The rats were exposed to ulcerogenic factors 1 h after the last injection. Control animals received distilled water according to the same schedule. The animals were deprived of food and bedding 24 h before ulcerogenic exposure. Ethanol (96% solution) and indomethacin in physiological saline (60 mg/kg) were administered intragastrically in single doses of 1 ml/200 g and 1 ml, respectively [7,8]. The rats were killed 1 and 6 h after administration of ethanol and indomethacin, respectively.

Acute ulcers were produced by ligation of the pylorus (H. Shay *et al*.) [9]. The surgery was performed under light ether anesthesia. The rats were killed by ether overdose 16 h after surgery. Secretory activity of the stomach was evaluated by the amount of gastric juice. In each sample pH was measured with an electronic ionometer. The concentration of active H^+ in the sample and output of free hydrochloric acid H^+ over 1 h were estimated by pH [3].

The stomachs were removed after autopsy. The count and type of ulcers were determined macroscopically. The degree of ulcerative destruction (Pauls index, PI) was determined. Antiulcer activity (AA) was calculated as the ratio between PI in control and experimental rats [4,7]. The preparation was considered to be effective, if AA≥2. The degree of GM damage was determined by measuring the total length and area of ulcers in each rat. The length of petechial destruction and large ulcers was 0.5 and 2 mm, respectively. AA activity of the preparation was determined by the severity of damages expressed in percents of the control level.

The results were analyzed by nonparametric Wilcoxon and Mann-Whitney tests and Fischer's angular transformation [1].

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TABLE 1. Effects of PAB-H on the Formation of Ulcers in the Stomach Produced by Various Damages to the Gastric Mucosa in Rats ($\bar{X}\pm m$)

Parameter	Ethanol		Indomethacin		Pyloric ligation	
	control (n=12)	PAB-H (n=14)	control (n=14)	PAB-H (n=16)	control (n=8)	PAB-H (n=7)
Number of rats with ulcers, %	100	93	100	100	100	71.4
Mean number of ulcers	14.0±1.0	7.5±1.1**	19.9±2.4	15.0±1.3***	3.4±0.8	1.4±0.6***
including						
petechiae	8.5±1.0	5.1±1.2***	7.6±1.7	5.5±0.8	1.1±0.5	0.1±0.1
strips	3.8±0.9	1.1±0.5***	6.9±1.2	6.3±0.8	0.3±0.2	0
large ulcers	1.9±0.7	1.2±0.5	5.5±1.1	3.3±0.9***	2.1±0.6	1.3±0.6
PI	14	6.98	19.93	15	3.38	1.02
AA	—	2.01	—	1.33	—	3.31

Note. Here and in Tables 2 and 3: *p<0.001, **p<0.01, and ***p<0.05 compared to the control.

RESULTS

PAB-H markedly decreased the number and severity of GM damages in rats with ethanol-induced ulcers (Table 1). The count of petechiae, strip-like damages, and large ulcers decreased by 40.6, 72.1, and 39.0%, respectively, compared to the control. Gastroprotective activity was manifested in a considerable decrease in the average number of ulcers (p<0.01 compared to untreated animals). The degree of GM damage decreased to 8.0±1.1 mm (*vs.* 43.0±10.6 mm in the control, p<0.01). The severity of damages was 17.5% of the control level.

In rats with indomethacin-induced ulceration PAB-H decreased the number of large ulcers and mean number of ulcers by 41 and 25%, respectively, compared to the control (Table 1). The total length of petechiae and strips tended to decrease. A decrease in the length of large ulcers was statistically significant (Table 2). The degree of GM damages decreased by 1.3 times compared to the control (p<0.05). The severity of damages was 35% of the control level.

Gastric damages modeled by the method of H. Shay [9] include neurodegenerative process and acid-peptic aggression. PAB-H decreased the number of animals with ulcers and count of damages (Table 1). The number of animals with large ulcers and count of these damages decreased by 18 and 39%, respectively, compared to the control. The number of animals with petechiae and nimber of these damages decreased by 49 and 88%, respectively, compared to the control (Table 1). PAB-H completely prevented the formation of strip-like ulcers. It should be emphasized that preventive treatment with PAB-H decreased the average number of ulcers and count of animals with ulcers by 2.4 times and 29.6%, respectively, compared to the control. The preparation decreased the severity of GM damage (Table 2).

Preventive treatment with PAB-H inhibited production of gastric acid and reduced acid-peptic aggression of the gastric juice (Table 3). H^+ concentration in the gastric juice and H^+ output over 1 h significantly decreased compared to the control (p<0.05, Table 3).

Our results show that preventive intragastric administration of PAB-H produces the gastroprotective effect in rats with ulcers induced by various factors. AA of the preparation was probably related to the stimulation of barrier functions of GM and synthesis of glycosaminoglycans, improvement of regional blood flow and re-epithelization of the mucosa, and activa-

TABLE 2. Effect of PAB-H on the Severity of GM Damage in Rats with Indomethacin-Induced Ulcers ($\bar{X}\pm m$)

Parameter	Indomethacin		Pyloric ligation	
	control (n=14)	PAB-H (n=16)	control (n=8)	PAB-H (n=7)
Length (area) of damages, mm^2	49.5±8.4	32.2±4.0***	9.81±2.32	3.99±1.94***
petechiae	3.8±0.9	2.8±0.4	0.22±0.08	0.03±0.03
strips	33.4±7.5	22.9±3.8	—	—
large ulcers	11.0±2.3	6.5±1.7***	9.3±2.4	4.0±2.0
Degree of GM damage	100	65**	100	40.7*

TABLE 3. Effect of Repeated PAB-H Administration (7 Times) on Gastric Acid Secretion in Rats After Pyloric Ligation ($\bar{X}\pm m$, n=5)

Parameter	Control	PAB-H
Volume of gastric juice, ml/h	0.87±0.14	0.80±0.17
Gastric juice pH	2.03±0.20	2.97±0.33**
Gastric juice H^+ concentration	17.21±6.80	4.71±3.70**
Output of free hydrochloric acid H^+ per 1 h (10^{-3} mmol/liter/h)	39.02±0.21	4.35±0.03**

tion of the antioxidant system [7,8]. The antiulcer effect of PAB-H is probably associated with the decrease in pH and gastric acidity. Previous studies revealed a correlation between histamine content, pH in the stomach, and activity of proteolytic enzymes [5,6]. It cannot be excluded that PAB-H modulate secretion and activity of histamine, which plays a key role in the pathogenesis of ulcer disease [2].

PAB-H hold much promise as an antiulcer drug.

REFERENCES

1. E. V. Gubler, *Computational Methods for Analysis and Recognition of Pathologic Processes* [in Russian], Leningrad (1978).
2. O. N. Minushkin, I. V. Zverev, G. A. Elizavetina, and L. V. Maslovskii, *Ulcer Disease* [in Russian], Moscow (1995).
3. V. G. Mysh, *Lab. Delo*, No. 4, 212-215 [1988].
4. Ya. I. Khadzhai and G. V. Obolentseva, *Farmakol. Toksikol.*, No. 4, 450-455 [1962].
5. Ya. S. Tsimmerman, *Permsk. Med. Zh.*, No. 4, 97-106 (1995).
6. A. A. Sheptulin, *Klin. Med.*, **74**, No. 8, 17-19 (1996).
7. Ch. F. Bon-Abbound, H. Wayland, G. Pauisen, and P. H. Guth, *Dig. Dis. Sci.*, **33**, No. 7, 872-882 (1988).
8. F. Bates, G. Buckley, and R. Strettle, *Br. J. Pharmacol.*, **14**, No. 11, 6-11 (1989).
9. H. Shay, S. A. Komarov, S. S. Fels, *et al.*, *Gastroenterology*, **5**, No. 1, 43-61 (1945).