Analgesic and Antiinflammatory Activity of Antibodies to Histamine under Experimental Conditions

S. G. Krylova, T. G. Razina, E. P. Zueva, E. N. Amosova, N. V. Shilova, Yu. L. Dugina*, and O. I. Epstein*

Ultralow doses of antibodies to histamine in produced an antiproliferative effect on experimental animals with inflammation. Analgesic activity of antibodies to histamine was revealed on the model of acetic acid-induced writhing.

Key Words: *antibodies to histamine; analgesic and antiinflammatory activity*

Inflammation, a general pathological process accompanying various diseases, is still an urgent medical problem [7]. Ulcer disease induced by various factors is accompanied by inflammation of the gastric mucosa preventing cicatrization of ulcers and epithelization of erosions [2,4]. Progressive inflammatory changes in the gastric mucosa contribute to the appearance or strengthening of pain syndrome. Regeneration of the gastroduodenal mucosa requires control of pain [1]. Persistent inflammation is the cause of recurrent ulcer disease. Moreover, inflammatory changes in the gastric mucosa promote autoimmune allergic processes [2].

The multistage pathogenesis of the disease dictates the use of specific drugs affecting various phases of ulceration [3]. The antiulcer effect is biologically complex and includes antiinflammatory and analgesic components [8].

Antibodies to histamine in ultralow doses possess antiulcer activity. Here we studied antiinflammatory and analgesic properties of these antibodies.

MATERIALS AND METHODS

Experiments were performed on 54 outbred mice and 25 rats 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.

Potentiated antibodies to histamine (PAB-H, mixture of homeopathic dilutions C12+C30+C200) were administered to mice in a daily dose of 0.3 ml for 7 days (for evaluation of analgesic activity of the preparation) or in a single dose of 0.3 ml (for evaluation of phlogolytic properties). The rats received PAB-H intragastrically in a daily dose of 0.5 ml for 8 days after implantation of cotton tampons. The last dose of PAB-H was given 1 h before ulcerogenic exposure. Control animals received distilled water according to the same scheme.

Analgesic activity of ultralow doses of PAB-H was studied on female outbred mice using the model of acetic acid-induced writhing. Acetic acid (0.2 ml, 3%) was injected intraperitoneally in a dose of 300 mg/kg [9]. Endogenous kinins formed in the medium with certain pH play a key role in the pathogenesis of pain. The response of mice to pain was estimated by the number of abdominal muscle cramps accompanied by stretching of hindlimbs and hyperextension of the back. Writhings were recorded over 20 min after administration of acetic acid. Indomethacin given perorally in a therapeutic dose of 10 mg/kg served as the reference drug. The efficiency of drugs was estimated by their ability to decrease writhings.

Antiexudative activity of PAB-H was evaluated routinely using the model of agar-induced edema. The development of this edema is associated with the activation of prostaglandin biosynthesis. The mice received subplantar injections of 1% agar (50 μl) into the hindlimb pad [11]. The animals were killed by cervical dislocation at the peak of agar-induced inflammation (5 h after administration of the phlogogen). Healthy and edematous limbs were dissected and weighted. The ability of preparations to reduce edema was estimated by the difference between the weights of edematous limbs in control and treated animals (percents of the control).

The effect of PAB-H on the proliferative phase of inflammation was studied in rats with cotton-induced granuloma [10]. Sterile cotton tampons (13 mg) were

Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences; "Materia Medica Holding" Research-and-Production Company

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TABLE 1. Effect of PAB-H on Pain Sensitivity in Female Outbred Mice with Acetic Acid-Induced Writhings ($\bar{X}\pm m$)

Parameter	Control (n=9)	Indomethacin (n=6)	PAB-H (n=9)
Number of writhings over 20 min	24.0±3.7	12.8±2.1**	6.9±1.7*
Suppression of pain sensitivity, %	—	46.5	71.3
Latency of writhings, min	3.8±0.3	4.0±0.7	4.5±0.8

Note. Here and in Table 2: *p<0.01 and **p<0.05 compared to the control.

implanted subcutaneously in the interscapular region of the back with needles for blood sampling (A-1-20×40-1.7 and 25). On day 8 the animals were killed by ether overdose. Tampons with granulation and fibrous tissues (GFT) were removed, weighted, dried in a thermostat at 37°C to a constant weight, and weighted again. The efficiency of treatment was estimated by inhibition of proliferation expressed in percents. The antiexudative effect was determined by the decrease in the weight of exudates.

The results were analyzed by nonparametric Mann-Whitney test and Fischer's angular transformation [5].

RESULTS

Analgesic activity is an essential component of pharmacological activity in antiulcer preparations. PAB-H decreased the number of writhings by 3.5 times compared to the control (Table 1). It should be emphasized that in 1 of 9 animals writhings were absent. The latency of writhings slightly increased in mice receiving PAB-H. Under these experimental conditions indomethacin reduced the number of writhings by 1.4 times (Table 1). Thus, PAB-H is superior to indomethacin by analgesic activity. Desensitization of pain receptors after administration of PAB-H is probably associated with inhibition of inflammatory transmitters.

Inflammation plays a role in the pathogenesis of ulcer disease. Studies of antiulcer preparations include evaluation of their influence on various antiinflammatory processes.

The animals receiving distilled water had pronounced edema. Five hours after agar injection the weight of edematous limbs in these animals was 148.5% of the control. Pretreatment with PAB-H decreased the volume of edema (by 18.6%, changes insignificant).

PAB-H decreased the initial weight of GFT by 1.4 times compared to the control (p<0.05, Table 2). It should be emphasized that PAB-H significantly suppressed the early proliferative phase of inflammation (by 24%). At this term the metabolism of sulfated glycosaminoglycans plays a key role in the formation of granulomas. The degree of exudation tended to decrease in animals receiving PAB-H. In these animals the weight of exudate decreased by 1.5 times compared to the control.

TABLE 2. Antiproliferative Activity of PAB-H in Rats with Cotton-Induced Granuloma ($\bar{X}\pm m$, n=12-13)

Parameter		Control	PAB-H
GFT weight, mg	initial	443.8±69.1	308.9±32.7**
	dry tissue	116.4±12.9	88.7±9.8**
Suppression of proliferation, %		—	24
Weight of exudate, mg		327.8±57.5	220.2±25.5
Suppression of exudation, %		—	30

Our results show that PAB-H exhibit potent analgesic (similarly to indomethacin) and antiproliferative activities. Analgesic activity of PAB-H probably attests to their involvement into regulation of the central and autonomic nervous system. Desensitization of pain receptors after administration of antibodies can probably associated with inhibition of algesiogenic compounds, including histamine, serotonin, kinins, and prostaglandins [6,8]. Taking into account the modern concept of regulation of inflammation, we hypothesize that antiproliferative activity of PAB-H is related to their effects on production of inflammatory transmitters histamine, neuropeptides, and endogenous opioid peptides [7].

REFERENCES

1. Kh. Brailski, *Klin. Med.*, **50**, No. 2, 87 [1972].
2. V. Kh. Vasilenko, A. L. Grebnev, and A. A. Sheptulin, *Peptic Ulcer Disease. Modern Notions about Pathogenesis, Diagnostics, and Therapy* [in Russian], Moscow (1987).
3. I. I. Goncharik *Gastrointestinal Diseases. Reference Book* [in Russian], Minsk (1994).
4. P.Ya. Grigor'ev, *Diagnosis and Therapy of Gastric and Duodenal Ulcers* [in Russian], Moscow (1986).
5. E.V. Gubler *Computational Methods for Analysis and Recognition of Pathologic Processes* [in Russian], Leningrad (1978).
6. I. I. Degtyareva and N. V. Kharchenko, *Peptic Ulcer Disease* [in Russian], Kiev (1995).
7. A. M. Dygai and N. A. Klimenko, *Inflammation and Hemopoiesis* [in Russian], Tomsk (1992).
8. O. N. Minushkin, I. V. Zverkov, G. A. Elizavetina, *et al.*, *Peptic Ulcer Disease* [in Russian], Moscow (1995).
9. C. Caschik, W. Dawson, and E. Ritchen, *J. Pharm. Pharmacol.*, No. 28, 330-336 (1977).
10. R. Meier, W. Schuler, and P. Dessulles, *Experimentia (Basel)*, No. 6, 469 (1950).
11. S. Moncada and J. Vane, *Pharmacol. Rev.*, **30**, 293-331 (1978).