

Histamine-Induced Activation of Human Lung Macrophages

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Key Words

Asthma · Histamine · β -Glucuronidase · Fexofenadine · Macrophages · IL-6 · Exocytosis

Abstract

Background: Histamine plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Increasing evidence suggests that histamine also regulates the function of inflammatory and immune cells. Macrophages are primarily involved in inflammatory diseases of the lung. We explored the ability of low concentrations of histamine to induce the release of proinflammatory mediators from human lung macrophages. **Methods:** Macrophages purified (>95%) from lung parenchyma by Percoll density gradients and adherence to polystyrene dishes were incubated (37°C, 2–24 h) with histamine (10^{-9} – 10^{-6} M). At the end of incubation, the release of β -glucuronidase and IL-6 was determined. **Results:** Histamine induced a concentration-dependent release of β -glucuronidase and IL-6 with a maximum release after 2 and 6 h of incubation, respectively. Exocytosis induced by histamine was noncytotoxic and was Ca^{2+} - and temperature-dependent. The effect of histamine was inhibited by the H_1 receptor antagonist fexofenadine but not by the H_2 antagonist ranitidine. **Conclusions:** These data indicate that histamine is an effective stimulus for exocytosis and cytokine production from human lung macro-

phages. These effects are inhibited by pharmacological concentrations of fexofenadine. Our results suggest that histamine may contribute to the long-term evolution of lung inflammation and tissue remodelling in allergic diseases by modulating the production of proinflammatory and immunoregulatory mediators by macrophages.

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Introduction

Allergic diseases are characterized by a complex interplay of dysregulated immune responses and an uncontrolled inflammatory reaction. Histamine is a major mediator of allergic inflammation synthesized and stored within cytoplasmic granules of human basophils and tissue mast cells [1, 2]. Activation of these cells induces the release of histamine at the sites of allergic reactions [3, 4]. The central role played by histamine in the pathophysiology of allergic disorders has been clearly established. Histamine exerts a variety of rapid inflammatory effects including bronchoconstriction, vasodilation, increased vascular permeability and stimulation of afferent sensory nerve terminals [5]. These effects are mostly mediated by interaction with H_1 receptors [6] and they are responsible for the majority of acute symptoms in bronchial asthma, allergic rhinitis and urticaria [7]. However, histamine may also influence immune responses by modulating the activity of T cells [8], monocytes [9] and macrophages

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1018–2438/01/1243–0249\$17.50/0

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[10]. These observations led to the hypothesis that the role of histamine in allergic disorders may not be limited to the acute inflammatory reaction but it may extend to the local dysregulation of immune responses.

In the last few years several studies have indicated that chronic release of histamine occurs in the airways of patients with bronchial asthma. For example, histamine has been detected in the bronchoalveolar lavage of patients with bronchial asthma even during remission [11, 12] and its concentrations have been correlated with asthma severity and bronchial hyperreactivity [13]. Basophils from patients with asthma have an increased capacity to release histamine *in vitro* as compared to those from healthy donors [12]. Finally, an increased number of degranulated mast cells and basophils has been documented in the airways of asthmatics even when biopsies were performed at a time distant from the acute attack [14]. These observations indicate that histamine is released in the airways of asthmatic patients even during asymptomatic periods and it may be involved in the pathogenesis of persistent inflammation and airway remodelling typical of chronic asthma [15].

The macrophage is the predominant cell in the airways and one of the most abundant in the lung parenchyma of both healthy individuals and asthmatic patients [16]. This cell plays a primary role in defense against infections and in the local modulation of immune and inflammatory responses. The macrophage synthesizes and releases a large spectrum of proinflammatory mediators, enzymes, cytokines and chemokines [17]. These molecules, in turn, may modulate the functions of T cells, epithelial cells, fibroblasts and infiltrating inflammatory cells such as eosinophils. Furthermore, macrophages express all three types of histamine receptors (H_1 , H_2 and H_3) [10, 18].

This study was designed to examine the role of low concentrations of histamine on the activation of human lung macrophages by evaluating its effect on exocytosis and IL-6 production.

Methods

Human Lung Macrophage Isolation and Cell Incubation

Human macrophages were obtained from the lung parenchyma of patients undergoing thoracic surgery for lung carcinoma, as previously reported [19]. Macrophages adherent to 24-well plates were incubated (37°C, 15 min to 18 h) in RPMI containing various concentrations of freshly prepared histamine. In some experiments, the cells were preincubated (37°C, 15 min) with various concentrations of fexofenadine or ranitidine before the addition of histamine. At the end of the experiment, the supernatant was removed, centrifuged twice (1,000 g, 4°C, 5 min) and stored up to 72 h at -80°C for subse-

quent determination of β -glucuronidase and IL-6 release. Selected experiments were performed by incubating the macrophages in the absence of extracellular Ca^{2+} and with EDTA (5 mM) or at 4°C. At the end of each experiment, an aliquot of cells was stained with trypan blue to determine cell viability. The cells remaining in the plates were lysed with 0.1% Triton X-100 for the determination of total cellular content of proteins and β -glucuronidase.

β -Glucuronidase and IL-6 Release

β -Glucuronidase release in cell-free supernatants or in cell lysates was measured by a colorimetric assay [20]. IL-6 release in the culture supernatant of macrophages was measured in duplicate determinations by a commercially available ELISA (Euro Clone, Torquay, UK) according to the manufacturer's instructions. The linearity of the assay ranged between 5 and 150 pg/ml. Since the number of adherent macrophages can vary in each well and in different experiments, the results were normalized for the total protein content in each well determined in the cell lysates (0.1% Triton X-100) by the method of Lowry et al. [21].

Results and Discussion

Initial experiments were performed to examine whether histamine induced the release of β -glucuronidase, a marker of exocytosis [22], from human lung macrophages. Figure 1 shows the results of six experiments indicating that incubation of macrophages with low concentrations of histamine (10^{-9} – 10^{-7} M) for 2 h results in the concentration-dependent release of β -glucuronidase. The effect of histamine is significant at a concentration of 10^{-9} M and it is maximum at a concentration of 10^{-7} M. At the latter concentration histamine induces release of β -glucuronidase that is 3-fold higher than that of unstimulated cells (7.2 ± 0.5 vs. 2.3 ± 0.3 ; $p < 0.01$).

To determine the type of receptor activated by histamine on macrophages, the cells were preincubated with fexofenadine, a second generation H_1 receptor antagonist [23], or with ranitidine, a selective H_2 antagonist [24], before the addition of histamine. Figure 1 shows that preincubation of macrophages with fexofenadine (10^{-5} and 10^{-4} M) for 15 min inhibits β -glucuronidase release induced by histamine. The inhibitory effect of fexofenadine is concentration-dependent and it has the pharmacological characteristics of a competitive antagonism, i.e. inhibition is reduced by increasing the concentrations of the agonist (histamine). In contrast, the H_2 antagonist ranitidine at concentrations up to 10^{-4} M had no effect on histamine-induced β -glucuronidase release (data not shown).

Histamine-induced β -glucuronidase release did not appear to be cytotoxic since the viability of macrophages, routinely assessed at the end of each experiment by trypan blue exclusion, was found to be always greater than 95%.

In addition, exocytosis induced by histamine did not occur when the cells were incubated in the absence of extracellular Ca^{2+} (and in the presence of EDTA) or at 4°C (data not shown).

Kinetic experiments indicated that the release of β -glucuronidase induced by histamine was relatively rapid, reaching a maximum between 30 min and 2 h after the addition of the stimulus and continuing for up to 18 h. These data indicate that concentrations of histamine such as those that can be found in the airways of asthmatic patients [11–13] are able to promote exocytosis from human lung macrophages. The release of lysosomal enzymes, such as β -glucuronidase, is thought to be an important event in inducing at least two features of airway remodelling such as the epithelial damage and basal membrane disruption [22, 25].

In addition, our results indicate that exocytosis induced by histamine is a process largely mediated by activation of the H_1 receptor on human macrophages. Pharmacological concentrations of the second generation H_1 antagonist fexofenadine competitively antagonize this effect of histamine.

Lung macrophages are an important source of cytokines regulating inflammatory and immune responses [17]. A major cytokine produced by lung macrophages is IL-6 [26], a multifunctional cytokine released in inflammatory diseases. This cytokine is a hematopoietic stem cell proliferation and differentiation factor; it has been implicated in human mast cell development and maturation and in the regulation of IgE synthesis [26]. Increased levels of IL-6 have been detected in the blood and bronchoalveolar lavage after bronchial challenge of patients with asthma [27] and bronchial biopsies of these patients reveal an increased expression of IL-6 [28].

Table 1. Effect of fexofenadine on IL-6 release induced by histamine from human lung macrophages

	None	Fexofenadine, <i>M</i>			
		10^{-7}	10^{-6}	10^{-5}	10^{-4}
Control	0.83 ± 0.18	–	–	–	–
Histamine 10^{-7} <i>M</i>	1.50 ± 0.33^a	1.25 ± 0.24	1.00 ± 0.16^b	0.95 ± 0.22^b	0.90 ± 0.15^b
Histamine 10^{-6} <i>M</i>	1.85 ± 0.46^a	1.53 ± 0.30	1.20 ± 0.32	1.06 ± 0.25^b	0.98 ± 0.28^b

Purified lung macrophages (>98%) were preincubated (37°C , 15 min) with buffer or with the indicated concentrations of fexofenadine and were subsequently incubated (37°C , 6 h) with the indicated concentrations of histamine. At the end of the incubation, the supernatant was removed and centrifuged (1,000 g, 4°C , 5 min). IL-6 release was determined by ELISA. The values are expressed as ng/mg of protein and they represent the mean $\pm$ SE of four experiments. ^a $p < 0.05$ vs. control; ^b $p < 0.05$ vs. corresponding samples incubated with histamine alone.

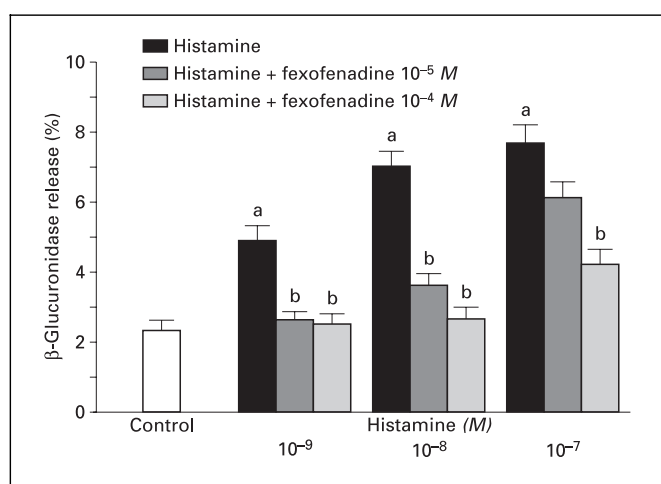


Fig. 1. Effect of histamine on β -glucuronidase release from human lung macrophages. Purified lung macrophages (>98%) were preincubated (37°C , 15 min) with buffer or with the indicated concentrations of fexofenadine and were subsequently incubated (37°C , 2 h) with the indicated concentrations of histamine. At the end of incubation, the supernatant was removed and centrifuged (1,000 g, 4°C , 5 min). β -Glucuronidase release was determined by a colorimetric technique [20]. The values are expressed as the percentage of the total cellular β -glucuronidase determined in cell aliquots lysed with 0.1% Triton X-100, and they represent the mean $\pm$ SE of six experiments. ^a $p < 0.01$ vs. control; ^b $p < 0.01$ vs. corresponding samples incubated with histamine alone.

In the next group of experiments we determined whether histamine induced the production of IL-6 from human lung macrophages. In these experiments, the cells were incubated with increasing concentrations of histamine for 6 h at 37°C . Table 1 shows that histamine (10^{-7} – 10^{-6} *M*) concentration-dependently increases the basal secretion of IL-6. The effect of histamine is signifi-

cant at 10^{-7} M and it is maximum at 10^{-6} M. At this concentration, histamine induces release of IL-6 that is 2-fold the basal secretion. Kinetic experiments revealed that histamine-induced potentiation of IL-6 release from macrophages required 4–6 h to become evident and it continued through 24 h of incubation. Table 1 also shows that preincubation (15 min, 37°C) with fexofenadine (10^{-7} – 10^{-4} M) concentration-dependently inhibits IL-6 release from lung macrophages induced by histamine. Therefore, histamine, in addition to β -glucuronidase release, significantly enhances the basal secretion of IL-6 from lung macrophages. Both effects of histamine are mediated by activation of histamine H₁ receptors.

Macrophages and mast cells are in close proximity in the airways of asthmatic patients. Our data demonstrate that low concentrations of histamine are able to activate human lung macrophages to release lysosomal enzymes

and IL-6. These results suggest that the macrophage may be a target cell for histamine chronically released from lung mast cells in the airways of asthmatic patients [11–13]. By activating the H₁ receptor present on these cells histamine may participate in the pathogenesis of several events occurring in chronic asthma such as the perpetuation of inflammation and airway remodelling. Our results also lead to the hypothesis that long-term treatment of asthmatic patients with H₁ antagonists, such as fexofenadine, may have a beneficial impact on the long-term evolution of chronic asthma. Further studies are needed to understand precisely the biochemical basis of the effect of histamine on lung macrophages and to define the potential role of H₂ and H₃ receptors on these cells. However, our results emphasize the possible relevance of histamine not only as a mediator of acute symptoms of asthma but also in the long-term evolution of this complex disease.

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