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Immunopharmacology and inflammation

In vitro study of histamine and histamine receptor ligands influence on the adhesion of purified human eosinophils to endothelium

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Histamine dihydrochloride (PubChem CID: 5818)
4-Methylhistamine dihydrochloride (PubChem CID: 215819)
Thioperamide maleate (PubChem CID: 11211984)
Diphenhydramine hydrochloride (PubChem CID: 8980)
Pyrilamine maleate (PubChem CID: 5284451)
Ranitidine hydrochloride (PubChem CID: 3033332)
Famotidine (PubChem CID: 3325)
JNJ7777120 (PubChem CID: 4908365)
Pitolisant (PubChem CID: 9948102)
N-formyl-Met-leu-Phe (fMLP) (PubChem CID: 443295)

ABSTRACT

It is a well-known fact that histamine is involved in eosinophil-dependent inflammatory responses including cellular chemotaxis and migration. Nevertheless, the relative role of histamine receptors in the mechanisms of eosinophils adhesion to endothelial cells is not known. Therefore the aim of presented study was to examine the effect of selective histamine receptors ligands on eosinophils adhesion to endothelium. For that purpose the highly purified human eosinophils have been isolated from the peripheral blood. The viability and functional integrity of isolated eosinophils have been validated in several tests. Histamine as well as 4-methylhistamine (selective H_4 agonist) in concentration-dependent manner significantly increased number of eosinophils that adhere to endothelium. Among the selective histamine receptors antagonist or H_1 inverse agonist only JNJ7777120 (histamine H_4 antagonist) and thioperamide (dual histamine H_3/H_4 antagonist) had direct effect on eosinophils adhesion to endothelial cells. Antagonists of H_1 (diphenhydramine, mepyramine) H_2 (ranitidine and famotidine) and H_3 (pitolisant) histamine receptors were ineffective. To the best of our knowledge, this is the first study to demonstrate that histamine receptor H_4 plays a dominant role in histamine-induced eosinophils adhesion to endothelium.

1. Introduction

Histamine is implicated in a variety of physiological and pathophysiological functions, including inflammatory processes (Mahdy and Webster, 2014). Four histamine receptors subtypes (histamine H_1-H_4 receptors) have been identified so far. All of them belong to the seven-transmembrane domain receptors, and are characterized with a different structure, function, distribution and affinity towards histamine (Leurs et al., 2009; Zampeli and Tiligada, 2009). The H_1 receptor is involved in: cellular migration, vasodilation, bronchoconstriction and nociception (Bakker et al., 2001), whereas the H_2 receptor modifies gastric acid secretion, vascular permeability and airway mucus production (Seifert et al., 2013). The H_3 receptor is playing important role in neurotransmission (Singh and Jadhav, 2013). Finally H_4 receptor is reported to be involved in inflammation (Tiligada, 2012). Histamine has been shown to activate immune cells, such as eosinophils, mast cells and dendritic cells. It is known that human eosinophils exhibit functional expression of three histamine receptors (H_1, H_2,

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H_4) (Ezeamuzie and Philips, 2000; Ling et al., 2004; Pincus et al., 1982; Reher et al., 2012). Nevertheless, the functional importance of each three histamine receptors, on human eosinophils has not been yet comprehensively characterized. This is mainly due to technical challenges in obtaining highly purified human eosinophils with preserved viability and functionality. Still using various methods of eosinophils isolation, it was demonstrated that activation of histamine H_4 receptors on human eosinophils results in intracellular calcium concentration flux, cytoskeleton and cellular shape change, adhesion molecules upregulation, cellular migration and chemotaxis (Buckland et al., 2003; Ling et al., 2004; Reher et al., 2012).

Migration, chemotaxis and trafficking of leukocytes, including eosinophils from the peripheral blood into the site of inflammation requires their adhesion to endothelial cells (Ley et al., 2007). Despite ongoing research, the histamine effect on eosinophils adhesion to endothelium cells has not been investigated yet. This may be important as eosinophils are considered pleiotropic, multifunctional, end-stage inflammatory granulocytes involved in allergy and asthma. During this inflammatory conditions the increased number of blood and tissue eosinophils are observed, contributing to the disease pathogenesis. Therefore several new strategies in treatment of eosinophilic disorders are developed that are aimed at blocking specific steps involved in eosinophil migration and adhesion (Rosenberg et al., 2013; Rothenberg and Hogan, 2006).

Here for the first time the role of histamine receptors in histamine-induced eosinophils interaction with endothelial cells was investigated. We provided detailed information on human eosinophils isolation from peripheral blood, using modified immunomagnetic separation technique, which represents combination of known cellular separation methods. In order to assure the reliable investigation, the purity, viability and functionality of human eosinophils was validated in detail. The involvement of histamine receptors in adhesion was studied using selective compounds (Fig. 1): H_1 antagonist/inverse agonist-diphenhydramine, mepyramine (Hill et al., 1997); H_2 antagonists-famotidine, ranitidine (Mahdy and Webster, 2014; Takagi et al., 1982); H_3 antagonist-pitolisant (Dauvilliers et al., 2013); H_4 antagonists-JNJ7777120 and thioperamide (Jablonowski et al., 2003; Liu et al., 2001). Additionally selective H_4 agonist 4-methylhistamine was also studied (Lim et al., 2005).

2. Materials and methods

2.1. Materials

For eosinophils isolation dextran from Leuconostoc spp. 500,000 (Sigma-Aldrich), Ficoll-Paque Plus d = 1.077 g/ml (GE Healthcare), ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich), and eosinophil isolation kit, mixture of: biotin-conjugated

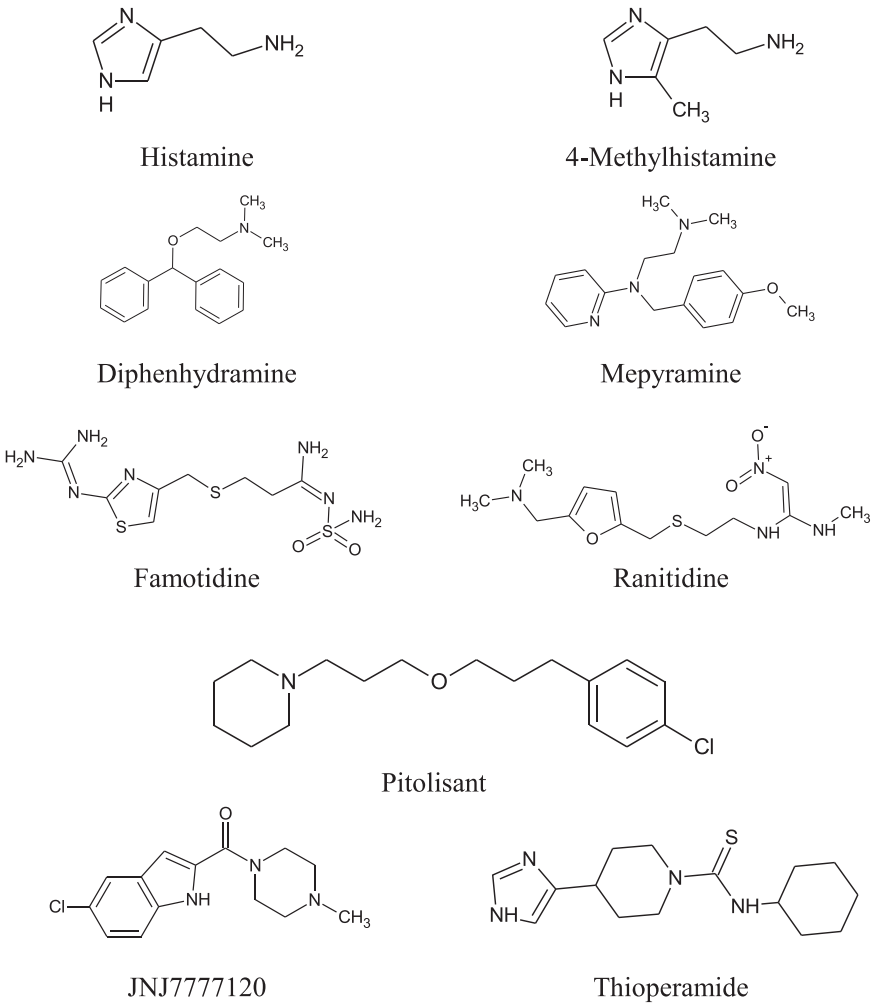

Fig. 1. Chemical structures of histamine receptor ligands used in this study: Histamine (natural agonist), 4-Methylhistamine (H_4 selective agonist), diphenhydramine (H_1 antagonist) and mepyramine (H_1 inverse agonist), famotidine and ranitidine (H_2 antagonists), pitolisant (H_3 antagonist), JNJ7777120 (H_4 antagonist) and thioperamide (H_3/H_4 antagonist).