

The clinical relevance of the anti-inflammatory properties of antihistamines

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The continuing worldwide increase in the incidence and prevalence of allergic diseases such as asthma, allergic rhinitis, and atopic dermatitis (1, 2) has created considerable research interest in the mechanisms responsible for the allergic response. It is to be hoped that this research will facilitate the development of more effective and more closely targeted therapies than those presently available. Allergic inflammation is characterized by an immediate response which is a consequence of IgE-dependent mediator release from mast cells or basophils. This is followed by a delayed and more prolonged infiltration of the site of allergic inflammation by leukocytes, significant numbers of which are eosinophils. Both resident and infiltrating cells are important sources of the mediators and cytokines which maintain and enhance the allergic inflammatory response. The mechanisms responsible for the accumulation of proinflammatory cells at tissue sites of allergic inflammation are complex but probably involve selective adhesion to postcapillary venular endothelium, followed by transmigration into the tissues under the influence of chemotactic factors including lipid mediators, cytokines, and chemokines. Once in the tissues, there is an elaborate interaction between infiltrating cells, resident cells, and the mediators and cytokines released as part of the inflammatory response. Important players in these processes include cells recruited to the inflammatory site, such as T cells, monocytes, eosinophils, basophils, and platelets, and resident cells including mast cells, antigen-presenting cells, epithelial cells, and endothelial cells.

T cells appear to play a central role in these responses due to their capacity to release an array of cytokines

which can attract and activate other proinflammatory cells. A number of studies have demonstrated that T cells from the blood or bronchoalveolar lavage (BAL) fluid of asthmatics are activated and express mRNA encoding the cytokines interleukin (IL)-4 and IL-5 (3). IL-4 and IL-13 are responsible for the isotype switch in B cells from IgG production to that of IgE, thereby leading to sensitization of mast cells and basophils (4), while IL-5 promotes the activation and differentiation of eosinophils (5). These cytokines are produced by a subset of T helper cells termed Th2 cells which are skewed toward the production of IL-4 and IL-5. In contrast, Th1 T cells produce IL-2 and interferon (IFN)- γ , which are associated with delayed-type hypersensitivity reactions (6). A T-cell response skewed toward the Th2 pathway is thought to be a prominent factor in the development of allergic inflammation (7). Mast cells and epithelial cells are also important mediator producers and are potent sources of a wide range of cytokines and chemokines together with prostaglandins and leukotrienes.

Normal skin and lung tissue contain few eosinophils, while, in contrast, their presence is often noted in patients with chronic allergies. For example, airway inflammation in allergic asthma is characterized by infiltration of the bronchial mucosa by large numbers of proinflammatory cells, among which the eosinophil is prominent despite its being a minority constituent of circulating leukocytes (8). Examination of endobronchial biopsies or BAL fluid from patients with mild or moderate asthma has demonstrated a correlation between disease severity and the number of activated eosinophils. Eosinophil granule-derived mediators,

such as major basic protein (MBP), are heavily involved in epithelial cell damage resulting in ciliary dysfunction and cell loss, both of which are thought to be central in the development of bronchial hyperresponsiveness (9, 10). In the skin, challenge of normal subjects with intradermal injection of platelet-activating factor (PAF) produced a cellular response composed primarily of neutrophils, while the same procedure performed in allergic subjects resulted in significant accumulation of eosinophils (11). In patients with atopic dermatitis, eosinophils infiltrate the dermis after a patch test antigen challenge (12), and the lesions of atopic dermatitis contain MBP deposits (13). In addition to their granule-derived preformed mediators, eosinophils have the ability to generate large quantities of the lipid mediators PAF and leukotriene (LT) C₄ (14). Furthermore, several studies have demonstrated that eosinophils are a source of up to 18 growth factors and regulatory or proinflammatory cytokines (15): for example, IL-2, IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor (GM-CSF), together with the chemokines eotaxin, macrophage inflammatory protein (MIP)-1 α , and RANTES. It can be appreciated, therefore, that the eosinophil has a potent array of mediators with considerable potential to initiate and sustain an inflammatory response.

The predominant preformed mediator released from 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 actions of histamine. However, it can be appreciated from the foregoing that antagonizing the effects of histamine only targets one aspect of allergic inflammation. An effective approach to the treatment of allergic disease might therefore require the inhibition of cell migration, mediator release, adhesion, and adhesion molecule expression. Thus, it is of interest that it has been recognized for some years that – in addition to being potent antihistamines – a number of the second-generation drugs appear to possess several antiallergic effects that cannot be explained by antagonism of the H₁ receptor. In general terms, these studies can be divided into those covering *in vitro* effects (16) on the function of isolated proinflammatory cells, and those studying *in vivo* effects where the impact of a given drug on inflammatory parameters is assessed in addition to its effects on symptom relief (17). Studies of this nature have demonstrated that several second-generation antihistamines inhibit inflammatory cell infiltration into allergen-challenged skin sites in sensitive individuals, downregulate mediator release, and have inhibitory effects on adhesion molecule expression by epithelial cells. The question of whether these additional attributes enhance the therapeutic effect of an antihistamine has been the subject of much debate. It is difficult to give a definitive answer owing to the problems inherent in isolating the many anti-inflammatory effects

from the H₁ blockade given by a given drug. Notwithstanding this limitation, demonstrable suppressive effects on allergic inflammation, additional to potent H₁ blockade, must be seen as a positive attribute for a drug.

In atopic patients, eosinophils and other cells are attracted by intradermal injection of allergen, PAF, or bradykinin. The accumulating cells can be identified and counted by either the skin-window technique or punch biopsies. Using the former technique, Fadel et al. demonstrated that cetirizine inhibited eosinophil accumulation at skin sites challenged with pollen (18) or PAF (19). The findings with the allergen-induced cutaneous late-phase reaction (LPR) were confirmed in a double-blind, placebo-controlled, crossover study (20) and also in a similar study in which cetirizine decreased eosinophil infiltration by approximately 80% in ragweed-sensitive subjects (21). This effect could not be attributed to cetirizine's H₁ activity since cetirizine does not block histamine-induced eosinophil infiltration, and neither of the potent H₁ antagonists promethazine and chlorpheniramine induced any alteration in allergen-induced inflammatory cell infiltration (22). Moreover, loratadine treatment had no effect on eosinophil influx in response to an intradermal injection of PAF in urticaria patients and did not influence the pollen-induced skin reactions or eosinophil influx seen in sensitive individuals (23). In addition, the skin-chamber technique failed to demonstrate a significant effect for loratadine treatment of atopic subjects on inflammation, histamine release, or recruitment of eosinophils or other leukocytes to skin sites challenged with anti-IgE (24). Considerable debate has been generated by more recent studies which have re-evaluated the effect of cetirizine on allergen-induced cutaneous LPR in atopic subjects. Two of these used punch biopsies to study the nature and extent of the cellular infiltrate. In neither study did pretreatment with cetirizine significantly inhibit the magnitude of the cellular infiltrate during the LPR, although both studies demonstrated a reduction in eosinophil numbers in some subjects. In contrast, pretreatment of these subjects with prednisolone significantly decreased both the magnitude of the LPR and eosinophil influx (25, 26). The discrepancies between the skin-window and biopsy studies might in part be explained by methodology; for instance, the quantity and purity of the allergens used to induce the LPR in all subjects. Furthermore, the skin-window technique enumerates and identifies cells that have undergone transendothelial migration and thus have left the dermal postcapillary venules, whereas biopsies measure the gross cellular content at the reaction site. This difference is a potentially important one since cetirizine might exert its effect by inhibiting eosinophil adhesion to, and/or transendothelial migration across, the microvascular endothelium (27).

These observations led to a number of *in vitro* studies which attempted to dissect the mechanisms by which cetirizine might exert its effect on cell migration. Loratadine (28), terfenadine (29), and cetirizine (30, 31) have all been reported to inhibit chemotaxis of eosinophils *in vitro*. In addition, cetirizine has been shown to inhibit neutrophil and monocyte chemotaxis (32). Interestingly, the antihistamine dexchlorpheniramine had no effect on f-Met-Leu-Phe (fMLP)- or PAF-induced eosinophil chemotaxis, an observation supporting the notion that inhibitory effects on eosinophil chemotaxis by second-generation drugs cannot be attributed to H₁ blockade. More recently, fexofenadine has been shown to inhibit eosinophil chemotaxis *in vitro* (33) but only at rather high concentrations which are unlikely to have physiologic significance. Moreover, *in vitro* PAF-induced eosinophil hyperadherence to cultured human umbilical vein endothelial cells has been shown to be significantly inhibited by cetirizine at concentrations as low as 10^{-9} M (34), and adhesion-dependent functions, such as PAF-induced neutrophil- and eosinophil-enhanced complement- or IgG-dependent rosette formation, were also inhibited by pretreatment of the cells with physiologically relevant concentrations of cetirizine (35). In contrast, fexofenadine had a significant inhibitory effect on eosinophil hyperadherence at only very high concentrations (33).

This raises an important consideration: examination of the literature reveals an almost bewildering number of studies on the anti-inflammatory effects of antihistamines *in vitro*, the majority of which examined the inhibitory effects of second-generation drugs on mediator release from diverse cell types including mast cells, basophils, eosinophils, and neutrophils (36). On closer examination, the usefulness of many of the mediator-release studies is debatable, as it is difficult to see the relevance – particularly in therapeutic terms – of any study reporting a significant effect on inflammatory cell function with drug concentrations which were hundreds or in some cases thousands of times higher than those that can possibly be achieved *in vivo*. Indeed, it could be argued that with very high concentrations it was drug toxicity which induced the inhibitory effect. This question is almost impossible to answer, as virtually none of these studies addressed the issue of the effect of the test drug on the viability of the cells under examination, and most failed to include a first-generation antihistamine as a control. A final consideration regarding the *in vitro* studies is that, of the currently available drugs, only acrivastine, fexofenadine, and cetirizine are not metabolized *in vivo*, a fact which raises additional doubts of the relevance of the *in vitro* testing of compounds reliant on biotransformation to the active metabolite.

Another interesting observation of the *in vitro* anti-inflammatory effects of second-generation drugs is that these actions are commonly seen only in cells isolated

from allergic, rather than normal, individuals. For example, therapeutically relevant concentrations of cetirizine were found to inhibit PAF-dependent superoxide generation by eosinophils isolated from allergic subjects. No significant inhibition was observed in cells isolated from normal subjects, and cetirizine did not have any effect on eosinophil peroxidase release in allergic subjects (37). Neutrophils are important sources of the potent chemotactic mediator LTB₄, which is produced from arachidonic acid by the action of the 5-lipoxygenase pathway (38). Neutrophils isolated from untreated patients with either asthma or allergic rhinitis produce greater quantities of LTB₄ after calcium ionophore stimulation than neutrophils isolated from normal controls (39). An *ex vivo* study by Cheria-Sammari et al. (40) showed that this enhanced LTB₄ production by neutrophils isolated from rhinitis patients could be markedly inhibited by cetirizine in that, after treatment, ionophore-stimulated LTB₄ production by their neutrophils was comparable to that of stimulated neutrophils from normal subjects. The effect of cetirizine was reproduced *in vitro* by demonstrating an inhibitory effect on LTB₄ production by isolated neutrophils from allergic subjects. The precise point(s) at which cetirizine inhibits the intracellular signalling pathways controlling eosinophil function remain to be elucidated. One study has demonstrated that low concentrations of cetirizine (1 µg/ml) induced a significant increase in the lipid order in the exterior part of the membrane and a decrease in eosinophil membrane heterogeneity. Moreover, cetirizine blocked the PAF-induced changes in membrane fluidity in eosinophils. In contrast, these effects were not observed in lymphocytes (41).

Epithelial damage may be a feature of atopic dermatitis, and damage to and the subsequent shedding of the bronchial epithelium is thought to make a major contribution to the pathogenesis of asthma (42). Epithelial adhesion molecule expression is considered to represent an important mechanism by which these cells interact with other proinflammatory cells, and their modulation might be of benefit clinically. For example, conjunctival challenge of allergic subjects resulted in an increase in epithelial cell intracellular adhesion molecule (ICAM)-1 expression and a large increase in the total number of inflammatory cells at the challenge site at 30 min; these effects persisted for up to 24 h, and the changes paralleled the increase in symptoms (43). In a double-blind, randomized, placebo-controlled trial, cetirizine reduced epithelial ICAM-1 expression, eosinophil and neutrophil numbers, and the clinical symptoms associated with both the early-phase reaction (EPR) and the LPR to a conjunctival challenge (44). However, this effect was not confined to cetirizine, as several other trials (45–47) have shown a protective effect by other second-generation antihistamines including oxatomide, loratadine, and terfenadine on the

allergic reaction induced by conjunctival challenge. Another double-blind, placebo-controlled, randomized, parallel study of children with allergic asthma associated with mite sensitivity showed that cetirizine treatment produced a significant reduction, and in some cases a total absence, of ICAM-1 expression by nasal epithelial cells which was associated with a trend toward a reduction in eosinophil and neutrophil numbers (48). Furthermore, Haye et al. (49) have demonstrated that, in nasal polyposis patients, treatment with cetirizine reduced nasal obstruction and ICAM-1 expression by nasal epithelial cells.

The concept of a minimal persistent inflammation (MPI) in allergic diseases including seasonal and perennial rhinitis may be likened to the inflammation which has been described in asthmatic subjects during clinical latency of their disease (50, 51). MPI has been shown to be present in asymptomatic allergic subjects and was manifested as a mild expression of ICAM-1 detectable on both conjunctival and nasal epithelium, together with the presence of inflammatory cells in the conjunctival and nasal scrapings. In contrast, relatives of the subjects and normal volunteers showed evidence of few inflammatory cells and no ICAM-1 expression on epithelial cells (52). As well as being an important adhesion molecule, ICAM-1 is the receptor for the major group of rhinoviruses. About 90% of the rhinoviruses use ICAM-1 as a receptor, their binding site being distinct from but overlapping with LFA-1 (53). Thus, a persistent inflammatory infiltrate, together with the changes in cell-surface adhesion molecule expression, particularly ICAM-1, by epithelial cells, may be of importance in determining susceptibility to rhinovirus infection of the upper airways. Although the relationship between viral infections of the respiratory tract and bronchospasm has been known for some time, the underlying mechanism(s) are still the subject of much debate (54). What is clear is that viral infections are involved in over 30% of asthma attacks in childhood in contrast to a rate of 3% in patients when free of symptoms. Furthermore, asthmatic children suffer from viral infections to a greater extent than normal controls (55). A low-grade exposure to allergen in some allergic individuals may therefore be insufficient to cause any obvious symptoms but may lead to enhanced epithelial cell expression of ICAM-1. This in turn might result in an increased susceptibility to infection with the rhinoviruses and consequently to an asthma attack (56). Moreover, a small but persistent epithelial ICAM-1 expression with a consequent higher risk of rhinovirus infection may at least partially explain the observation that allergic rhinitis may be a risk factor for asthma (57).

A double-blind, placebo-controlled, randomized, parallel-group study in 20 asymptomatic children with mite-allergic asthma looked at the effect of cetirizine on ICAM-1 expression by nasal epithelial

cells. Nasal scrapings were taken at the beginning and end of the study and evaluated for the presence of eosinophils and neutrophils and ICAM-1 expression by epithelial cells. Compared with the placebo group, cetirizine-treated children showed a significant reduction and in some cases a total absence of ICAM-1 expression by nasal epithelial cells. There was also a trend toward a reduction in eosinophil and neutrophil numbers (58). These observations suggest that, in the presence of MPI, treatment should be long-term, not simply administered during symptomatic periods. Cetirizine may prove to be useful for the prolonged treatment of allergic children, including episodes of clinical latency, but additional studies are required to define further the usefulness of cetirizine in this role.

Histamine is an additional mediator implicated in asthma pathogenesis, causing smooth-muscle contraction, mucus hypersecretion, and increased vascular permeability leading to mucosal oedema; moreover, bronchial hyperresponsiveness to histamine is a well-known feature of asthma. Inhalation of histamine will produce dose-dependent bronchial constriction that is greater in asthmatics than normals. Histamine concentrations in BAL from allergic asthmatics are higher than those of allergic rhinitis or normals, and histamine can contribute to the hyperreactivity seen in asthmatic patients (59, 60). Many asthmatic patients show an enhanced capacity for spontaneous histamine release in the peripheral circulation and lungs compared with normals as a result of increased peripheral basophil and mast-cell degranulation. Those patients receiving effective asthma medication release less histamine than those not receiving treatment (61). Segmental antigen challenge of normals and asthmatics demonstrated increased histamine levels and increased bronchoconstriction in the challenged area in the asthmatic group (62), and increased levels of histamine in BAL fluid were associated with increased hyperresponsiveness after methacholine challenge (63). The potential therapeutic efficacy of antihistamines in asthma would therefore appear to be considerable. However, the use of the early antihistamines in asthma treatment was limited because they were not effective at the doses recommended for treatment of allergic rhinitis, while higher doses resulted in intolerable side-effects. There is evidence that some of the newer antihistamines might be useful in asthma therapy (64), particularly as it is now recognized that inflammation of the airways is a key pathogenic event in asthma and, as discussed above, many of the second-generation drugs have documented anti-inflammatory effects.

Although it is acknowledged that antihistamines cannot be considered to be first-line medications for asthma therapy, a number of studies have evaluated the potential of some second-generation antihistamines as adjunct therapies. For example, Wood-Baker & Holgate (65) compared the inhibition of bronchospasm

due to inhalation of histamine or methacholine by astemizole, brompheniramine, cetirizine, chlorpheniramine, clemastine, cyproheptadine, terfenadine, and placebo in a single group of patients with asthma. Compared with placebo, all the antihistamines provided significant protection against histamine-induced bronchoconstriction, as determined by the histamine PC₂₀, with cetirizine giving the greatest protection. None of the drugs tested protected against the effects of methacholine-induced constriction. In atopic asthma patients, ebastine (66), cetirizine, and loratadine (67) effectively reduced the bronchial response to histamine challenge.

In contrast, a double-blind, placebo-controlled, crossover study with 10 mildly asthmatic subjects found that cetirizine had no significant effect on PAF-induced bronchoconstriction (68). Cetirizine inhibited cellular recruitment in dual asthmatic responders 24 h after allergen inhalation challenge in terms of total numbers of inflammatory cells in the bronchial wash and with a particular effect on eosinophil recruitment (69). Wasserfallen et al. (70) found that cetirizine had no effect on the early reaction to antigen but did have a significant protective effect against the late-phase asthmatic response to allergen bronchial challenge. Treatment of subjects with pollen-associated asthma with cetirizine 15 mg daily for 2 weeks produced a marked lowering of pulmonary symptoms associated with a drop in the use of other medications including β_2 -agonists and steroids compared to the placebo group (71). Bousquet et al. found cetirizine (twice daily at 10 or 15 mg) to be more effective than terfenadine (60 mg twice daily) in the control of seasonal asthma symptoms in 97 grass-pollen-allergic patients with recent onset mild asthma (72). A similar study also concluded that cetirizine could prevent exacerbation of seasonal asthma caused by grass pollen (73). In contrast, Rafferty et al. were unable to demonstrate a significant protective effect by either oral or inhaled cetirizine on allergen-induced early bronchoconstriction in a double-blind, randomized, placebo-controlled trial (74), while a similar study demonstrated only a nonsignificant tendency for cetirizine to protect against the allergen-induced immediate asthmatic response (75). Azelastine treatment (4 mg a day for 3 months) of 13 asthmatic patients resulted in significant improvements in asthma symptoms accompanied by a reduction of activated eosinophils and T cells in the bronchial mucosa compared with the placebo-treated group, and significant correlations were found between clinical data and immunohistochemical parameters (76). Moreover, azelastine, 6 mg twice a day, reduced the need for inhaled corticosteroids in patients with chronic bronchial asthma and did not lead to a deterioration in pulmonary function (77). Additionally, a single 4-mg dose of azelastine in patients with chronic asthma resulted in rapid onset of bronchodilating activity with

a 12-h duration of action, as demonstrated by a clinically and statistically significant mean per cent improvement in FEV₁ (78). Grant et al. recently demonstrated that cetirizine significantly improved asthma symptoms in patients with seasonal allergic rhinitis and concomitant asthma, although it had no statistically significant effect on lung function as measured by peak flow or FEV₁ (79).

These findings are similar to those from a number of other studies (80–82). Two randomized crossover studies of terfenadine and placebo reported improvements in symptoms and lung function in treated patients with atopic asthma (83, 84). In an uncontrolled study which looked at the effect of loratadine on 25 patients with allergic asthma, Kroll et al. (85) found that a dose of 10 mg daily for 6 weeks was associated with improved pulmonary function, reduced asthma symptoms, and reduced bronchodilator use. However, loratadine administered to 17 perennial asthmatics failed to have an effect on symptoms or peak flow (86) and, when given as an adjunct to standard asthma therapy, had little or no effect in a double-blind, crossover study of 35 patients with moderate-to-severe asthma (87). A more recent study using 193 patients compared a combined medication consisting of 5 mg loratadine and 120 mg pseudoephedrine sulphate (Claritin-D) with placebo. Claritin-D was found to improve patients' pulmonary function and both rhinitis and asthma symptoms significantly (88). Thus, antihistamines have some potential as safe and useful adjunct therapies in asthma patients who suffer from rhinitis. Cetirizine appears to be the leader in this regard, especially in the light of a recent study demonstrating its usefulness in preventing the development of asthma in at-risk atopic children (89).

Conclusion

An obvious question that arises from these observations regarding the additional anti-inflammatory effects of the newer drugs is whether such properties are shared by all members of the second-generation antihistamines; that is, whether this is a class effect or is confined to certain members of the second-generation drugs. Only carefully conducted, large-scale, comparative studies can answer this question with confidence. An additional question is whether such effects are therapeutically relevant and, if so, whether they may enable us to distinguish further between these compounds. One final issue that remains to be clarified is the disclosure of the mechanisms by which these drugs exert their anti-inflammatory effects, information which might facilitate the development of more effective anti-inflammatory therapy. However, there is a paucity of data which would allow a definitive answer with regard to the underlying cellular mechanisms responsible for

these *in vitro* anti-inflammatory effects by several second-generation drugs.

Discussion

Timmerman: Thank you very much for your comprehensive review. Any questions?

Levander: This is a general point, actually. In your second to last slide, you showed 5% significance. How does that compare with clinical significance? Was that a large study? I know Cohen defined in 1977 a small clinical effect as 0.2 standard deviation, a moderate clinical effect as 0.5 standard deviation group difference, and a large effect as 0.8 or greater. How large is the effect in terms of standard deviation?

Walsh: In my opinion, it was not particularly large. The trouble with all these studies is that they tend to be done with relatively small numbers.

Levander: They are small numbers? So that means that there is a good match between clinical and statistical significance?

Walsh: The challenge studies tend to be done with smaller numbers, and the Grant et al. study, for example, used about 100 patients in each group.

Levander: Still, 100 patients in each group means that you get a statistically significant effect for quite a small clinical effect. I would say that 30 or 40 subjects in each group gives a good match between clinical and statistical significance.

Walsh: I take your point and I think that what is needed is more studies of the Grant type, and these ideally would be multicentre studies with large numbers of patients comparing several different antihistamines, to accurately define how large the clinical benefit really is. The trouble is that, until recently, antihistamines were contraindicated for patients with asthma, particularly in the USA, and it's only recently that people have become aware of the potential of the second-generation drugs as adjunct therapies. That is why, to my knowledge, there have only been two or three studies of this nature.

Timmerman: You presented a large number of lab-based studies and also evidence that there are clinical effects unrelated to H₁ blockade. You told us about studies which looked at the release of mediators or effects on functions such as the adhesion and chemotaxis of proinflammatory cells. I'm very interested in a more molecular question: what is the mechanism of action given these diverse abilities?

Walsh: The last round-table held in Vienna in 1996 tried to answer that question. I'm afraid that your question is a difficult one, and there is no easy answer. I take your point that cetirizine in particular seems to have all these anti-inflammatory effects on a wide range of anti-inflammatory cells, so is that a specific effect? It doesn't seem to be a class effect, in that the anti-

inflammatory effects appear to be independent of cetirizine's potent H₁-blocking ability. Other antihistamines seem to have additional effects, but to a lesser degree compared with cetirizine. I know Jean-Pierre [Rihoux] has been doing some studies looking at the effects of cetirizine on the lipid profiles of the cell membrane, for example, to try to dissect what is different about this molecule and why it has these effects. The thing that I am very struck by is that in some of the *in vitro* studies very low concentrations of cetirizine have potent inhibitory effects on several parameters of cell activation or function.

De Vos: That's right; 10⁻⁹ and even 10⁻¹⁰ molar sometimes.

Walsh: The point here is that there are an awful lot of studies which demonstrate at least some anti-inflammatory effect by any second-generation drug you care to mention. However, the majority of these needed antihistamine concentrations of 10⁻⁶, 10⁻⁵, and even 10⁻⁴ molar before a significant inhibitory effect was observed. This raises two points: firstly, were toxic effects on the cells under test or taken into account, the answer is almost never, so inhibition was seen because the cells were poisoned; and, secondly, I fail to see the point of testing drugs for anti-inflammatory effects at concentrations which are impossible to achieve *in vivo*.

Timmerman: Have you looked at D and L isomers of cetirizine, because you might be able to distinguish between anti-inflammatory and H₁ blockade using this approach?

Rihoux: We are starting some experiments now with the D and L cetirizine, but we will have to wait for the new data. In one recent study on endothelial cells, cetirizine had a modulating effect on NFκB activity. Of course, that's not an explanation of everything; also, these data are not easy to understand since there is a very poor penetration by cetirizine into the cells.

Timmerman: That was my next question because a lot of these things are likely caused by intracellular effects, but we have heard yesterday that cetirizine has very poor cell penetration.

Rihoux: It's probably possible to reach some intracellular targets without massive penetration into the cells. Or perhaps there is a second messenger inducing intracellular effects.

Walsh: We did see some data in Vienna 18 months ago that showed an effect on G-proteins in a variety of cell types including epithelial cells, PMN, platelets, and mononuclear cells. I'm not sure if cetirizine had a direct effect on the G-proteins in these cells (90).

Rihoux: Yes, it is difficult to say if there was a direct effect. All we can say is there was modulation but nobody knows if it is direct or indirect. We have some other experiments running on that at the moment, so I am also waiting for the new data. I would also like to

add that we have new data about the adhesion molecule VCAM-1 in patients with atopic dermatitis who had a patch test on their skin with DPT. We measured the expression of VCAM-1 after 6 h, 24 h, 48 h using the biopsy technique and found that there was a significant decrease of VCAM-1 expression following cetirizine treatment. So cetirizine's effect is not confined to ICAM-1.

Tillement: It is clear from the situation with β -receptors, for instance, that an antagonist penetrates more deeply into the brain than an agonist. The second point is that when we discuss distribution, in normal situations the distribution may be restricted, as I showed you yesterday. But when you have an inflammatory state you have an increase of permeability. It may be a real advantage for a drug to enter cells where there is an inflammatory process and which would not enter when there is no inflammatory process. The distribution is defined by that particular state.

Walsh: That's a good point because if you look at the *ex vivo* studies with cetirizine, there is this phenomenon that in the cells from allergic patients an inhibitory effect was observed, but in those cells from normal

subjects there was no effect. For example, cetirizine only inhibited LTB₄ production by neutrophils from allergic patients, but not in cells from normal donors. Presumably, this is because the neutrophils are primed in the allergic subjects as a result of the mediators that are present as part of the inflammatory response. So perhaps cetirizine has an anti-inflammatory effect because it gets into stimulated cells but is unable to get into resting cells. This might also explain why we see so many antiallergic effects with cetirizine despite the fact that it exhibits poor cellular penetration.

De Vos: The same situation is seen with inhibition of eosinophil superoxide production.

Walsh: Absolutely. Cetirizine only inhibited eosinophil superoxide production if the cells were from allergic patients. A similar effect was observed in an *in vitro* study which looked at human eosinophil adhesion to endothelial cells. In these studies, we saw no effect by cetirizine on resting adhesion, but when we enhanced the adhesive ability of the eosinophils with platelet-activating factor, cetirizine gave very significant inhibition at very low concentrations (34). I think that this is a very important point for understanding cetirizine's anti-inflammatory mode of action.

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