

# Antihistamines as Important Tools for Regulating Inflammation

E. Nettis\*, M.C. Colanardi, A. Ferrannini and A. Tursi

Department of Medical Clinic, Immunology and Infectious Diseases – Division of Allergy and Clinical Immunology – University of Bari –Italy

**Abstract:** Allergic disorders are characterized by typical symptoms and an infiltrate of cells, including Th2 lymphocytes, eosinophils and mast cells. Activated mast cell mediators cause the early appearance of symptoms, and cytokines induce a cascade of inflammatory events. Both resident and infiltrating cells are important sources of those mediators and cytokines which maintain and enhance the allergic inflammatory response.

The predominant preformed mediator released by mast cells and basophils is histamine, which binds to specific cell receptors to produce its clinical effects. Therapeutic intervention in allergic disease has thus commonly focused on blocking the action of histamine. Ever since Arunlakshana demonstrated, in 1953, the ability of antihistamines to inhibit histamine release by mast cells, numerous studies have been conducted, both *in vivo* and *in vitro*, to determine the H1 antihistamines additional properties which contribute to their clinical efficacy in the treatment of allergic disease. It has been reported that some antihistamines can also regulate the expression and/or release of cytokines, chemokines, adhesion molecules, and/or inflammatory mediators. Such properties make these agents important tools for the continuous long-term regulation of both early and late-phase allergic reactions. It appears likely that antihistamines exert these anti-inflammatory effects by means of both receptor-dependent and receptor-independent mechanisms. The receptor-dependent mechanisms seem to involve inhibition of the generation of NF- $\kappa$ B dependent cytokines and adhesion proteins. The latter mechanisms, which require higher drug concentrations, appear to include the release by inflammatory cells of pre-formed mediators, such as histamine and eosinophil proteins as well as eicosanoid generation and oxygen free radicals production.

Herein, we review the current state of knowledge of the anti-inflammatory properties of antihistamines and their mechanisms.

**Key words:** anti-inflammatory effects, antihistamines, histamine, urticaria.

## ALLERGIC REACTIONS

Allergic reactions in the upper and lower airways and skin represent an overexuberant immune response to the allergen, resulting in common pathological mechanisms comprising early-phase (EP) and late phase (LP) reactions.

The early-phase response occurs within minutes of antigen exposure and subsides after 30-90 minutes. In contrast, the late-phase reaction begins around 4-8 hours after the early-phase response, continues for several days and may lead to the development of chronic inflammatory disease. LP reactions are characterized by the infiltration of the site of allergic inflammation by leukocytes, a significant number of which are eosinophils [1-2].

Type I allergy is initiated by a specific immune reaction to antigen that results in the preferential differentiation and activation of Th2 (CD4+) lymphocytes. The subsequent release of interleukin 4 (IL4) and IL13 from the Th2 cells induces B-cell synthesis of antigen-specific IgE antibodies. These reactions are triggered by binding of an allergen to a specific IgE found on the surface of mast cells or basophils;

depending on the target organ affected, the signs and symptoms of the specific allergic condition will ensue.

Mast cells are tissue cells, whereas basophils are blood cells. Mast cells are normally distributed throughout connective tissue, especially in the areas of the skin and mucous membranes of the respiratory, gastrointestinal and genitourinary tract, and adjacent to blood and lymph vessels. This location places mast cells near surfaces that are exposed to antigens.

Both mast cells and basophils have granules that contain potent mediators of allergic reactions. These mediators are preformed in the cell or activated through enzymatic processing. During sensitisation, the allergen-specific IgE antibodies attach to receptors on the surface of mast cells and basophils. On subsequent exposure, the sensitising allergen binds the cell-associated IgE and triggers a series of events that ultimately lead to degranulation of the mast cells or basophils, causing release of their allergy-producing mediators and also sustained synthesis and release of cytokines, chemokines and growth factors. In turn, these activate leukocytes, endothelial and epithelial cells.

The preformed mediators of allergic reactions include histamine, acetylcholine, adenosine, chemotactic mediators and neutral proteases (e.g. tryptase). In addition, biochemical pathways are activated that metabolise a component of cell

\*Address correspondence to this author at the Cattedra di Allergologia e Immunologia Clinica, Padiglione Chini – Policlinico, Piazza Giulio Cesare, 70124 Bari (Italy); Tel: 0039-080-5592821; Fax: 0039-080-5478780; E-mail: e.nettis@allergy.uniba.it

membranes called arachidonic acid. This can be metabolised down two major pathways by two different enzyme systems: cyclooxygenase and lipoxygenase. The end products of these pathways are called prostaglandins and leukotrienes, that can enhance the inflammatory process typical of allergic reactions.

All these mediators collectively produce chemical symptoms by increasing vascular permeability, inducing bronchospasm and activating nociceptive neurons linked to parasympathetic reflexes. Simultaneously, some mast cells mediators up-regulate the expression on endothelial cells of adhesion molecules for leukocytes, which are key elements in the late-phase allergic response. Endothelial activation results in the expression of endothelial adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1). Epithelial activation is associated with the generation and release of a number of chemokines such as “regulated on activation, normal T-cell expressed and secreted” (RANTES), macrophage inflammatory protein (MIP)-1, monocyte chemoattractant protein (MCP)-1, IL8 and eotaxin, which are chemoattractants for eosinophils, mast cells, lymphocytes, neutrophils and basophils.

All these events result in intravascular trapping of leukocytes and their migration from blood into the inflamed tissue site. The importance of this reaction is underscored by the fact that these cells, especially eosinophils, release numerous charged polypeptides (e.g. ECP), leukotrienes, pro-inflammatory cytokines and cytolytic enzymes that disrupt the epithelium and reinforce the inflammatory reaction [3-4].

Collectively, these responses culminate in the cardinal allergic features of the LP reaction in allergic rhinitis and asthma (nasal congestion, increased mucus secretion and airway smooth muscle cell hyper-reactivity) and skin (erythema, pruritus and oedema). Following EP and LP reactions the allergic response is perpetuated by infiltrating lymphocytes and macrophages that, by continuing to release proinflammatory cytokines, promote the development of a chronic inflammatory lesion, characterized by tissue remodelling, as demonstrated by the increased release of matrix metallo-proteinases.

## HISTAMINE

Histamine, an autacoid, has been recognized as a major mediator promoting allergic reactions and diseases [5]. It is produced and stored in cytoplasmic granules in tissue mast cells and basophils. The most important mechanism for histamine release is in response to immunological stimulus; in particular, it is released in large quantities by non cytotoxic mechanisms during the early phase of IgE-mediated reactions.

Other agents that induce mechanical/chemical histamine release comprise physical stimuli (e.g. cold, trauma), C3a and C5a (anaphylatoxins), interleukin-1, PAF and other histamine-releasing factors from polymorphonuclear cells (PMN), monocytes, platelets [6]. In addition, common drugs such as morphine or tubocurarine can displace histamine from granule storage sites.

The formation of histamine occurs by means of decarboxylation of amino acid L-histidine. It is metabolized to N-methylhistamine by an N-methyl-transferase or to acetic imidazole by monoamine oxidase.

Very little histamine is excreted unchanged as a result of these metabolic steps. Some exceptions include the cases of neoplastic disease or the following diseases: systemic mastocytosis, gastric carcinoid syndrome and urticaria pigmentosa.

The synthesis of histamine was identified in 1907 and it was characterized in 1910 by Barger and Dale as a substance (“beta-1”) capable of constricting guinea pig ileum [7].

In 1927, Lewis reported that a histamine-like substance was released from mast cells in the skin by the interaction of antigen and antibodies [8]. The connection between histamine and anaphylactoid reactions was made rapidly by Dale in 1929 [9], whereas the link to mast cells was not traced until 1952 [10] and the link to basophils in 1972 [11].

Histamine, like many other transmitters, mediates responses via receptors. H1 receptors are found in the smooth muscle of the intestines, bronchi, and blood vessels. In general, allergic responses are mediated by means of H1 receptors [12]. The H1 receptors possess all of the structural features of G-protein-coupled receptors, including 7 transmembrane domains, aminoterminal glycosylation sites, protein kinase A and protein kinase C phosphorylation sites [13]. They are preferentially stimulated by 2-methylhistamine and are encoded on chromosome 3. Activation of the H1 receptor stimulates the inositol phospholipid signalling pathways, resulting in formation of inositol-1,4,5-triphosphate (InsP3) and diacylglycerol (DAG) as well as an increase in intracellular calcium. The increase in intracellular calcium explains the wide variety of pharmacological effects of H1-receptor stimulation. For instance, histamine-induced mobilization of intracellular calcium might liberate arachidonic acid from phospholipids, leading to the generation of arachidonic acid metabolites. Similarly, it has been suggested that elevation in intracellular calcium leads to increases in cyclic adenosine monophosphate (cAMP) levels.

H2 receptors are located on the gastric mucosa, uterus, and brain. They are also expressed on lymphoid and inflammatory cells and on bronchial epithelium. Although activation of the H2 receptor is primarily important in augmenting gastric acid secretion from parietal cells, it also contributes to increase respiratory mucus secretion, nasal airways resistance [14-16]. H2 receptors tend to act as negative feedback receptors and to turn off the allergic reaction.

H2 receptors are stimulated preferentially by 4-methylhistamine and mediate an intracellular response characterized by elevations in cAMP.

H3 receptors are present in the brain and in bronchial smooth muscle [17]. They have been implicated in autocrine regulation of histamine synthesis and release from nerve tissue. In addition, the demonstration that H3 receptors are expressed in postganglionic cholinergic nerves in human bronchi has suggested that their stimulation may act as a protective mechanism against excessive bronchoconstriction.

H<sub>3</sub> receptors are preferentially stimulated by (R/N)-methylhistamine. Their activation is characterized by increases in IP<sub>3</sub> and intracellular calcium concentrations but has the additional effect of decreasing cAMP levels [18].

A new subclass of histamine receptor, H<sub>4</sub>, has been both functionally and pharmacologically characterized, and it has recently been cloned [19-21]. It is a G-coupled receptor encoded on chromosome 18 and its expression has been observed on peripheral blood mononuclear cells, neutrophils, eosinophils, mast cells, and CD4<sup>+</sup> T lymphocytes. Although only few functional studies on this receptor have been conducted, its distribution suggests that it may also have a role in immune/inflammatory responses.

There is also a fifth category including ill-defined histamine receptors such as an intracellular receptor labelled Hic. Its existence has so far only been inferred by the presence of small amounts of histamine in cells not traditionally thought to contain histamine [22].

The interaction of histamine with the histamine H<sub>1</sub> receptors mediates a variety of pathophysiological effects typical of allergic disease. Histamine causes vasodilatation, vascular permeability due to its ability to induce phosphorylation of an intercellular adhesion protein, called VE-cadherin, found on vascular endothelial cells. In addition, histamine causes smooth muscle contraction, mucus secretion, which lead to bronchial obstruction in asthma, pruritus, nasal blockage, sneezing, itching and discharge in rhinitis, and itchy skin wheals/flare in urticaria [23,24].

Considering all the above roles of histamine on H<sub>1</sub> receptors, it is clear that anti H<sub>1</sub>antihistamines are the most common drugs used to treat allergic diseases. Their history goes back to 1937 when Bonet and Straub produced the first drug with an antihistamine activity. However, the toxicity of this compound precluded its clinical use in humans. In 1942, Holpern described antergan, the first antihistamine used clinically in humans [25]. Many new antihistamines followed in rapid succession, classified as first or second-third generation. The first generation of antihistamines, like chlorpheniramine, promethazine, dihydrochloramine are very potent H<sub>1</sub>-receptor antagonists capable of reducing the symptoms of allergic rhinitis and those of chronic idiopathic urticaria. However, these potent drugs are not selective antagonists for the H<sub>1</sub> receptor, but bind to other receptors (acetylcholine, serotonin, calcium channels). In addition, their chemical structures are such that they possess lipophilic properties which allow them to cross the blood-brain barrier and to induce some side-effects in the central nervous system, such as decreased alertness, impairment of reaction times and vigilance, and sedation. In the 1970s, new drugs emerged. They consisted of second-generation agents (e.g. loratadine, cetirizine) and their metabolites, the so-called third generation antihistamines (e.g. fexofenadine, desloratadine, levocetirizine). These drugs possess many of the clinical effects of those of the first generation but lack significant central nervous system and anticholinergic side-effects.

Traditionally, all antihistamines have been considered as “antagonists” of the H<sub>1</sub> receptor. However, this theory has

recently been modified. In fact, they are now considered as “inverse agonists”. In accordance with this theory the H<sub>1</sub> receptors are present in two conformations, the inactive and active forms, which coexist in equilibrium. So, even in the absence of stimulation by histamine, constitutive receptor activation occurs. In this model, histamine acts as an agonist, by combining with and stabilizing the activated conformation of H<sub>1</sub>-receptors to shift the equilibrium towards the inactive state [26].

Traditionally, the efficacy of H<sub>1</sub>-antihistamines in allergic disorders is attributed primarily to their effects on histamine. However, these drugs also have anti-allergic and additional anti-inflammatory effects. In fact, since Arunlakshnanor and Schild demonstrated in 1953 that these drugs are able to inhibit histamine release from mast cells, numerous studies have been conducted, both *in vitro* and *in vivo*, to determine whether H<sub>1</sub>-antihistamines possess such properties, which might contribute substantially to the clinical efficacy of antihistamines in the treatment of allergic diseases [27].

They could exert anti-inflammatory effects by means of both receptor- dependent and receptor- independent mechanisms.

Receptor-dependent mechanisms involve the stabilization of the histamine receptor in its inactive conformation. A possible site where they could exert an anti-inflammatory activity is at the level of the transcription factors, NF-κB and GATA 3. This would lead to inhibition of these factors-dependent cytokines and adhesion molecules. This could explain, for instance, the findings by Scroeder *et al.* [28], that low concentrations of desloratadine inhibit the production of Th<sub>2</sub> cytokines IL4 and IL13. This would mean that all antihistamines could inhibit the production of cytokines and adhesion molecules due to a receptor-dependent effect. So the receptor-dependent effects of antihistamines may actually be clinically relevant.

Receptor-independent effects consist in inhibition of mast cell and basophil histamine release and inhibition of inflammatory cell activation.

Several studies examining the effects of H<sub>1</sub>-antihistamines on mediator secretion showed clearly that they have the capacity to inhibit histamine release. These studies established that there was no correlation between the concentrations of drugs which inhibited histamine release and their H<sub>1</sub>-antihistamines potency and that these drugs act on calcium channels; leading a reduction of the inward of calcium current activated by intracellular store depletion.

However, this effect requires high drug concentrations, not corresponding to therapeutic dosages, for example, it has been evaluated that the concentrations of H<sub>1</sub>-antihistamines required to prevent histamine release *in vitro* are 1-10μM, higher than those likely to occur *in vivo* [29].

As above mentioned, receptor-independent effects consist in inhibition of inflammatory cell activation too. This includes the de novo generation of proinflammatory products such as superoxide radicals, the arachidonic acid products LTB<sub>4</sub> and LTC<sub>4</sub>, and the release of granule associated products, such as neutrophil elastase and eosinophil cation protein (ECP). Also in these cases the suggested mechanism

is the inhibition of calcium mobilization and of the activity of membrane-associated enzymes such as inhibition of protein kinase C and NADPH oxidase. Also these actions require high drug concentrations (e.g. 10 $\mu$ M), not corresponding to therapeutic dosages.

So these anti-inflammatory properties related to receptor-independent effects remains uncertain [30].

Extensive studies have been conducted to clarify the mechanisms underlying the anti-inflammatory actions of antihistamines. These actions consist of the downregulation of adhesion molecules expression, mediator release, superoxide generation, chemotaxis, and cytokine expression, and the upregulation of the number and function of  $\alpha_2$  adrenoceptors. Below we would explain the more relevant anti-inflammatory actions of antihistamines

### 1. Inhibition of Adhesion Molecules Expression

It has been shown that epithelial cells play a crucial role during allergic inflammatory processes. In fact, several studies have suggested that in addition to providing a natural self-cleansing barrier against inhaled bacteria, particulates and allergens, the epithelial cells synthesize and release a large variety of inflammatory cytokines and adhesion molecules, that influence the activity of eosinophils, mast cells, macrophages and lymphocytes, which play important roles in the allergic reaction [31]. The ability to inhibit cell adhesion molecule expression by epithelial cells could limit leukocyte recruitment and thus have significant implications for the treatment of allergic diseases.

Adhesion molecules are expressed both by epithelial and endothelial cells. Among the adhesion molecules, intracellular adhesion molecule-1 (ICAM-1) plays a pivotal role [32-34]. Thus, ICAM-1 might be used as a reliable marker of clinical and even subclinical inflammation. In addition, ICAM-1 is a selective receptor for human rhinoviruses. Because rhinoviral infections are the most frequent event preceding asthma attacks in children, the continuous expression of ICAM-1 on epithelial cells in symptom-free allergic subjects should be regarded as a possible triggering event for asthma attacks.

The modulation of adhesion molecules expression by antihistamines has been demonstrated *in vitro* and *in vivo*.

In particular, by means of *in vivo* nasal and conjunctival challenge models, it has been demonstrated that several of the newer antihistamines reduce both ICAM-1 expression on nasal and conjunctival epithelial cells, and inflammatory infiltration. This effect has been observed both after allergen challenge and during natural exposure [35]. Ciprandi *et al.* were among the first authors to propose that modulation of ICAM-1 expression represents an important therapeutic target in allergic disease, and to investigate the effect of treatment with several antihistamines, including azelastine, cetirizine, levocabastine, loratadine, oxatomide and terfenadine, on natural or experimental allergen exposure-induced changes in the expression of ICAM-1 on nasal and conjunctival epithelial cells in pollen-sensitive rhinitis. The authors demonstrated that while treatment with cetirizine for a period of 2.5 days was sufficient to attenuate allergen-challenge-induced expression of ICAM-1 and inflammatory

cell infiltration into the conjunctiva of asymptomatic patients [36], 2-4 weeks treatment was required to attenuate natural allergen-induced expression of ICAM-1 on the nasal epithelial cells of these individuals [37,38].

More recently, these authors have compared the effect of continuous and on-demand treatment with cetirizine, over a 4-week period of natural allergen exposure, in patients with seasonal allergic rhinitis. They demonstrated that not only did continuous treatment provide significantly better symptom relief than on-demand treatment, but it also significantly decreased the numbers of eosinophils and neutrophils in the nasal mucosa, compared with on-demand treatment [39]. This study suggests that antihistamines are likely to be clinically useful in the management of inflammation in allergic disease, and also as prophylactic treatment. The same authors have evaluated the action of azelastine eye drops for ongoing allergic reactions [40]. They administered azelastine eyedrops 30 minutes after an allergen-specific conjunctival challenge and demonstrated reduced expression of ICAM-1. The supposed mechanisms underlying this downregulation were modulation of the NF- $\kappa$ B transcription factor (that is necessary for adhesion molecule expression), an action on mast cells by reducing the release of cytokines [such as tumour necrosis factor alpha (TNF- $\alpha$ ) and IL1], which upregulate ICAM-1 [41] and an action on eosinophils by reducing the release of eosinophil cationic protein, which stimulates ICAM-1 expression [36].

Other studies have been performed to investigate the effect of different antihistamines on ICAM-1 expression.

To study the effects of terfenadine on epithelial cell expression of ICAM-1 a double-blind, placebo controlled study was performed in 20 subjects with seasonal allergic rhinitis to *Parietaria judaica* [42]. Patients received 120mg terfenadine or placebo daily for 7 days during the allergy season and some objective parameters of the allergic response were monitored. Among them, the positivity of ICAM-1 staining on epithelial cells was evaluated. Compared to placebo, treatment with terfenadine resulted in a significant reduction of ICAM-1 positivity on nasal epithelial cells.

Paolieri *et al.* [43] evaluated the *in vitro* effect of fexofenadine on ICAM-1 expression of a human continuously cultured conjunctival epithelial cell line (WK) and a fibroblast cell line (HEL). They found that a concentration of 50 $\mu$ /ml of this drug significantly decreased ICAM-1 basal expression on WK cells, sICAM-1 levels in INF- $\gamma$ -stimulated WK cells, and INF- $\gamma$ -induced ICAM-1 upregulation on HEL.

Other studies have demonstrated that loratadine and its metabolite desloratadine have an antiinflammatory effect by modulating ICAM-1 expression on epithelial cells. Vignola *et al.* [44] investigated the effect of loratadine and desloratadine on the expression of ICAM-1 and human leukocytes class II antigen HLA-DR on nasal epithelial cells cultured from nasal turbinates and polyps, and demonstrated that both compounds significantly decreased histamine-induced expression of ICAM-1 on nasal epithelial cells *in vitro*. In addition, Papi *et al.* [45] recently reported that desloratadine and loratadine inhibit the expression of ICAM-

1 caused by rhinovirus infection on cultured primary bronchial or transformed epithelial cells. Most importantly, the authors noted that activation of the ICAM-1 gene-promoter region by the rhinovirus infection was completely inhibited by desloratadine. The nuclear factor NF- $\kappa$ B is a key inducer of ICAM-1 gene transcription via a specific binding site on the ICAM-1 gene promoter region.

Ciprandi *et al.* [46] conducted a randomised, double-blind, parallel study in 20 seasonal allergic rhinitis subjects, examining the effect of 2 weeks treatment with loratadine 10 mg daily on cellular infiltration and the expression of adhesion molecules after natural allergen exposure. Loratadine significantly reduced symptoms, and objective parameters such as eosinophil infiltration, levels of ECP and histamine in nasal lavage fluid, ICAM-1 expression on nasal epithelial cells, compared to the baseline values. A reduction of ICAM-1 expression on the conjunctival epithelium by loratadine was also shown in a challenge study of allergen-specific conjunctival epithelium [47].

## 2. Inhibition of Mediator Release

As described above, although the release of histamine is the principle mechanism by which mast cells and basophils affect the allergic reaction, they also influence the reaction through the release of other proteins, metabolites, and cytokines [3].

Proteins such as tryptase, chymase are released through degranulation, as proteoglycans including heparin and chondroitin sulphate [48]. Arachidonic acid metabolites, including leukotrienes and prostaglandins as well as platelet-activating factor (PAF), are synthesized *de novo* after the activation of membrane phospholipids [3,49]. These mediators include leukotriene C4 (LTC4), LTB4, and prostaglandin D2, LTC4 and PGD2 are both powerful bronchoconstrictors and vasodilators inducing a resultant increase in vascular permeability. They also have significant effects on leukocytes, being reported to activate eosinophils (PGD2, LTB4, PAF) and to have chemotactic properties for neutrophils (PGD2, PAF, LTB4) [50,51].

Early studies examining the effects of H1-antihistamines on mediator secretion using guinea-pig and rat mast cells showed clearly that these drugs are able to inhibit histamine release [52]. This was confirmed in later studies which also established that there was no correlation between the concentrations of drugs inhibiting histamine release and their H1 antihistamine potency [53,54].

Perhaps the most likely explanation of the mechanisms by which H1-antihistamines exert these effects derives from the studies performed [55,56], in which the authors tested a series of lipophilic cationic drugs with local anaesthetic properties.

They suggested, and other authors have concurred, that the physical nature of these drugs, namely lipophilic molecules which carry a positive charge, allows them to associate with the cell membrane and competitively inhibit the binding of calcium, thereby reducing the activity of calcium-dependent enzymes, such as calmodulin. [55-58].

More recent studies, however, have suggested a direct inhibitory effect of H1-antihistamines on calcium channels, reducing the inward Ca<sup>++</sup> current activated by intracellular Ca<sup>++</sup> store depletion [59-61].

*In vitro* studies have consistently established that essentially all tested H1 antihistamines inhibit the release of mediators from both mast cells and basophils. However, *in vivo* studies evaluating the effect of antihistamines on nasal lavage fluid after challenge have yielded more varied responses.

Using a double-blind placebo-controlled crossover design, Naclerio *et al.* [62,63] showed that therapeutic amounts of cetirizine and terfenadine reduced antigen-induced sneezing. Patients receiving terfenadine exhibited a significant, dose-dependent reduction in histamine concentrations recovered from nasal lavage fluid compared with those treated with placebo, whereas those receiving cetirizine did not. Pretreatment with cetirizine inhibited LTC4 expression in nasal lavage after allergen challenge, but failed to reduce histamine or PGD2 [63].

Shin and colleagues duplicated these findings with the use of a single administration of oral azelastine, showing inhibition of the release of LTC4 and kinins, but not of histamine or PGD2 [64].

It appears that suppression of LTC4 release can be achieved with lower levels of antihistamine than are necessary to attenuate the release of histamine. The effect of cetirizine on mast cell mediator release during cutaneous LPR was studied *in vivo* in ragweed-allergic subjects using a skin chamber challenge model [50].

As in the nasal lavage model, histamine release was not affected by pre-treatment with cetirizine, although PGD2 production was significantly reduced.

Jacobi *et al.* [51] were able to show a reduction in both histamine and tryptase in nasal lavage fluid after challenge in patients treated for 1 week with oral cetirizine or intranasal azelastine. Similar studies with loratadine and azatadine have revealed suppression of antigen-induced histamine in nasal lavage fluid [65,66].

In 14 individuals allergic to pollen, Bousquet *et al.* [67] performed a three-way crossover study comparing the effect of 1-week treatment with loratadine, terfenadine, or placebo on mediator release during the immediate response to nasal challenge with the allergen. Both active drugs suppressed symptoms and the release of histamine and PGD2, suggesting that they affected mast cells activation. Using a similar model, Andersson [68] showed a significant reduction in the release of these mediators during the early allergic reaction after loratadine treatment. These studies show that loratadine is able to block the release of mediators during the immediate nasal allergic reaction; however, it did not significantly inhibit the histamine concentrations after allergen administration to the skin [69,70].

Baroody *et al.* [71] performed another double-blind, placebo-controlled, three way crossover study comparing the effects of 1 week pre-treatment with loratadine, terfenadine or placebo on the early response to nasal challenge with allergen, the subsequent cellular influx and the increased

responsiveness to methacholine 24 h later. Both loratadine and terfenadine treatment resulted in significant reductions in the levels of histamine in the nasal lavage, and in other subjective and objective parameters, with no significant differences between the two treatments. Neither treatment decreased the levels of tryptase, PGD2 or LTC4.

Shin *et al.* [72] performed a double-blind, placebo-controlled, crossover study comparing the effect of treatment with oral azelastine or placebo on the early phase reactions to nasal challenge with allergen in 13 asymptomatic allergic subjects. Pretreatment with a single oral dose of azelastine did not show a significant reduction of histamine and PGD2 compared to placebo. On the contrary, azelastine inhibited leukotriene production. This finding reflects the fact that the concentration of azelastine *in vivo* was sufficient to inhibit only LTC4, but not histamine release, because *in vitro* studies have shown that azelastine inhibited LTC4 release at a lower dosage than the one required to inhibit histamine release [73,74].

Another possible explanation could be that azelastine may have some 5-lipoxygenase inhibitory activity that leads to a preferential reduction of leukotriene generation in nasal mast cells [75,76].

In general, *in vivo* studies reveal that concentrations of antihistamines three or four-fold those required for histamine receptor blockade are needed to achieve an antiallergic effect.

Lichtenstein and Gillespie [77] evaluated the *in vitro* inhibition of antigen-induced histamine from human leukocytes in response to increasing concentrations of promethazine, chlorphenizamine, or diphenhydramine. In each of these drugs concentration-dependent inhibition of histamine release of differing degrees of intensity was observed, confirming that higher concentrations than those used for histamine-receptor blockade may be necessary to inhibit this antiallergic response.

Through their effect on mast cells and basophils, many of the newer and more selective antihistamines including fexofenadine, cetirizine, desloratadine, loratadine, terfenadine and azelastine have been shown to have an anti-allergic activity *in vitro*. De Paulis *et al.* [78] assessed the effect of fexofenadine on the release of histamine and LTC4 from basophils purified from lung parenchyma. After preincubation with fexofenadine for 35 minutes, basophils were challenged with anti-IgE antibodies or dust-mite antigen. Fexofenadine inhibited the release of histamine and LTC4 in a dose-dependent manner. It has thus been shown that fexofenadine inhibits the release of preformed mediators from mast cells and basophils, as well as mediators such as leukotrienes, that are synthesized *de novo* from membrane phospholipids.

To evaluate the efficacy of loratadine and desloratadine, basophils and lung tissue and skin mast cells were preincubated with loratadine or desloratadine to evaluate their effects on mediators release after immunological challenge with Der p 1 or anti-Fc RI [79]. Desloratadine and loratadine inhibited the release of histamine and LTC4 from basophils in a concentration-dependent fashion. Both antihistamines also reduced the release of histamine, LTC4

and PGD2 from skin mast cells in a concentration-dependent fashion.

Genovese *et al.* [79] showed that preincubation of human mast cells with desloratadine inhibited anti-Fc RI induced release not only of histamine but also of tryptase and PDG2 from human mast cells.

Additional studies have confirmed that histamine release from basophils challenged with anti-IgE, Der p 1, concavalin A, and calcium ionophore is attenuated by preincubation with desloratadine in a similar fashion [80,81].

Recently, Schroeder *et al.* [28] have assessed the ability of desloratadine *in vitro* to inhibit histamine and LTC4 release by human basophils. The antiallergic activity of azelastine has also been evaluated *in vitro*. Azelastine was shown to inhibit the release of histamine from anti-IgE-stimulated human basophils in a concentration-dependent manner [82].

Studying cultured human mast cells, Shichijo *et al.* [83] showed that azelastine had an inhibitory effect on the release of histamine, LTC4 and PGD2 when added at the time of anti-IgE antibody challenge. Although the effect of cetirizine on histamine release has been inconsistent *in vivo*, it has also been reported to inhibit anti-IgE-induced degranulation of human basophils *in vitro* [84].

Overall, the above studies support an antiallergic effect of antihistamines by revealing that all drugs in this category attenuate the release of mediators from mast cells and basophils, most frequently in a concentration-dependent manner, which is one of the reasons that could explain why this property has often not been confirmed in studies *in vivo*. In addition, the different results of the different studies could be explained by considering some variables such as the heterogeneity of mast cells, concentration and/or duration of the treatment antihistamines used.

## OTHER ANTI-INFLAMMATORY EFFECTS

Now, briefly, we will explain the influence of H1 antihistamines on eosinophils and superoxide generation. There is increasing evidence suggesting that eosinophils and then their mediators play an important role in the pathogenesis of such allergic conditions as seasonal allergic rhinitis (SAR) and asthma [85-89]. *In vivo* studies have demonstrated that eosinophils are readily detectable in the nasal secretions of subjects with allergic rhinitis after exposure to allergen and that there is a correlation between the number of eosinophils and the severity of the rhinitis symptoms during the pollen season [90-92].

Studies have demonstrated that new antihistamines attenuate the recruitment of eosinophils into the sites of allergic inflammation via the inhibition of ICAM-1 expression or, for instance, of platelet-activating-factor (PAF) into the skin [93,94]. Antihistamines such as cetirizine at therapeutic concentrations have been shown to reduce PAF-induced eosinophil chemotaxis [95]. Other antihistamines have also been examined against eosinophil chemotaxis.

In particular, ketotifen has been reported to reduce PAF-induced eosinophil chemotaxis *in vitro* at a lower

concentration than the one needed to suppress LTC<sub>4</sub> production.

In a study by Eda *et al.* [96], both loratadine and terfenadine have been shown to inhibit chemotaxis of human eosinophils induced by PAF at concentrations equivalent to or marginally above those which would be expected to be found in the blood after a single oral dose.

*In vitro* studies using eosinophils have demonstrated that desloratadine can downregulate platelet-aggregation factor-induced chemotaxis by up to 36% [97]. These *in vitro* effects on eosinophils have recently been extended to human studies. Normally, a decrease in the circulating progenitors of eosinophils is seen during the allergy season, as trafficking is believed to occur preferentially from the circulation to nasal mucosa under the influence of local and systemic allergic inflammatory mediators. A randomised, double-blind placebo-controlled study of the effect of desloratadine on peripheral blood progenitors of eosinophils was performed [98]. Progenitor cells from 45 patients with symptomatic SAR were studied during 4 weeks' treatment with desloratadine or placebo. Peripheral blood eosinophil progenitors reduced more with placebo than with desloratadine after 14 days. These results provide evidence that desloratadine can influence systemic trafficking of eosinophil precursors during the allergic response in patients with SAR, although the underlying mechanism is uncertain.

An additional anti-inflammatory action is played by antihistamines on neutrophil functions. Neutrophils are well known to generate superoxide anions which cause the tissue damage. Studies have demonstrated that new antihistamines inhibit superoxide generation [99].

An early study performed on human neutrophils demonstrated that superoxide radical production stimulated by calcium ionophore A23187, concavalin A and fMLP, was strongly suppressed by ketotifen and that this effect required lower drug concentrations than did inhibition of chemotaxis. In contrast, it has been reported that cetirizine inhibits neutrophil superoxide radical production only at concentrations above 35 mg/mL, higher than those required for suppression of chemotaxis. Since then, many reports have confirmed the inhibitory effects of antihistamines on neutrophils.

Van Epps *et al.* [100] reported that cetirizine can prevent the release of neutrophil lysosomal enzymes, whereas Werner *et al.* [101] found that azelastine, and astemizole inhibit fMLP-induced release of neutrophil elastase.

Paubert-Braquet *et al.* [102] have reported similar inhibitory effects of desloratadine on superoxide production in neutrophil and monocyte preparations, although these effects may be more relevant in lung/bronchial inflammation than in allergic inflammation of the nasal mucosa.

Finally, we have reported upregulation of the number and function of  $\beta_2$ -adrenoceptors. This effect leads to enhancement of the power of  $\beta_2$ -adrenergic bronchodilators used to treat bronchial asthma. In fact  $\beta_2$ -adrenoceptor desensitization may occur during long-term treatment of bronchial asthmatics with basophils-adrenergic agonists, and may limit the efficacy of  $\beta_2$ -adrenergic agonists.

The density of  $\beta_2$ -adrenoceptor on circulating lymphocytes has been used as a model to study  $\beta_2$ -adrenoceptor function in man. Ketotifen increases  $\beta_2$ -adrenoceptor density on lymphocytes from bronchial asthmatics who have been treated with  $\beta_2$ -adrenergic bronchodilators. This was accompanied by a significant increase in peak expiratory flow rate in response to inhaled salbutamol [103].

In addition, the number of  $\beta_2$ -adrenoceptors was higher in azelastine and terbutaline-treated guinea pig lung than in lung treated with terbutaline alone, showing that azelastine may prevent  $\beta_2$ -adrenergic agonist-induced downregulation of the number of  $\beta_2$ -adrenoceptors [104].

T lymphocytes play a critical role in the modulation of the immune response in allergic reactions. Th2 cells secrete various cytokines including IL4, IL5, IL 6, IL 9, IL 10 and IL 13. The release of IL 4 and IL 5 is of particular significance because these cytokines have been shown to contribute to the activation of basophils and eosinophils [3] IL 4 and IL13 also play an important role in the inflammatory response through their involvement in the proliferation and differentiation of B cells into plasma cells that secrete IgE.

As the secretion of cytokines from lymphocytes, particularly the Th2 subset of lymphocytes, appears to be central to the establishment and maintenance of allergic inflammation, it seemed pertinent to examine the effects of antihistamines on these cytokines production by T cells.

Antihistamines such as azelastine, terfenadine and ketotifen inhibit IL 2, IL 3, IL 4 and IL 5 production by mitogen-stimulated peripheral blood lymphocytes [105].

Nori *et al.* [106] have recently evaluated the effect of ebastine on the production of Th2-type cytokines. Using T cells derived from healthy non-atopic volunteers, they showed that ebastine inhibited the secretion *in vitro* of IL 4, IL 5 but not IL2 and INF  $\gamma$ . This effect was not observed with ketotifen.

However, antihistamines could interfere with cytokine-basophil and epithelial cells too. In particular, Schroeder *et al.* [28] found that desloratadine was an inhibitor of IgE-mediated IL 4 and IL 13 secretion from human basophils. As is well known, such regulation is important because IL 4 and IL13 control IgE production, mast cell growth and development, expression of adhesion molecules such as ICAM-1, VLA-4 and B-cell growth and development [107].

The effect of antihistamines on cytokines production by epithelial cells was investigated by Arnold *et al.* [108]. They demonstrated that cetirizine reduced the release of IL 8 from A549 cells stimulated with PMA and TNF  $\alpha$ . IL 8 is a chemokine that possesses chemotactic activity for neutrophils, and, as a consequence, plays a causative role in the pathogenesis of many acute inflammatory reactions.

Azelastine, too, has been shown to inhibit IL 1, IL 6 and TNF [109].

There are several possible mechanisms by which antihistamines act on inflammatory reactions:

- By inhibiting calcium influx into the cells, it is likely that these drugs may modulate the activity of genes encoding for proinflammatory mediators by influencing the signal transduction pathways primed by changes in cytosolic calcium [110-114].
- By modulating intracellular cAMP levels, an increase in which prevents intracellular mobilization and interferes with calcium dependent reactions.
- By inhibiting protein kinase C (PKC) and its dependent pathway.
- By inhibiting the G-protein function that regulates intracellular signals and cell function.
- By inhibiting NF- $\kappa$ B binding, that allows the expression of several genes.

The anti-inflammatory actions described have highlighted several potentially new properties of antihistamines. However, some of them do not appear to be clinically relevant, or at least, have not yet been demonstrated to be so, because they require high drug concentrations. In addition to the above-described properties, a new aspect should be considered: the analgesic properties of antihistamines. In fact, preclinical and clinical models have displayed that these drugs display analgesic efficacy [115]. This effect could enhance the properties of these versatile and eclectic drugs, leading to a closer focus on actions other than their simple anti-histamine effects.

#### ABBREVIATIONS

EP	=	Early-phase
LP	=	Late phase
IL	=	Interleukin
ICAM-1	=	Intercellular adhesion molecule-1
VCAM-1	=	Vascular adhesion molecule-1
RANTES	=	Regulated on activation, normal T-cell expressed and secreted
MIP-1	=	Macrophage inflammatory protein
MCP-1	=	Monocyte chemotactic protein
InsP3	=	Inositol-1,4,5-triphosphate
DAG	=	Diacylglycerol
cAMP	=	Cyclic adenosine monophosphate
LTC4	=	Leukotriene C4
PGD2	=	Prostaglandin D2
SAR	=	Seasonal allergic rhinitis
INF	=	Interferon
TNF	=	Tumour necrosis factor
PMN	=	Polymorphonuclear
PAF	=	Platelet activating factor
PKC	=	Protein kinase C
ECP	=	Eosinophil cation protein

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