

REVIEW

Pharmacological modulation of human mast cells and basophils

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Introduction

Mast cells and basophils are the only cells expressing the tetrameric ($\alpha\beta\gamma_2$) structure of high affinity receptor for IgE (Fc ϵ RI) and synthesizing histamine in humans [1]. Tissue mast cells and circulating basophils differ morphologically, ultrastructurally, immunologically, biochemically and pharmacologically [2, 3]. Basophils, like other granulocytes, differentiate and mature in the bone marrow, circulate in the blood, and are not found in normal connective tissues. In contrast, mature mast cells are normally distributed throughout connective tissues, where they are often adjacent to blood and lymph vessels, near or within nerves, and beneath epithelial surfaces that are exposed to such environmental antigens as those of the respiratory and gastrointestinal systems and the skin [4, 5]. Mast cells originate from CD34⁺ pluripotent cells present in bone marrow and in fetal liver [6]. There is evidence that mast cells do not originate from circulating basophils, monocytes and lymphocytes, but rather from a specific lineage of the haematopoietic system. These and many other differences suggest that basophils and mast cells play different roles in various types and phases of immune responses involving the respiratory tract [7] and the skin [8].

A feature of basophils is their ability to migrate into such inflamed tissues as the sites of late-phase reactions [7, 9]. Mast cells are instead predominantly involved in immediate allergic reactions [7, 8, 10].

Human mast cells and basophils play a role in the pathogenesis not only of allergic diseases but also of a variety of immunological disorders through the elaboration of proinflammatory and fibrogenic mediators [11–13]. Moreover, mast cells [11, 14–20] and basophils [12, 13, 21, 22] can generate various cytokines and chemokines. In addition, activated Fc ϵ RI⁺ cells express the ligand for CD40 (CD40L), which can provide the cell contact signal required for IgE synthesis by human B cells [23]. Mast cells and basophils may therefore play a key role in allergic disorders, not only by producing inflammatory and fibrogenic mediators, but also by directly (CD40L) and indirectly (IL-4 and IL-13) regulating IgE synthesis independently of T cells. Consequently, it is not unlikely that elucidation of the intrinsic signalling pathways underlying Fc ϵ RI⁺ cell activation and their pharmacological modulation will give rise to novel immunomodulation strategies for the treatment of disorders involving these cells.

Several studies have been carried out on the *in vitro* effects of drugs that modulate the release of mediators from these cell

types. This approach has helped to clarify the complex biochemical mechanisms underlying the release of mediators in response to immunological and non-immunological stimuli [24–26]. Significant differences have been documented: (a) between mast cells and basophils as regards the pharmacological agents that modulate the release of preformed (histamine) and *de novo* synthesized (LTC₄ and PGD₂) mediators; (b) between mast cells isolated from different anatomical sites; and (c) between the pharmacological effects of compounds of the same class of drugs [26–28].

Several excellent reviews have focused on the molecular consequences of mast cell activation and their pharmacological modulation [29–32]. In this brief review we shall concentrate on agents that modulate *in vitro* the release of mediators from human mast cells and basophils that appear to hold promise for the treatment of inflammatory diseases.

Phosphodiesterase inhibitors and adenylate cyclase activators

In a variety of cell systems cyclic AMP (cAMP) is an important second messenger and elevations of intracellular cAMP down-regulate the functions of human inflammatory cells [33].

Phosphodiesterases (PDEs) are responsible for cAMP hydrolysis. At least 11 different classes of cAMP PDE isoenzymes have been proposed, each the product of a distinct gene or a family of genes [34]. These PDE isozymes differ in: (i) kinetic characteristics; (ii) physical characteristics; (iii) tissue distribution; (iv) endogenous activators and inhibitors (e.g. Ca²⁺/calmodulin, cGMP); and (v) subcellular distribution [34]. A variety of PDE inhibitors are available as pharmacological probes; these are of two types: (i) standard non-specific inhibitors of PDE activity such as theophylline and 3-isobutyl-1-methylxanthine; and (ii) selective inhibitors of individual PDE isozymes. The latter comprise compounds such as siguazodan, a selective PDE3 inhibitor, rolipram, a selective PDE4 inhibitor, and zaprinast, a selective inhibitor of cGMP-specific PDE (PDE5) [34].

Both cAMP PDE3 and PDE4 have been identified in purified basophils and mast cells [35]. PDE4 has attracted particular attention and four genes (or splice variants) have been identified (PDE4A, PDE4B, PDE4C and PDE4D) [36]. The four gene products of family 4 of the PDEs are characterized by selective, high affinity hydrolysis of cAMP and sensitivity to inhibition by rolipram, an archetypal inhibitor of this enzyme family.

Rolipram inhibits PAF- and anti-IgE-induced mediator release and increases cAMP levels in basophils [37, 38]. In addition, it enhances cAMP accumulation and inhibition of

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mediator release by forskolin, a direct activator of adenylate cyclase [39]. Inhibitors of PDE4 also regulate mast cell functions [40]. In contrast, mediator release was not inhibited by compound SK & F 95654 or zaprinast, respectively PDE3 and PDE5 inhibitors. It thus appears that a rise in intracellular cAMP inhibits mediator release from basophils and that PDE4 is the isoenzyme mainly responsible for regulating cAMP content in these cells.

Selective inhibitors of PDE isoenzymes may prove useful in the modulation of FcεRI⁺ cells in the near future. Interestingly, pentoxifylline, generally considered non-selective in its activity on PDE enzymes, exerts several anti-inflammatory and immunoregulatory effects [41]. We found that pharmacological concentrations of pentoxifylline concentration-dependently inhibit IgE-mediated release of proinflammatory mediators from human basophils but not from human lung mast cells [42].

Isoproterenol and other catecholamines inhibit IgE-mediated histamine release from basophils, by binding to β₂-adrenergic receptors [33]. Fenoterol, a β₂-adrenergic agonist, rapidly inhibited antigen-induced histamine secretion from human lung mast cells and basophils [43] and the dose–response inhibition curve was paralleled by a fenoterol-induced increase in cAMP levels. Unfortunately, the use of fenoterol has been reported to be associated with an increased risk of death or near death from asthma [44]. Salmeterol and formoterol, two long-acting β₂-adrenergic agonists, also modulate the release of mediators from lung mast cells [45].

Non-steroidal anti-inflammatory drugs

The cyclooxygenase pathway leads to prostaglandin and thromboxane generation from endogenous arachidonic acid (AA) released from membrane phospholipids. At least two classes of phospholipases A₂ (PLA₂s) are responsible for AA mobilization in human inflammatory cells: a cytosolic, high molecular weight PLA₂ (cPLA₂) and secretory, low molecular weight PLA₂s (sPLA₂s) [46]. Cyclooxygenase (COX) exists in two isoforms (COX-1 and COX-2). COX-1 is the constitutive isoform mainly, but not exclusively, responsible for maintaining 'physiological' levels of prostanoids in the gastric mucosa, kidney, platelets and bone tissue. COX-2 is an inducible isoform expressed in inflammatory cells upon exposure to cytokines or growth factors [47]. COX-2 is primarily involved in prostanoid generation in inflammatory areas.

Non-selective COX inhibitors inhibit both COX-1 and COX-2, thus simultaneously inhibiting fever, pain and inflammation and the physiological functions of prostanoids. Selective COX-2 inhibitors preferentially suppress the production of prostaglandins by inflammatory cells, although not completely, given the contribution of COX-1.

Aspirin and other non-steroidal anti-inflammatory drugs (NSAID) are non-selective inhibitors of COX-1. Such NSAID as indomethacin, meclofenamic acid and acetylsalicylic acid enhance antigen- and anti-IgE-induced histamine release [48, 49], and potentiate the *de novo* synthesis of LTC₄ from basophils [50]. This appears to be a property of the majority of NSAID tested [51], and their potency in enhancing IgE-mediated histamine secretion was significantly correlated with their capacity to inhibit the cyclooxygenase pathway [51]. However, it should be mentioned that immunological

activation of human basophils does not generate any known COX product.

Nimesulide (4-nitro-2-phenoxy methane sulphonanilide) is a NSAID chemically unrelated to other compounds of the same class, its functional acid group being sulphonanilide [52]. We found that nimesulide and its active metabolite 4-hydroxynimesulide concentration-dependently inhibited the release of histamine from basophils [53]. Nimesulide also inhibited histamine, LTC₄ and PGD₂ release from lung and skin mast cells [54]. This drug and its metabolite, differently from other NSAIDs, inhibits the release of preformed and *de novo* synthesized proinflammatory mediators from human FcεRI⁺ cells challenged with IgE- and non-IgE-mediated stimuli. Nimesulide appears to be the only NSAID found so far with these properties, which might explain why it is well tolerated in patients with acetylsalicylic acid (ASA) idiosyncrasy [55].

We have compared the effects of nimesulide and other NSAIDs (piroxicam and diclofenac) on the immunological release of mediators from human synovial mast cells [26]. These cells differ from mast cells isolated from other human tissues [56]. Nimesulide concentration-dependently inhibited the release of histamine, tryptase, PGD₂ and LTC₄ from immunologically activated synovial mast cells, whereas piroxicam and diclofenac had no effect. It is feasible that the inhibition of mediator release from synovial mast cells might contribute, at least in part, to the efficacy of nimesulide in the treatment of inflammatory arthritis [57].

There is now evidence that nimesulide preferentially inhibits COX-2 [58, 59]. It is still unknown if the inhibitory effects of nimesulide on human FcεRI⁺ cells are related to selective COX-2 inhibition or whether the pharmacological effects of this drug may be more complex than those of a NSAID.

Glucocorticoids

Glucocorticoids play a central role in the modulation of the immune system and they are one of the therapeutic mainstays in most inflammatory and immune-mediated disorders. It is well established that glucocorticoids exert multiple biological and pharmacological effects [60].

Intravenously injected glucocorticoids in man cause rapid basopenia, whereas skin tissue histamine remains unchanged [61]. In contrast, prolonged treatment with topical glucocorticoids reduced skin mast cell density and histamine content and inhibited the allergen-induced wheal-and-flare response [62]. Prolonged (12–24 h) *in vitro* incubation with glucocorticoids inhibited IgE-mediated histamine release from basophils [63, 64]. In contrast, incubation for up to 24 h of mast cells isolated from lung parenchyma, intestine or skin with glucocorticoids did not alter their immunological release of histamine, PGD₂ or LTC₄ [65]. Figure 1 shows the effects of preincubation (18 h, 37 °C) of human basophils and lung mast cells with increasing concentrations (10⁻⁹–10⁻⁶ M) of dexamethasone on the IgE-mediated release of histamine. The latter example emphasizes the pharmacological heterogeneity of human FcεRI⁺ cells.

The fact that human basophils [12, 13, 21, 22] and mast cells [11, 14–20] are a major source of cytokines raised the important question as to whether corticosteroids can modulate, *in vitro* as well as *in vivo*, the synthesis of cytokines in immunologically

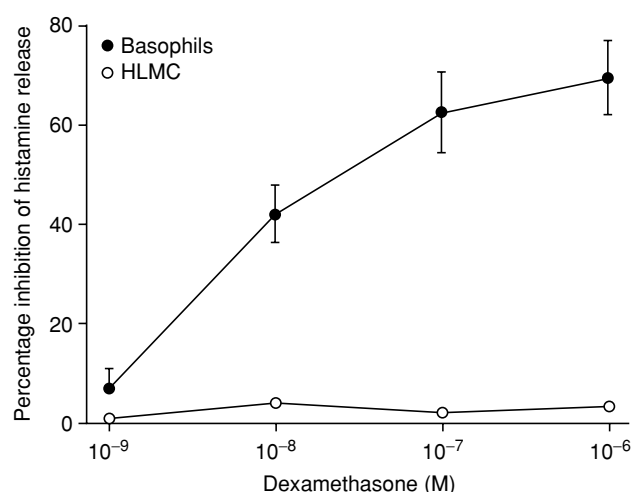


Fig. 1. Effects of dexamethasone on IgE-mediated release of histamine from human peripheral blood basophils (●) and lung mast cells (HLMC) (○). Cells were pre-incubated for 18 h at 37 °C with the indicated concentrations of dexamethasone and then challenged (45 min, 37 °C) with anti-IgE (basophils, 0.1 µg/mL; mast cells, 2 µg/mL). The data are expressed as the percentage inhibition of histamine release from control cells. Each point represents the mean $\pm$ SEM of three experiments (control histamine release in basophils, $22.3 \pm 3.7\%$; in HLMC, $18.2 \pm 5.3\%$).

activated FcεRI⁺ cells. Short pre-incubation (c. 1 h) of human basophils with several glucocorticoids (dexamethasone, deflazacort and hydrocortisone) inhibited the immunological release of IL-4 [66]. In this experimental condition glucocorticoids did not exert any inhibitory effect on the release of histamine from basophils. These results suggest that the mechanisms controlling the release of cytokines (IL-4 and IL-13) differ from those controlling the immunological release of histamine from basophils.

Protein kinase C inhibitors

To date, several PKC isoenzymes are known and they are divided into three major groups: the calcium-dependent or conventional PKC (α , β I, β II and γ), the calcium-independent isoenzymes (δ , ϵ , ϵ' , η , θ and μ), and the atypical PKC (ζ and λ) [67]. There are marked differences in tissue distribution among the PKC isoenzymes. The relative proportion of PKC δ to other PKC isotypes in basophils is greater than observed in some other cell types (D.W. MacGlashan Jr, personal communication). PKC plays a role in signal transduction by phosphorylating different proteins, including ion channels, receptors, other kinases and cytoskeletal proteins. Phorbol esters (TPA) and bryostatins, which are thought to directly activate PKC enzymes, can induce histamine release from basophils [68, 69]. This does not occur in human lung or skin mast cells. This observation suggested that the signalling pathway that induces degranulation in basophils and mast cells might be regulated by PKC in a different way.

Bryostatins and TPA can activate basophils to release histamine, but not LTC₄ [68, 69]. Anti-IgE- and TPA-induced activation of basophils is accompanied by a rapid rise in membrane-associated PKC [70]. Staurosporine, a non-specific PKC inhibitor, inhibits TPA-induced mediator release from

basophils [70]. Both TPA and bryostatins inhibit anti-IgE-induced mediator release from skin and lung mast cells [69, 71]. This only apparently contrasts with the observation that TPA and bryostatins do not activate human mast cells and suggests that, although PKC activation alone is not sufficient to cause mediator release from mast cells, it might contribute to the release induced by cross-linking of surface IgE.

Recently, Miura and MacGlashan have demonstrated that PKC β I, β II, δ and ϵ were expressed in human basophils [72], whereas PKC γ and η were not. There is also evidence that PKC activation plays different roles in the various biochemical pathways induced by different secretagogues. It has been reported that the effects of staurosporine and other putative PKC inhibitors differ with different secretagogues [73]. However, studies with bis-indolylmaleimide and its analogs showed inhibition of TPA-induced, but not anti-IgE-induced, histamine release from basophils [72]. Moreover, the PKC inhibitor Ro-31-8220 did not inhibit IgE-mediated histamine and LTC₄ release from basophils [74]. These pharmacological studies suggest that PKC do not play a prodegranulatory role in IgE-mediated secretion in basophils.

Several isoforms of PKC differing in their subcellular localization and their requirements for activation have been identified [67, 75]. It is unclear whether specific inhibitors of PKC isoenzymes in human FcεRI⁺ cells will help to identify novel therapeutic agents that modulate basophil- and mast cell-mediated inflammatory disorders.

Tyrosine kinase inhibitors

Tyrosine kinases are important in signal transduction mechanisms in many cell types. In rodent mast cells and mast cell lines IgE cross-linking leads to an increase in tyrosine phosphorylation [76], suggesting that tyrosine kinases may be involved in IgE-induced responses. There is some evidence that the tyrosine kinases Lyn, Syk and Zap-70 are present in human basophils [77]. Four inhibitors of tyrosine kinases were tested on IgE-dependent histamine release from human lung mast cells and basophils [78]. Genistein and tyrphostin 25 inhibited histamine secretion from basophils, but had much less effect in lung mast cells. Methyl 2, 5 dihydroxycinnamate did not inhibit mediator release from basophils, but proved to be an effective inhibitor of IgE-mediated degranulation in lung mast cells. Finally, laven Dustin A failed to inhibit histamine release from either basophils or mast cells. The tyrosine kinase inhibitor piceatannol has little or no effect on Lyn activation in basophils, but blocks the activity of both Syk and Zap-70, and abolishes anti-IgE-mediated histamine secretion [77]. A recent study has concluded that piceatannol does not inhibit secretion from basophils by inhibiting the activity of Syk kinase [79].

In summary, additional *in vitro* studies using more specific inhibitors of tyrosine kinases should provide a better picture of whether this class of compounds can be used to modulate the release of mediators from human mast cells and basophils.

Immunophilin ligands

Handschumacher et al. first identified a family of proteins with high affinity for cyclosporin A (CsA) [80]. This protein, named

cyclophilin (CyP) displayed high affinity for CsA and low affinity for inactive analogs such as CsH. There is now evidence that CyP belongs to a family of intracellular proteins, the immunophilins, which include the FK-binding proteins (FKBPs) [81]. Two natural macrolides, FK-506 (tacrolimus) and rapamycin (RAP) bind with high affinity to FKBP [81].

CsA binding to CyP, and FK-506 to FKBP, is essential in their immunosuppressive activity [28]. CsA-CyP complexes, as well as FK-506-FKBP complexes, bind to a protein identified as calcineurin [64]. Calcineurin (Cn) has a catalytic A subunit (CnA) and a regulatory B subunit (CnB). The CnA subunit has a binding site for calmodulin (CaM) and for the CnB subunit. Complexes of CsA or FK-506 and their respective intracellular binding proteins inhibit the CaM-dependent protein phosphatase 2B, which appears to be essential in the signal transduction pathway for lymphocytes [82], basophils [24, 83] and mast cells [64, 83, 84]. RAP binds to FKBP, but the molecular mechanism of its immunosuppressive action is different from that of FK-506 [85].

Low concentrations of CsA concentration-dependently inhibited histamine and LTC₄ release from basophils challenged with antigen or anti-IgE [24, 83]. CsH, which has extremely low affinity for CyP, had no effect on the immunological activation of basophils. CsA and its analogs inhibit the release of preformed and *de novo* synthesized mediators from basophils, by interacting with CyP [83], because there is a significant correlation between the concentrations of several cyclosporins (CsA, CsG, CsC and CsD) that inhibited histamine release and their affinity for CyP. CsA, but not CsH, also inhibited the immunological release of histamine, LTC₄ and PGD₂ from human lung and skin mast cells, by interacting with CyP [64].

A single oral dose of CsA in normal volunteers caused rapid and significant inhibition of histamine release from basophils obtained *ex vivo* and provide a rare example of how an *in-vivo*-administered drug can modulate basophil releasability *ex vivo*.

Compound FK-506 also inhibits histamine and LTC₄ release from mast cells and basophils challenged with antigen and anti-IgE, whereas RAP has little or no effect. Interestingly, RAP acts as a competitive antagonist of FK-506, presumably at the level of FKBP [24, 84]. Thus, binding to FKBP is necessary, but not sufficient in itself to deliver the inhibitory signal for the release of mediators from basophils and mast cells.

More recently, we have demonstrated that low concentrations of CsA and FK-506 are potent inhibitors of the *de novo* synthesis of IL-4 and IL-13 from basophils immunologically activated by several stimuli [86, 87]. FK-506 (tacrolimus) ointment is rapidly effective in the treatment of patients with atopic dermatitis [88]. In this condition skin mast cells and infiltrating basophils play a prominent role [89]. FK-506 exerts potent anti-inflammatory effects by inhibiting the release of histamine and eicosanoids from these cells [24, 84]. These findings, together with the rapid clinical efficacy of tacrolimus in atopic dermatitis, suggest that the anti-inflammatory properties of this compound are largely responsible for its beneficial effects *in vivo* [90]. It is likely that the anti-inflammatory properties of immunophilin-binding drugs could be beneficial in the treatment of other allergic and autoimmune disorders of the skin and respiratory tract.

H₁ receptor antagonists

H₁-antihistamines antagonize the effects of histamine at the H₁ receptor and are widely used in the treatment of several allergic conditions [91]. It has been known since 1975 that high concentrations of first generation antihistamines inhibit *in vitro* antigen-induced histamine release from human basophils [92]. In these studies, no correlation was seen between the potency of H₁ receptor-blocking activity and the inhibition of mediator release, suggesting that a mechanism other than receptor antagonism was involved. Other *in vitro* studies have shown that antihistamines prevent the release not only of histamine, but also of other proinflammatory mediators such as LTC₄, PAF and PGD₂ [93–97]. For example, oxatamide inhibits the release of both preformed (histamine and tryptase) and *de novo* synthesized mediators (LTC₄ and PGD₂) from mast cells and basophils [94, 97]. It is important to emphasize that not all H₁ antagonists have such anti-inflammatory activity *in vitro*. For instance, cetirizine does not prevent the release of histamine and lipid mediators from human basophils and mast cells, whereas another H₁ antagonist, mizolastine, inhibits the *de novo* synthesis of LTC₄ from basophils, but not the secretion of preformed histamine (Triggiani et al., unpublished observation).

Loratadine and desloratadine inhibit histamine release from human basophils induced by both IgE-dependent and IgE-independent mechanisms [95]. These results were extended to the human skin and lung mast cells in which desloratadine inhibits preformed (histamine and tryptase) and *de novo* synthesized mediators (LTC₄ and PGD₂) [95]. Figure 2 illustrates the results of a typical experiment showing the inhibitory effects of desloratadine on the immunological release of histamine and tryptase from skin mast cells. More recently, it has been shown that desloratadine also inhibits IL-4 and IL-13 synthesis induced by anti-IgE in human basophils [98].

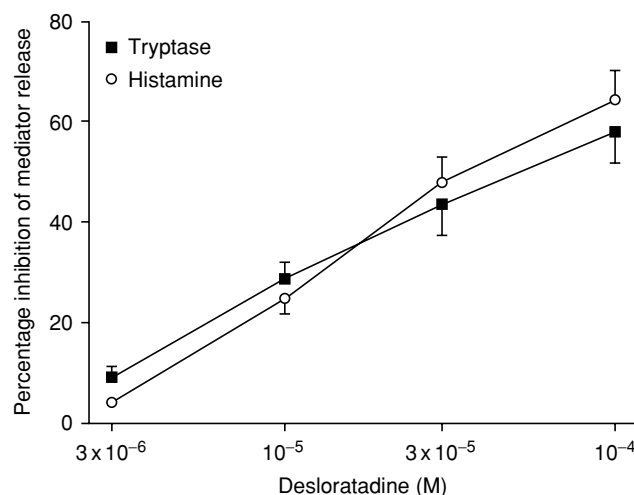


Fig. 2. Effects of desloratadine on IgE-mediated release of histamine and tryptase from human skin mast cells. Cells were pre-incubated for 15 min at 30 °C with the indicated concentrations of desloratadine and then challenged with anti-IgE (2 µg/mL). Cells were then incubated for 30 min at 30 °C. Cell-free supernatants were then analysed for histamine and tryptase content. Values are the mean $\pm$ SEM of triplicate determinations obtained in a typical experiment performed with purified (86%) human skin mast cells. Control values were $16.3 \pm 1.9\%$ histamine release and 14.9 ± 2.4 µg of tryptase/ 10^7 mast cells.

Table 1. Overview of the effects of several classes of pharmacological agents on the release of mediators from human basophils and mast cells

Selective phosphodiesterase (PDE) inhibitors of isoenzymes present in human mast cells and basophils may prove useful in the modulation of FcεRI ⁺ cells.
Short-term and long-acting β ₂ -agonists are potent inhibitors of the immunological release of mediators from human mast cells and basophils.
Non-selective cyclooxygenase inhibitors enhance IgE-mediated histamine release from human basophils, but not from mast cells.
Prolonged <i>in vitro</i> incubation with glucocorticoids inhibits IgE-mediated histamine release from basophils, but not from mast cells. Glucocorticoids also inhibit the immunological release of IL-4 and IL-13 from basophils.
Several isoforms of protein kinase C (PKC) have been identified in human basophils and mast cells. It is unclear whether specific inhibitors of PKC isoenzymes in human FcεRI ⁺ cells will be useful as therapeutic agents.
More specific inhibitors of tyrosine kinases will provide a clearer picture of whether these compounds can be used to modulate the activation of human FcεRI ⁺ cells.
Such immunophilin ligands as cyclosporin A and FK-506 are potent inhibitors of the release of proinflammatory mediators and cytokines from human basophils and mast cells.
Certain H ₁ receptor antagonists inhibit mediator release from human FcεRI ⁺ cells. This is not a class-specific effect.

Together these results indicate that the inhibitory effect of certain H₁ receptor antagonists on mediator release from human FcεRI⁺ cells is not a class-specific effect and it is not a general property of all antihistamines.

Closing thoughts

In this brief review, we have concentrated on the eight main classes of pharmacological agents that have been indicated as modulators, *in vitro* and *in vivo*, of the release of proinflammatory mediators and cytokines from human basophils and mast cells. Table 1 provides a synthetic overview of the effects exerted by each of these eight classes.

An overwhelming body of evidence suggests that human mast cells and basophils differ biochemically, immunologically and pharmacologically. The heterogeneity of pharmacological modulation of FcεRI⁺ cells is presumably related to the complexity and diversity of signal transduction pathways activated in these cells. There is also evidence that different immunological stimuli (e.g. IgE- and non IgE-mediated) can trigger distinct signalling events leading to the release of selective mediators and cytokines. It is therefore not surprising that certain pharmacological agents can selectively modulate the release of mediators induced from human FcεRI⁺ cells by some, but not all, stimuli.

Human mast cells [11, 14–20] and basophils [12, 13, 21, 22] synthesize and release several cytokines in addition to various proinflammatory and fibrogenic mediators [20, 56]. It is of interest that one may modulate selectively the release of preformed or *de novo* synthesized mediators from immunologically activated FcεRI⁺ cells. An additional aspect of interest and complexity is the fact that varying the pre-incubation time with glucocorticoids can lead to either selective inhibition of cytokine release or complete inhibition of mediator secretion. Thus, a drug can exert different effects on the release of cytokines and proinflammatory mediators from the same cell depending on time of pre-incubation.

Another interesting feature is that immunophilin-binding drugs (e.g. CsA and FK-506) also exert potent pharmacological effects on human FcεRI⁺ cells. The inhibitory effect of these compounds on basophils extends to IL-4 and IL-13 secretion and indicates that immunophilin-binding drugs might play a

dual role by inhibiting the release of proinflammatory mediators and the synthesis of some immunoregulatory cytokines.

The inhibitory effects of certain H₁ receptor antagonists on mediator release seen in *in vitro* studies is intriguing. This is not a class-specific effect and it is not a general property of all antihistamines. These anti-inflammatory effects were mainly observed *in vitro* and they generally require doses higher than those recommended for symptom-relief in allergic disorders. Therefore, it is uncertain whether the anti-inflammatory effects seen in *in vitro* studies have any relevance to the clinical efficacy of these drugs. However, the recent observation that certain second generation antihistamines also modulate the release of important cytokines (IL-4 and IL-13) deserves further study.

The availability of effective methodologies for the purification of nearly homogeneous basophils from peripheral blood and of enriched preparations of mast cells from different tissues should encourage further studies of the signalling mechanisms and their pharmacological modulation in human FcεRI⁺ cells.

Efforts to gain insight into the complex biochemical events occurring during the immunological activation of human FcεRI⁺ cells could lead to the identification of novel biochemical targets to improve the pharmacological modulation of the release of different mediators from human mast cells and/or basophils.

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