Inhibition of Tryptase Release from Human Colon Mast Cells by Histamine Receptor Antagonists

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SUMMARY The main objective of this study was to investigate the ability of histamine receptor antagonists to modulate tryptase release from human colon mast cells induced by histamine. Enzymatically dispersed cells from human colon were challenged with histamine in the absence or presence of the histamine receptor antagonists, and the tryptase release was determined. It was found that histamine induced tryptase release from colon mast cells was inhibited by up to approximately 61.5% and 24% by the H_1 histamine receptor antagonist terfenadine and the H_2 histamine receptor antagonist cimetidine, respectively, when histamine and its antagonists were added to cells at the same time. The H_3 histamine receptor antagonist clobenpropit had no effect on histamine induced tryptase release from colon mast cells at all concentrations tested. Preincubation of terfenadine, cimetidine or clobenpropit with cells for 20 minutes before challenging with histamine did not enhance the ability of these antihistamines to inhibit histamine induced tryptase release. Apart from terfenadine at 100 μg/ml, the antagonists themselves did not stimulate tryptase release from colon mast cells following both 15 minutes and 35 minutes incubation periods. It was concluded that H_1 and H_2 histamine receptor antagonists were able to inhibit histamine induced tryptase release from colon mast cells. This not only added some new data to our hypothesis of self-amplification mechanisms of mast cell degranulation, but also suggested that combining these two types of antihistamine drugs could be useful for the treatment of inflammatory bowel disease (IBD).

It was reported that an increase in mast cells and mast cell degranulation are closely associated to several gastrointestinal diseases including idiopathic inflammatory bowel disease,[1] chronic ulcerative colitis,[2] Crohn's disease,[3-5] gastritis,[5] collagenous colitis,[6,7] irritable bowel syndrome[8,9] and chronic inflammatory duodenal bowel disorders.[10] Through releasing their proinflammatory mediators including histamine, tryptase, chymase, heparin and some cytokines,[11] mast cells actively participate in the pathogenesis of these intestinal diseases.

Histamine has been found to have a close relationship with IBD. For example, the secretion rate of histamine was increased in patients with active Crohn's disease compared to normal controls, and the secretion of histamine was related to the disease activity;[12] highly elevated mucosal histamine levels were observed with allergic enteropathy and ulcerative colitis[13] and an enhanced histamine metabolism was found with collagenous colitis and food allergy.[14]

Histamine is a primary amine synthesized from histidine in the Golgi apparatus, from where it is transported to the granules for storage in ionic associa-

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tion with the acidic residues of the glycosaminogly-can side chains of heparin and with proteinases. As only mast cells contain histamine in man (apart from histaminergic nerves and a few basophils in human tissues), histamine can be used as a marker for mast cell degranulation.

It was found recently that histamine was able to stimulate tryptase release from human colon mast cells,[15] which may represent a unique self-amplification mechanism of mast cell degranulation. However, the mechanism by which histamine acts on mast cells remains unclear. To date, a total of three histamine receptors H_1, H_2, and H_3 have been discovered in the human gut,[16,17] and they are all expressed on human mast cells.[18] This suggests that histamine may act on mast cells through these receptors. Since tryptase is a unique marker of mast cell degranulation,[19] we investigated the effects of histamine receptor antagonists on histamine induced tryptase release from human colon mast cells in the current study.

MATERIALS AND METHODS

Dispersion of mast cells

Human colon tissue was obtained from patients with carcinoma of the colon at colectomy. Only macroscopically normal tissue was used for the study. After removing fat, the tissue was washed and chopped finely with scissors into fragments of 0.5-2.0 mm^3, and then incubated with 1.5 mg/ml collagenase (Sigma) and 0.75 mg/ml hyaluronidase (Sigma) in MEM containing 2% fetal calf serum (FCS; 1 g colon/10 ml buffer) for 70 minutes at 37°C. Dispersed cells were separated from undigested tissue by filtration through nylon gauze (pore size 100 μm diameter), washed and then maintained in MEM (Gibco) (containing 10% FCS, 200 U/ml penicillin, 200 μg/ml streptomycin) on a roller overnight at room temperature. Mast cell purity, as determined by light microscopy after staining with Alcine blue, ranged from 3.5 to 5.4%.

Mast cell challenge

Dispersed cells were resuspended in HEPES buffered salt solution (HBSS, pH 7.4) with $CaCl_2$ and $MgCl_2$ (complete HBSS), and 100 μl aliquots containing 4-6 x 10^3 mast cells were added to 50 μl histamine (Sigma), the calcium ionophore A23187

(CI, Sigma), or a histamine receptor antagonist in complete HBSS and incubated for 15 minutes or 35 minutes at 37°C, respectively. The reaction was terminated by addition of 150 μl ice cold incomplete HBSS and the tubes were centrifuged immediately (500 x g, 10 minutes, 4°C). All experiments were performed in duplicate. Supernatants were stored at -20°C until tryptase concentrations were determined.

Inhibition of the tryptase release

For some experiments, the histamine receptor antagonist was preincubated with the cells for 20 minutes before histamine was added. For other experiments, the histamine receptor antagonist and histamine were added to the cells at the same time (no preincubation period). Histamine and the antagonist were then incubated with the cells at 37°C for 15 minutes. Data were expressed as the percentage inhibition of tryptase release, taking into account tryptase release in the presence and absence of the antihistamine. Based on our previous experiments, histamine concentrations at 100 μg/ml and 1,000 μg/ml were taken as the standard concentrations throughout the study.[15]

Tryptase measurement

Tryptase concentrations were measured with a sandwich ELISA procedure with a specific polyclonal antibody against human tryptase as the capture antibody and AA5, a monoclonal antibody specific for human tryptase as the detecting antibody.[20]

Statistical analyses

Statistical analyses were performed with SPSS software. Data were expressed as mean ± SEM. Where analysis of variance indicated significant differences between groups with ANOVA, for the preplanned comparisons of interest, Student's t test was applied. For all analyses, $p < 0.05$ was taken as significant.

RESULTS

Effects of secretagogues and anti-histamine on tryptase release from mast cells

At 15 minutes following incubation, histamine at 100 μg/ml and 1,000 μg/ml and CI at 1 μg/ml were able to induce 25.7 ± 5.8, 42.4 ± 13.8 and 27.9 ±

3.6 ng/ml tryptase release from colon mast cells respectively, whereas at the same time point spontaneous tryptase release (buffer alone) was 19.4 ± 4.7 ng/ml. Following a 35 minutes incubation period, 100 μg/ml and 1,000 μg/ml histamine were able to provoke 23.8 ± 5.1 and 26.7 ± 5.1 ng/ml tryptase release, respectively. The H_1 histamine receptor antagonist terfenadine at 100 μg/ml induced significant tryptase release following both 15 minutes and 35 minutes incubation periods. The H_2 histamine receptor antagonist cimetidine and the H_3 histamine receptor antagonist clobenpropit at all concentrations tested had no stimulatory effect on colon mast cells (Table 1).

Effects of anti-histamines on histamine induced tryptase release from mast cells

The concentration dependent inhibition of histamine induced release of tryptase from colon mast cells was observed when 1,000 μg/ml histamine and various concentrations of terfenadine were added to the cells at the same time (Fig. 1). Approximately 61.5% inhibition of histamine induced tryptase release was achieved with 100 μg/ml terfenadine (Fig. 1). As little as 1.0 μg/ml terfenadine was able to sig-

nificantly inhibit histamine induced tryptase release when histamine and terfenadine were added to the cells at the same time. Similarly, but to a much lesser extent, cimetidine at a concentration of 100 μg/ml was able to reduce histamine induced tryptase release by approximately 24%. Clobenpropit had little effect on histamine induced tryptase release. Terfenadine and cimetidine were also able to inhibit 100 μg/ml histamine induced tryptase release (data not shown). Preincubation of terfenadine, cimetidine or clobenpropit with cells for 20 minutes before challenging with histamine did not enhance the ability of these anti-histamines to inhibit histamine induced tryptase release (Fig. 2).
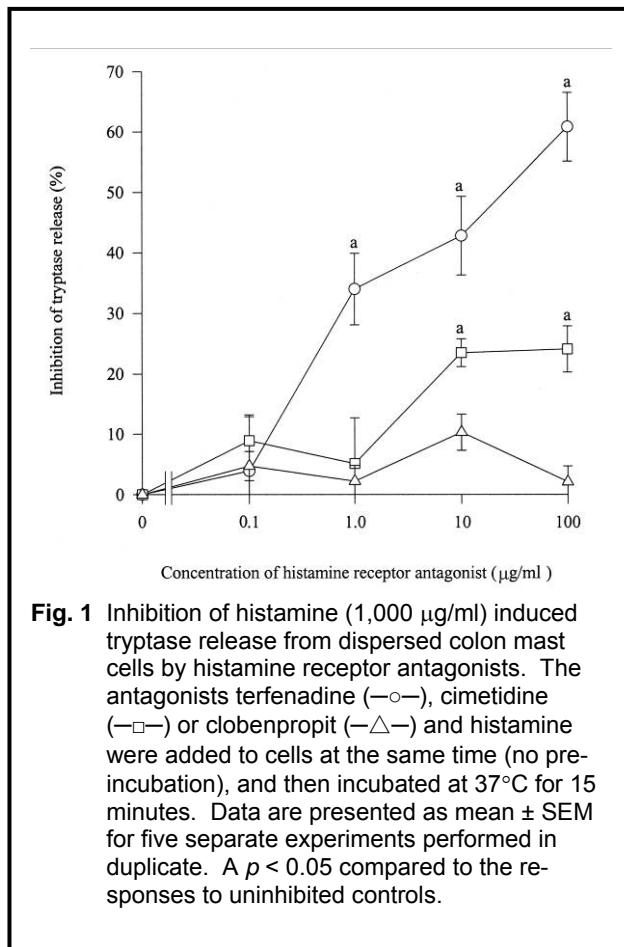
DISCUSSION

It was interesting to learn that the histamine H_1 receptor antagonist terfenadine was able to abolish more than 60% histamine induced tryptase release from colon mast cells, which indicated strongly that histamine induced tryptase release from colon mast cells were mainly through the H_1 receptor. Approximately 24% histamine induced tryptase release was inhibited by the histamine H_2 receptor antagonist cimetidine implicating that activation of mast cells

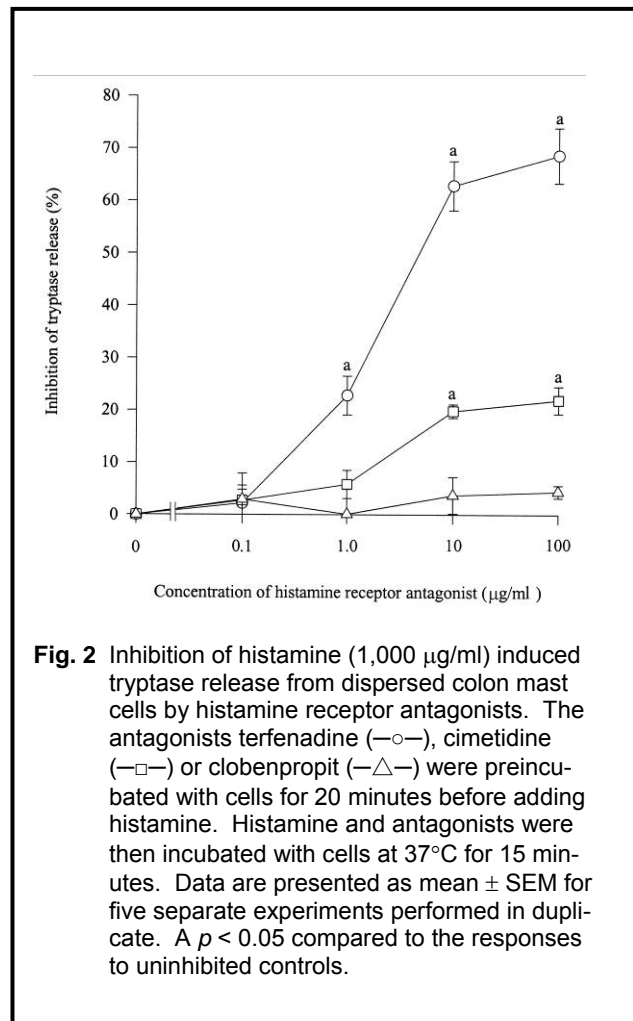**Table 1** Effect of histamine and its receptor antagonists on tryptase release from human colon mast cells

Compound (μg/ml)		Tryptase released (ng/ml)	
		15 minutes	35 minutes
Buffer alone		19.4 ± 4.7	17.8 ± 3.0
Terfenadine	0.1	19.5 ± 4.1	22.4 ± 3.7
	1.0	24.3 ± 4.9	16.2 ± 1.3
	10	24.2 ± 5.1	22.1 ± 4.9
	100	26.7 ± 2.1^a	26.5 ± 3.9^a
Cimetidine	0.1	24.1 ± 4.3	20.1 ± 4.9
	1.0	23.3 ± 3.5	18.9 ± 3.6
	10	22.3 ± 3.7	21.4 ± 3.2
	100	17.4 ± 4.0	16.9 ± 2.2
Clobenpropit	0.1	20.3 ± 3.8	21.6 ± 4.3
	1.0	20.6 ± 5.1	15.3 ± 2.9
	10	20.0 ± 4.2	20.4 ± 4.0
	100	23.0 ± 4.9	17.9 ± 6.5
Histamine	100	25.7 ± 5.8^a	23.8 ± 5.1^a
	1000	42.4 ± 13.8^a	26.7 ± 5.1^a
CI	1.0	27.9 ± 3.6^a	32.4 ± 3.9^a

The values are mean ± SEM for five separate experiments. a $p < 0.05$ compared with buffer alone control (Student's t test). CI = calcium ionophore.

Fig. 1 Inhibition of histamine (1,000 μg/ml) induced tryptase release from dispersed colon mast cells by histamine receptor antagonists. The antagonists terfenadine (—○—), cimetidine (—□—) or clobenpropit (—△—) and histamine were added to cells at the same time (no pre-incubation), and then incubated at 37°C for 15 minutes. Data are presented as mean ± SEM for five separate experiments performed in duplicate. A $p < 0.05$ compared to the responses to uninhibited controls.

Fig. 2 Inhibition of histamine (1,000 μg/ml) induced tryptase release from dispersed colon mast cells by histamine receptor antagonists. The antagonists terfenadine (—○—), cimetidine (—□—) or clobenpropit (—△—) were preincubated with cells for 20 minutes before adding histamine. Histamine and antagonists were then incubated with cells at 37°C for 15 minutes. Data are presented as mean ± SEM for five separate experiments performed in duplicate. A $p < 0.05$ compared to the responses to uninhibited controls.

by histamine was partially through H_2 receptors. The phenomenon that histamine acts on both H_1 and H_2 receptors for one pathophysiological event was observed previously when a pyrilamine and cimetidine combination was able to completely abolish histamine induced microvascular leakage in a guinea pig model.[21] In contrast to H_1 and H_2 receptors, H_3 receptors seemed to play no role in histamine induced activation of mast cells, as its antagonist clobenpropit did not show any effect on histamine induced tryptase release.

Cimetidine by itself did not stimulate tryptase release from mast cells indicating that within the doses tested cimetidine may be safe to be used as a mast cell stabilizer, whereas the dose of terfenadine should not exceed 10 μg/ml. Since the histamine content of mast cells is 2 to 5 pg/cell, 1,000 μg/ml histamine could only be achieved at the nearby surrounding area of the degranulating mast cells, which indirectly suggested that the potent inhibition of his-

tamine induced tryptase release by terfenadine should efficiently block histamine induced mast cell degranulation. The rapid blockage of histamine induced mast cell activation by anti-histamine drugs and the close relationship between histamine and IBD suggested that a combination of H_1 and H_2 histamine receptor antagonists might be useful for the treatment of IBD.

The evidence that actions of histamine on mast cells was through H_1 and H_2 histamine receptors added some new data in our hypothesis of self-amplification mechanisms of mast cell degranulation,22 which is that degranulated mast cells release histamine, histamine then either acts on its surrounding mast cells (paracrine mechanism) or back on its host mast cells (autocrine mechanism) through H_1 and H_2 histamine receptors.

In conclusion, the H_1 and H_2 histamine receptor antagonists were able to inhibit histamine induced tryptase release from colon mast cells, indicating that combining these two types of anti-histamine drugs may be useful for the treatment of IBD.

ACKNOWLEGEMENTS

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