

Multiple action agents and the eye: do they really stabilize mast cells?

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Purpose of review

Multiple action drugs, such as azelastine, epinastine, ketotifen and olopatadine, have recently been suggested to combine antihistaminic effect, mast cell stabilization and anti-inflammatory action. This pharmaceutical class is, therefore, rapidly becoming the first choice for prevention and treatment for allergic conjunctivitis.

Recent findings

Increasing in-vitro studies have been performed to investigate the mast-cell-stabilizing effect of multiple action drugs. Most of the study results agree that these drugs are able to inhibit histamine and several neoformed mediators, including cytokines and arachidonic acid-derived products, from mast cells. However, the mechanisms of action have not yet fully been elucidated. Most of the results from clinical trials as well as the in-vivo experimental studies, including the conjunctival provocation model, support the evidence of a stabilizing effect of these drugs.

Summary

Evidence of a different inhibitory effect of multiple action compounds on the pro-inflammatory mediators released from the mast cells suggests the possibility to target different phases of the allergic reaction, leading to a potential improvement in the management of allergic patients.

Keywords

azelastine, epinastine, ketotifen, mast cells, olopatadine

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Introduction

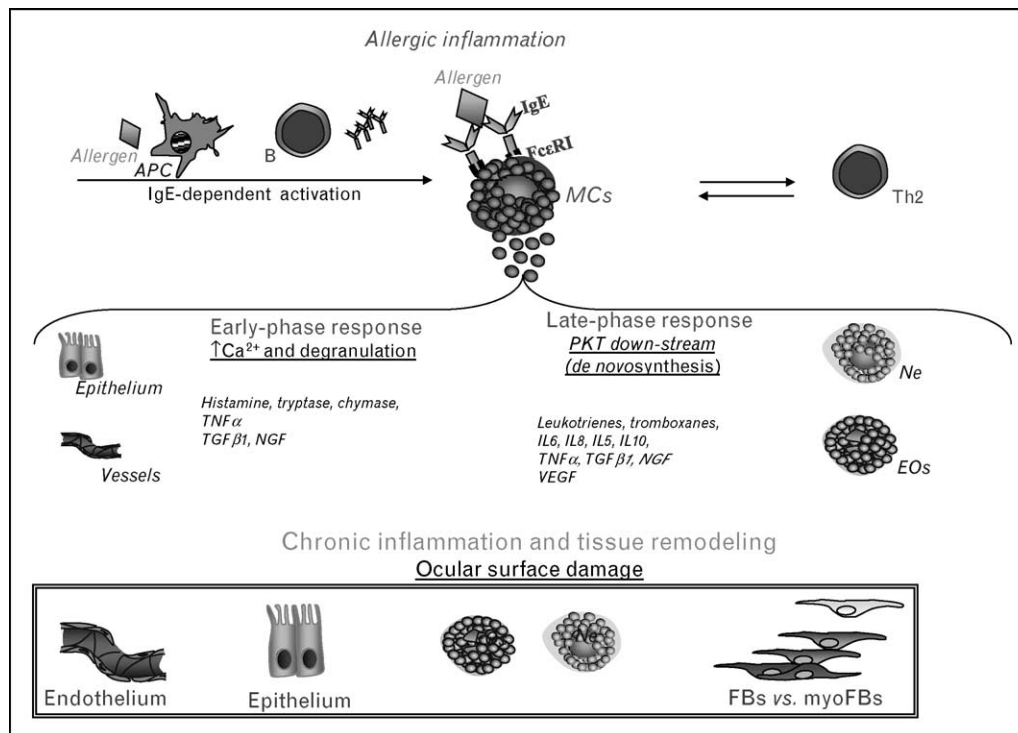
In the normal conjunctiva, mast cells are normally present in the substantia propria under the basement membrane [1]. In allergic conjunctivitis, mast cells increase in the substantia propria and eventually infiltrate the epithelium [2]. Mast cells represent the pivotal cells of allergic conjunctivitis and are classified as tryptase-positive and tryptase/chymase-double-positive cells. Tryptase/chymase-positive mast cells are the major subtype in conjunctiva, and they can be found in 95% of healthy individuals, 96% of vernal keratoconjunctivitis patients and 100% of patients with giant papillary conjunctivitis [3].

Once in the tear film, allergens bind to immunoglobulin E (IgE) receptors on the conjunctival mast cell, causing mast cell activation and subsequent degranulation, with release of preformed and neoformed pro-inflammatory mediators (Fig. 1) [4]. Preformed mediators are stored in granules and are secreted within minutes. These mediators include biogenic amines (histamine, serotonin), neutral proteases (tryptase, chymase, carboxypeptidase), acid hydrolases (b-hexosaminidase, b-glucuronidase, b-D-galactosidase),

proteoglycans (heparin, chondroitin sulfate), eosinophil chemotactic factor (ECF) and neutrophil chemotactic factor (NCF). Histamine is recognized as the primary mediator of clinical signs and symptoms of ocular allergy: the activation of neuronal histamine (H1) receptors induces itching, whereas activation of vascular endothelial cells through the action of both H1/H2 receptors induces hyperemia due to vasodilation and eyelid swelling and chemosis due to transudation of fluid [5]. This early phase of the allergic reaction may be targeted by several drugs acting on mast cells and histamine and/or histamine receptors.

The synthesis of newly generated mediators starts after mast cell activation, and their release occurs within hours. These mediators include interferons, tumor necrosis factor- α (TNF- α), granulocyte and macrophage-colony stimulating factor (GM-CSF), platelet activating factor (PAF), lipoxygenase and cyclooxygenase products (leukotrienes and prostanoids) and are associated with increased mucus secretion and cellular infiltration [6]. Mast cell-derived cytokines initiate and boost the Th2 immunological reactions as well as a broad spectrum of pro-inflammatory, growth promoting and chemotactic

Figure 1 Mast cell activation in allergic inflammation



Schematic representation of the mast cell activation, starting from the common IgE-dependent stimulation (referring to sensitization and challenge), early-phase and late-phase responses. Early and late-phase products act at different time points on endothelium, epithelium, eosinophils/neutrophils recruitment and on fibroblast/myofibroblast remodeling. The chronic mechanism might result in ocular complications and impairments of visual function due to corneal damage. IgE, immunoglobulin E; MC, mast cell; NGF, nerve growth factor; VEGF, vascular endothelial growth factor.

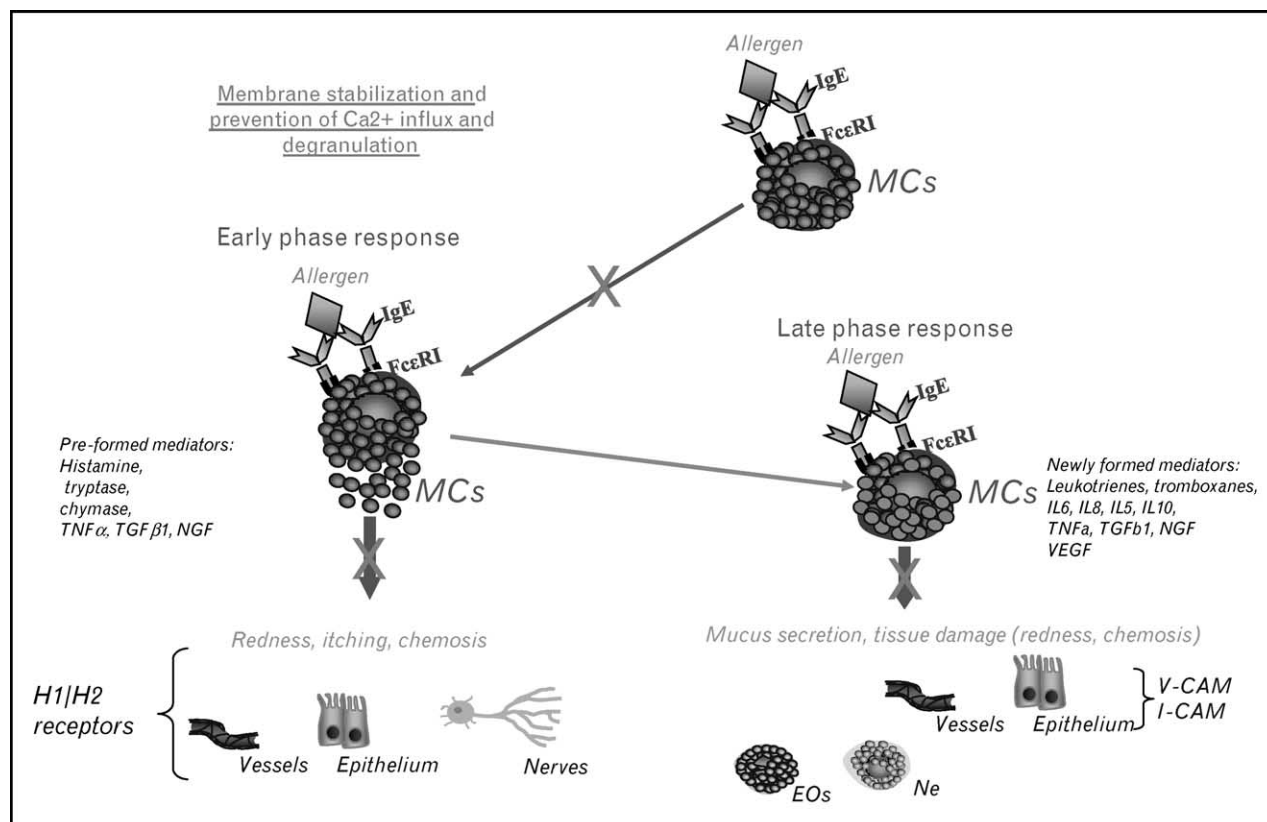
responses from several cell types. These mediators include interleukins (IL)1, 3, 4, 5, 6, 8, 10, 13, 16, TNF- α , GM-CSF, transforming growth factor- β (TGF- β), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), nerve growth factor (NGF) and macrophage chemotactic protein-1, regulated upon activation normal T cell expressed and secreted (RANTES), macrophage inhibitory proteins and lymphotactin [7]. Cytokines, chemokines and growth factors trigger a cascade of inflammatory events, including the increased expression of different adhesion molecules (ICAM-1 and VCAM-1) on epithelial cell, leading to a late-phase response with recruitment of eosinophils and neutrophils, responsible for the ocular surface damage.

Several classes of ophthalmic medications are available for treating clinical signs and symptoms of allergic conjunctivitis by inhibiting specific mechanism of the allergic inflammatory cascade [8]. Multiple action drugs, such as azelastine, epinastine, ketotifen and olopatadine, have recently been suggested to combine antihistaminic effect, mast cell stabilization and anti-inflammatory effect, acting on several immune cells (Fig. 2) [9]. This pharmaceutical class is, therefore, quickly becoming the

first choice for prevention and treatment of allergic conjunctivitis. In fact, acting as antihistaminic drugs, they antagonize histamine H1/H2 receptor binding, providing immediate relief of early phase symptoms; acting as mast cell stabilizers, they inhibit mast cell degranulation; acting on the immune cells of late-phase reaction, they reduce ocular surface damage. However, a critical review of the literature highlights that several mechanisms of action of these multiple action drugs are still unknown. Moreover, different compounds exert different actions on mast cells, and contrasting data exist in supporting their effects on conjunctival mast cell stabilization.

In-vitro evidence

Antigen-induced cross-linking of IgE antibodies on the mast cell surface induces tyrosine phosphorylation of Fc ϵ RI subunits by Src family kinase Lyn, which is followed by an activation of Syk/Zap family kinase Syk. Activated kinases phosphorylate and activate other enzymes [phospholipase C γ isoforms, phosphatidylinositol-3 kinase (PI3K) isoforms, protein kinase C (PKC) isoforms, Bruton's tyrosine kinase (Btk) and others], adaptors (linker for activation of T cells, Cbl, Grb2 and others) and guanosine-5'-triphosphate (GTP) exchange

Figure 2 Clinical and pharmacological effects of multiple action antiallergic agents

This figure summarizes the pathways of action of multiple action antiallergic drugs, which include membrane activation and cell degranulation (early phase reaction) and de-novo synthesis (late-phase reaction). Drugs acting at the membrane level also interfere with the subsequent steps of intracellular Ca^{2+} influx and PTK signal transduction pathway. IgE, immunoglobulin E; MC, mast cell; NGF, nerve growth factor; PTK, protein-tyrosine kinase; VEGF, vascular endothelial growth factor.

factors/GTPases (Vav, Ras, Rho and others) and subsequently induce the mobilization of stored/extracellular Ca^{2+} and cytoskeletal changes required for intracellular granule release. These events lead within seconds or minutes to the secretory response and later to the production of cytokines/chemokines. Intracellular Ca^{2+} increase represents a necessary step for the activation of these kinases and early mediator secretion [10–12]. One suspected mechanism of mast cell stabilizers is the blocking of IgE-regulated calcium channels, stabilizing the cell and preventing the release of histamine and related mediators. Without intracellular Ca^{2+} , histamine vesicles cannot fuse to the cell membrane and degranulate. On the other hand, inhibition of intracellular Ca^{2+} elevation leads to inhibition of Ca^{2+} -dependent PKC, which is also critical in mast cell activation or inhibition of exocytosis or both [12–14].

Consequently, the ability of a drug to stabilize mast cells can rely on the measurements of preformed and neoformed mediators released *in vitro* [15]. As described in detail below, multiple action drugs (azelastine, epinas-

tine, ketotifen and olopatadine) affect, at least *in vitro*, multiple and selective mechanisms of mast cell activation/degranulation [16–24,25**].

Azelastine, a phthalazinone derivative, is a second-generation H1 receptor antagonist, proved to be clinically effective in relieving allergic symptoms after oral/topical administration [26]. Azelastine inhibits the early allergic response and histamine release from rat peritoneal mast cells after IgE-dependent and IgE-independent stimuli [27–29]. Azelastine significantly inhibits the release of histamine, leukotrienes and PGD_2 from human cord blood-derived mast cells (CBMCs) [18]. Azelastine also inhibits the release of tryptase and IL6, and this inhibitory effect appears more potent than that of olopatadine [7,16,17]. Azelastine inhibits IL8, TNF-α and, to a lesser extent, IL6 secretion from human CBMCs as well as human leukemic mast cells in a dose-dependent fashion [10]. A recent study [10] demonstrated that this mediated inhibition of exocytosis occurs via selective interfering of Ca^{2+} influx and nuclear factor-κB (NFκB) activation. However, in this study, more than 99% of the investigated cells had

tryptase-positive granules, and only 20–30% had tryptase/chymase-double-positive granules, resembling mast cells populating the human lung and gastrointestinal mucosa, rather than the conjunctiva. Kurosawa *et al.* [30] also demonstrated that azelastine is able to inhibit mediator release from rat mast cells through controlling phosphatidylinositol turnover and cAMP generation. Finally, azelastine inhibits leukotriene synthesis and superoxide generation by neutrophils and eosinophils and down-regulates ICAM-1 expression in human conjunctiva [26,31].

Emedastine is a relatively selective blocker of H1 receptors without any effect on adrenoceptors, dopamine or serotonin receptors [32]. In addition, emedastine possesses an inhibitory effect on IgE-mediated histamine release from rat peritoneal mast cells, an inhibitory effect on substance P-induced histamine release from guinea pig mast cells, an inhibitory effect on LTB₄-elicited or PAF-elicited eosinophil migration and a potent/selective inhibitory effect on eosinophil chemotaxis [19,32–34].

Epinastine is a potent H1/H2 receptor antagonist with mast cell-stabilizing and anti-inflammatory properties [9]. Epinastine has no effect on membrane fluidity, and yet it inhibits Ca²⁺ uptake and release from intracellular stores and suppresses calmodulin dose-dependently in guinea pig skin mast cells exposed to compound 48/80 and substance P [35]. Epinastine inhibits VEGF, TNF- α and keratinocyte-derived chemokine (a murine homolog of IL8) IgE-stimulated production from murine mast cells through a mechanism presently unclear [20^{••}]. The Fc ϵ RI-mediated expression of TNF, IL6 and IL8 transcripts in mast cells strictly depends on the activation of NF κ B [36,37]. Therefore, the possibility that epinastine inhibits NF κ B activation through Ca²⁺-independent mechanisms and results in suppression of angiogenic factor production from mast cells cannot be ruled out.

Ketotifen, a benzocycloheptathiophene, has multiple mechanisms of action, possessing both mast cell-stabilizing and antimediator properties, including strong H1 receptor antagonism and inhibition of leukotriene formation [9]. In experimental and clinical conditions, ketotifen reduced antigen-induced mast cell degranulation and decreased the release of histamine, proteases, myeloperoxidase, leukotrienes, PAF and various prostaglandins. Apart from its antihistaminic action, ketotifen exhibited a stabilizing effect on mast cell membrane regarding calcium permeability [21,38,39]. As compared with the other multiple action drugs, ketotifen has lower inhibitory effect on cytokine synthesis and secretion after stimulation (IL8, IL10, TNF- β) [25^{••}]. Lastly, a biphasic effect of ketotifen on histamine release (inhibition at low concentrations and stimulation at higher concentrations)

has been demonstrated on several mast cell types, including human conjunctival mast cell [25^{••},40–42].

Olopatadine hydrochloride is a tricyclic compound, showing H1 receptor antagonist and mast cell-stabilizing effects, as well as modulatory action on immune cell recruitment [9]. Olopatadine inhibits antigen-induced histamine and cytokines, leukotriene and prostaglandin release from sensitized mast cells [43,44]. Olopatadine also inhibits IgE-mediated histamine and TNF- α release from human conjunctival tryptase/chymase-containing mast cells and inhibits upregulation of ICAM-1 expression on epithelial cells [22–24]. This last effect seems to be mediated through a TNF- α -specific mechanism. Inhibition of mast cell activation and reduced ICAM-1 expression lead to a decrease of inflammatory cell recruitment/infiltration (eosinophils and neutrophils). In addition, Ikemura *et al.* [45] demonstrated that olopatadine suppressed leukotrienes and thromboxane A₂ release as well as PAF formation in neutrophil-enriched human polymorphonuclear leukocytes (PMNs) exposed to calcium ionophore, by reducing arachidonic acid release from membrane phospholipids, probably by interference with phospholipases. Olopatadine has also been shown to inhibit calcium influx, which correlates with the degree of degradation and IL4 expression in a basophil cell line [46].

Nedocromil was originally thought to be only a mast-cell stabilizer, but it is now comprised among the multiple action compounds [47], for its H1 receptor antagonist properties and inhibitory effects on various allergic inflammatory cells, mast cells and eosinophils [48]. However, conflicting results exist regarding the inhibition of neutrophil migration by nedocromil [49,50]. In-vitro, nedocromil has been shown to abolish the expression of human leukocyte antigen (HLA)-DR and to reduce ICAM-1 expression by human conjunctival epithelia [51]. In addition, nedocromil suppresses early and late-phase conjunctival hyperemia, chemosis, eyelid edema and eosinophil infiltration in sensitized guinea pigs [52]. Lastly, nedocromil eye drops reduce tear concentrations of histamine and PGD₂ and the number of IL4-secreting mast cells in an ocular allergen-challenge model [53].

In a recent study, Galatowicz *et al.* [25^{••}] compared the stabilizing effects of the different multiple action drugs on both human CBMC and conjunctival-derived mast cells. Histamine secretion by Fc ϵ RI-stimulated CBMC was differentially inhibited by all the above-mentioned drugs in a dose-dependent fashion, with nedocromil being the most effective CBMC stabilizer. All drugs significantly reduced TNF- α and, with the exception of olopatadine, all drugs decreased IL5 production; IL8 was inhibited by epinastine and azelastine; IL10 was decreased by epinastine and olopatadine; and finally, TNF- β was reduced by azelastine and olopatadine. How-

ever, these data were not confirmed in conjunctival-derived mast cells in which epinastine and olopatadine did not induce a significant decrease of histamine, IL8 and TNF- β release. It is noteworthy to highlight that in-vitro studies of human conjunctival mast cells are severely hampered by the low numbers of cells recoverable from biopsy specimens. Table 1 summarizes the in-vitro studies of the stabilizing effects of multiple action drugs.

Evidence in animal models

All multiple action drugs have been investigated in animal models of the allergic reaction, demonstrating effectiveness in inhibiting one or more mechanisms of the inflammatory process. In these in-vivo studies, it is difficult to distinguish the mast cell-stabilizing effect from the antihistamine action or from the direct/indirect anti-inflammatory activity. However, several interesting data on the mast cell-stabilizing action can still be extrapolated from these in-vivo studies [25^{••},54–57,58[•]].

Azelastine has been reported to inhibit antihistamine-resistant, leukotriene-mediated allergic bronchoconstriction in guinea pigs through inhibition of leukotriene C4/D4 synthesis [59]. In line with this observation, treatment with azelastine significantly inhibited both the release of antigen-induced leukotrienes and the increases of nasal vascular permeability in sensitized guinea pigs [60]. In addition, Lytinas *et al.* [17] demonstrated that azelastine, more than olopatadine, prevents Evan's blue extravasation following intradermal injection of 48/80 in rats through the inhibition of mast cell degranulation. Moreover, azelastine treatment significantly inhibited both immediate-phase response and late-phase response [mainly due to LTB(4) and PAF] in sensitized mice challenged with dinitrofluorobenzene [61].

Ketotifen was also extensively investigated in different animal models. In a rat model of induced mast cell degranulation by mechanical stimulation of the external auditory canal, administration of ketotifen significantly inhibited mast cell degranulation and tissue swelling [62]. In rats, ketotifen also blocked both granuloma formation and angiogenesis by inhibiting mast cell protease-5 release [63]. However, in a model of histamine-induced and antigen-induced conjunctivitis in guinea pigs, ketotifen failed to inhibit increase of tear histamine levels following allergen challenge [54].

Emedastine was evaluated for its antiallergic activity in histamine-stimulated and antigen-stimulated animal models of conjunctivitis. In this study, emedastine caused a concentration-dependent inhibition of histamine-induced conjunctival vascular permeability, similarly to ketotifen and more potent than 'pure' antihistamine drugs; and in a passive conjunctival anaphylaxis model

in guinea pigs, emedastine significantly inhibited the allergic response when administered prior to antigen challenge [55].

Epinastine was effective in inhibiting the swelling of rat foot pad induced by a subcutaneous injection of antirabbit antiserum and the early phase in active Arthus reaction in guinea pigs [64]. In addition, epinastine was more effective than 'pure' antihistamine compounds, such as cetirizine and terfenadine, in inhibiting scratching behavior and cutaneous reactions induced in mice by dinitrofluorobenzene painting, passive cutaneous anaphylaxis and substance P injection [65].

Olopatadine, systemically and topically administered, has been shown to inhibit antigen-induced conjunctivitis in actively sensitized guinea pigs [56]. Studies performed on other animal models of allergic reaction suggest that olopatadine exerts more than a 'pure' antihistamine effect. Oral administration of olopatadine significantly reduced the sneeze response, the nasal rubbing response and the increase in the nasal vascular permeability in actively sensitized rats [56,66], where both histamine H1 receptor antagonists and leukotriene receptor antagonists have only partial effects [67]. Systemic administration of olopatadine significantly inhibited clinical signs of both rhinitis and asthma associated with a decrease of eosinophils infiltration in actively sensitized guinea pigs [44,66,68]. In addition, olopatadine significantly prevented the increase in histamine and tended to inhibit the increase of leukotrienes and TXB2 concentrations in nasal lavage fluid following the intranasal antigen challenge in actively sensitized guinea pigs [69].

A recent study [25^{••}] has compared the effectiveness of different multiple action antiallergic drugs (epinastine, olopatadine and nedocromil) in an animal model of allergic conjunctivitis. Epinastine, olopatadine and nedocromil significantly reduced conjunctival redness, while both nedocromil and epinastine decreased tearing and only epinastine reduced lid edema. All drugs significantly decreased the conjunctival eosinophil numbers, whereas neutrophil counts were selectively decreased by epinastine. In line with this data, Beauregard *et al.* [57] showed that olopatadine, epinastine, azelastine and ketotifen inhibit histamine-induced vascular permeability in guinea pigs for up to 4 h, whereas only olopatadine action lasts for 16 h following a single topical application. However, these results are apparently in contrast with the experimental data obtained by Sanchis *et al.* [58[•]], who showed that in guinea pigs sensitized with intraperitoneal egg albumin and aluminum hydroxide, sparglumic acid and emedastine, but not ketotifen, olopatadine and azelastine, inhibit Evans blue ocular extravasation following conjunctival allergen challenge. Table 2 summarizes the in-vivo studies on the ocular effects of multiple action drugs.

Table 1 In-vitro studies of the stabilizing effects of azelastine, emedastine, epinastine and olopatadine on mast cells

Drug	System	Methodology (in-vitro steps)	Comparator	In-vitro parameters	Notes	Reference
Azelastine	HMC1	1 h-preincubation with drug 24 h-incubation PMA/ionophore	Loratadine Cetirizine Ranitidine Dexamethasone Placebo	TNF- α , IL3, IL6, IL8, GM-CSF; $[Ca^{2+}]$	Dose-dependent inhibition as follows: TNF- α > IL8 >> IL6 >> IL3 No effect on GM-CSF	[7]
Azelastine	CBMCs	72 h-cell priming		Histamine, tryptase	Almost 100% tryptase-labeled cells; $\downarrow$ cytokine production/release; $\downarrow$ intracellular iCa^{2+} flux and NF κ B	[10,21,22]
Olopatadine		5 min drug preincubation 6 h-stimulation with anti-IgE		IL6, TNF- α , IL8, NF κ B $[Ca^{2+}]$		
Azelastine	CBMCs	IgE priming with IgE-rich atopic patient's serum	Disodium cromoglycate	Histamine, LTs, PGD ₂	Almost 100% tryptase-labeled cells (>99% tryptase-containing cells/25% chymase-containing cells); $\downarrow$ histamine release and de-novo synthesis/release	[20**]
Emedastine	RtMCs	Challenged with anti-IgE Substance P-stimulated mast cells (IgE-independent stimulation)	None	Histamine	$\downarrow [Ca^{2+}]$ release/influx	[27]
Epinastine	MoMCs	Ovalbumin presensitized and challenged cells	None	Cytokine production and release	Complete $\downarrow$ VEGF and TNF- α	[30]
Olopatadine	HuMCs	Mono-dispersed HuMCs	Ketotifen	Histamine, tryptase, PI turnover, PGD ₂	$\downarrow$ in a dose-dependent fashion	[35,42,43,44]
Azelastine	HuMCs	IgE primed and stimulated cells HuMCs were isolated and SCF-exposed	Levocabastine Nedocromil versus all others	Histamine and cytokine production/secretion	CBMCs were CD34 ⁺ stem cells, tryptase/chymase-containing cells) No drugs were better than nedocromil as a MC stabilizer	[36]
Epinastine	CBMCs	CBMCs were exposed to SCF/IL6 and finally IL3			Differential drug-related effects $\downarrow$ histamine release in the supernatant	
Ketotifen Olopatadine		Both cell types were presensitized and challenged			$\downarrow$ in a dose-dependent fashion	

CBMCs, cord blood mast cells; GM-CSF, granulocyte and monocyte-colony stimulating factor; HMC1, human leukemic mast cells; HuMCs, human conjunctival mast cells; IgE, immunoglobulin E; LTs, leukotrienes; MoMCs, mouse mast cells; MC, mast cells; PGD₂, prostaglandin D₂; PMA, phorbol myristate acetate; RtMCs, rat mast cells; SCF, stem cell factor; VEGF, vascular endothelial growth factor.

Table 2 In-vivo studies on the ocular effects of multiple action agents

Drug	System	Methodology	Comparator	In-vivo parameters	Notes	Reference
Epinastine	Mice	Ragweed pollen sensitization	Versus each other	Clinical scores	Epinastine and olopatadine pretreatment significantly ↓ the clinical scores and EO infiltration; epinastine ↓ Ne infiltration	[36]
Olopatadine		Topical challenge		Conjunctival immunostaining		
Ketotifen	Guinea pigs	Drug pretreatments Histamine-induced conjunctivitis	Levocabastine; amlexanox; chlorpheniramine	Tear histamine levels	Ketotifen and chlorpheniramine failed to reduce the rate of increase of allergen-induced histamine tear levels	[59]
Emedastine	Guinea pigs	Antigen-induced conjunctivitis Histamine-induced conjunctivitis	Ketotifen; brompheniramine; chlorpheniramine; climastine; pyrilamine; levocabastine; pheniramine; diphenhydramine; antazoline	Vascular permeability	Equipotent to ketotifen and more potent than other antihistamines	[60]
Olopatadine	Guinea pigs	Antigen-induced conjunctivitis Histamine-induced conjunctivitis	Ketotifen	Clinical scores	Equipotency of the two drugs	[63]
Olopatadine	Guinea pigs	Antigen-induced conjunctivitis	Epinastine; azelastine; ketotifen	Vascular permeability	Olopatadine was effective for up to 16 h	[68]
Emedastine	Guinea pigs	Histamine-induced conjunctivitis Egg albumin sensitization	Ketotifen; olopatadine; azelastine; spaglumic acid	Vascular permeability; clinical scores	Emedastine and spaglumic acid significantly decrease vascular permeability	[69]
		Challenge in the conjunctival sac Drug pretreatments				

EO, eosinophil.

Evidence in patients

Several clinical trials have been performed to demonstrate the safety and efficacy of multiple action drugs in improving signs and symptoms of allergic conjunctivitis. Some evidence of a stabilizing effect of these drugs can also be extrapolated from clinical trials or from studies performed with the conjunctival provocation test (CPT). In fact, the extent of mast-cell stabilization exhibited by a drug can be determined by the quantification of mast cell-derived mediators and immune cells in the conjunctiva/tear up to 6 h after CPT, as mast-cell degranulation is responsible (through the release of inflammatory mediators) for the conjunctival cellular infiltrate occurring after allergen challenge.

Azelastine has been demonstrated to be effective in the relief of ocular signs and symptoms of allergic conjunctivitis [9,70] and to significantly reduce itching, conjunctival redness, tearing and chemosis following specific allergen challenge when compared with placebo-treated eyes [71]. Interestingly, in a randomized, placebo-controlled, double-blind, parallel-group study [72] performed on 20 patients, azelastine significantly reduced symptom scores, number of inflammatory cells and ICAM-1 expression during the early-phase and late-phase reaction following specific allergen challenge. Evidence that azelastine differs from 'pure' antihistamine drugs can be obtained also by studies in other allergic diseases. Using nasal provocation test, Pipkorn *et al.* [73] demonstrated that azelastine, as well as olopatadine 0.2%, inhibit nasal symptoms, histamine, albumin and lysozyme levels. In addition, azelastine, but not cetirizine, improves bronchial asthma and decreases the number of activated eosinophils and lymphocytes in bronchial biopsies, in line with previous data on the different efficacy of the two drugs on mast cell cytokine release *in vitro* [74,75]. However, clinical trials on patients with allergic conjunctivitis demonstrated a similar clinical efficacy of levocabastine and azelastine with a significantly higher number of patients improving and a more rapid effect in those taking azelastine [76,77].

Emedastine significantly decreases itching in the CPT model of acute allergic conjunctivitis and is more effective than nedocromil in alleviating redness and itching 3 and 10 min after allergen challenge [78]. In CPT studies, emedastine has been shown to be significantly more effective than levocabastine in reducing ocular itching and to be equivalent in controlling the conjunctival redness [79]. In addition, in a double-blind, randomized study [80] involving 222 patients, emedastine eye drops were more effective than levocabastine eye drops in the prevention and treatment of signs and symptoms of allergic conjunctivitis in adults and children. These results were confirmed by other clinical trials comparing the effectiveness of emedastine and levocabastine for

Table 3 Evidence of multiple actions in patients

Drug	Disease	Methodology	Comparator	In-vivo parameters	Notes	Reference
Azelastine	SAC	CPT Randomized, double-blind, parallel group	Placebo	Clinical parameters Number of inflammatory cells; ICAM-1 expression	Effective for all the evaluated parameters	[72]
Azelastine	SAC	14 days' treatment Open-study, parallel group	Levocabastine	Clinical parameters	Significantly more responders with azelastine	[76]
Azelastine	SAC	14 days' treatment Randomized, multicenter, parallel group	Placebo (Double-blind) Levocabastine (open)	Clinical parameters	Azelastine more effective than placebo and equipotent to levocabastine	[77]
Emedastine	SAC/PAC	CPT	Placebo	Clinical parameters	More effective for itching than levocabastine	[79]
Emedastine	SAC/PAC	Randomized, double-blind, contralateral eye 6 weeks' treatment	Levocabastine	Clinical parameters	levocabastine	[80]
Emedastine	SAC/PAC	Randomized, double-blind, multicenter, parallel group 42 days' treatment	Levocabastine	Clinical parameters	Emedastine superior to levocabastine	[81]
Emedastine	SAC	Randomized, double-blind, parallel group 42 days' treatment	Levocabastine	Clinical parameters	levocabastine in controlling chemosis, redness, itching, eye lid swelling	[82]
Epinaestine	SAC	Randomized, double-blind, parallel group 8 weeks' treatment	Placebo	Clinical parameters	Emedastine superior to levocabastine in controlling chemosis, eye lid swelling, redness, itching	[84]
Ketotifen	SAC/PAC	Randomized, double-blind, parallel group 30 days' treatment	Levocabastine Baseline	Clinical parameters	Equipotent to levocabastine	[87]
Ketotifen	SAC	Open, uncontrolled 30 days' treatment	Placebo	Clinical parameters	Improvement of signs and symptoms associated with decreased CD29 and eotaxin expression	[88]
Ketotifen	SAC	Randomized, double-blind, parallel group	Olopatadine	ICAM-1, VCAM-1, HLA-DR, CD29 expression by epithelium	Both drugs were effective for the evaluated parameters	[93]
Ketotifen	SAC	4 weeks' treatment Randomized, double-blind, multicenter, parallel group	Placebo Levocabastine	Clinical parameters	Ketotifen superior to levocabastine	[94]
Olopatadine	SAC	CPT Randomized, double-blind, parallel group	Systemic desloratide	Clinical parameters	Ketotifen more effective in controlling itching and redness	[98]
Olopatadine	SAC	CPT Randomized, double-blind, contralateral eye	Placebo	Clinical parameters Tear histamine level, number of immune cells, ICAM-1/CD54 expression by epithelium	Effective for all evaluated parameters	[99]
Olopatadine	SAC/PAC	CPT Randomized, double-blind, contralateral eye	Levocabastine	Clinical parameters	More effective for itching and redness than levocabastine	[99]

CPT, conjunctival provocation test; PAC, perennial allergic conjunctivitis; SAC, seasonal allergic conjunctivitis.

the treatment of patients with allergic conjunctivitis [81,82].

Epinastine significantly decreased ocular itching, eyelid swelling, chemosis and conjunctival, episcleral and ciliary hyperemia following specific allergen challenge in a randomized, double-masked, vehicle-controlled study [83] performed on 67 patients with allergic conjunctivitis. In addition, an environmental clinical trial [84] showed that epinastine eye drop treatment is similar or superior to levocabastine in the relief of ocular itching and hyperemia.

Ketotifen has been used for several years as an antiallergic drug for the management of bronchial asthma, atopic dermatitis, seasonal or perennial rhinitis, allergic conjunctivitis, acute or chronic urticaria and food allergy [38]. In large clinical studies [85,86] performed on patients with allergic conjunctivitis, ketotifen was more effective in inhibiting ocular itching and hyperemia following allergen challenge when compared with placebo. The improvement of signs and symptoms induced by ketotifen eye drop treatment was associated with a decreased expression of inflammatory markers, such as ICAM, CD29+ and eotaxin+ epithelial cells [87,88].

However, ketotifen failed to inhibit histamine, leukotriene and kinin production induced by nasal allergen challenge [89]. In addition, Phillips *et al.* [90] demonstrated that ketotifen had lower inhibitory effect on skin weals induced by allergen than on weals induced by histamine, suggesting that ketotifen does not possess any additional mast cell-stabilizing action, at least for human skin mast cells.

In addition, contrasting data are described on the mast cell-stabilizing effect of ketotifen based on the different allergic diseases investigated. In fact, though ketotifen reduces clinical severity and inhibits cold-evoked histamine release in patients with cold urticaria [91], Mallet *et al.* [92] showed that ketotifen treatment was ineffective in lowering urinary histamine and its metabolite in patients with mastocytosis. However, it is conceivable that histamine release from mast cells of mastocytosis patients may be regulated differently when compared with cells of patients with cold urticaria or with conjunctival mast cells in patients with allergic conjunctivitis. In fact, ketotifen is well known to be more effective in controlling signs and symptoms of patients with allergic conjunctivitis when compared with 'pure' antihistamine drugs administered topically or systemically [93,94].

Olopatadine has been shown to be significantly more effective than placebo in relieving itching and redness in patients with allergic conjunctivitis [9,95]. In two double-masked, randomized, placebo-controlled studies [96,97], olopatadine was significantly effective in preventing itch-

ing and redness in respectively 98 and 169 patients with allergic conjunctivitis, immediately and 8 h after drug administration. A following study [98] using CPT confirmed the positive effect of olopatadine in reducing signs and symptoms (including itching and redness) in patients with allergic conjunctivitis. In this study, the clinical efficacy of olopatadine was associated with a significant decrease of tear histamine levels, conjunctival number of immune cells (including eosinophils, neutrophils and lymphocytes) and ICAM-1 expression after allergen challenge when compared with placebo. The results of this study support the in-vitro evidence that olopatadine combines a mast cell-stabilizer action with the antihistamine activity in eyes of allergic patients. In fact, it is plausible that the decrease of signs and symptoms is due to a reduced release of mediators from conjunctival mast cells, but an anti-inflammatory effect due to inhibition of histamine receptor of the conjunctival epithelial cells cannot be excluded. In line with these results, it has been shown that olopatadine eye drop treatment is superior to 'pure' antihistamine drug administered topically or systemically [99,100]. Table 3 summarizes the clinical evidence of mast cell-stabilizing effects of multiple action drugs.

Conclusion

In-vitro and in-vivo experimental data as well as clinical trials support the evidence that multiple action antiallergic drugs may affect the allergic reaction at different levels: inhibiting histamine receptor(s), inhibiting release of mast cell mediators (including histamine and cytokines) and exerting direct/indirect actions on immune cells. However, most of the in-vitro studies were performed on cord blood-derived mast cells or leukemic mast cells that significantly differ from conjunctival mast cells. Interestingly, multiple action drugs inhibit the release of neoformed mast cell mediators in different ways. The mechanisms of these stabilizing effects have not yet been completely elucidated, but the selective action of the different drugs open new perspectives for a more specific targeting of the different phases of the allergic reaction. However, an in-vitro study performed on conjunctival mast cells, obtained from pterigium, failed to demonstrate a significant effect of multiple action drugs in stabilizing the conjunctival mast cells. On the other hand, animal models of different allergic diseases, including conjunctivitis, showed that multiple action drugs may exert a stabilizing action on mast cells. Human studies performed by conjunctival allergen provocation test also suggested a significant mast cell-stabilizing activity of these drugs. In clinical trials, the superior effectiveness of multiple action drugs as compared with both antihistamines and mast cell stabilizers further support the concept of a multiple action of these molecules. In fact, multiple action agents not only provide faster and superior relief than systemic and topical 'pure'

antihistamines, but also they possess a longer duration of action suggesting a combined action of histamine receptor blockage, inhibition of mast cells mediator release and modulation of immune cells recruitment/activation.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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