

Consensus group on new-generation antihistamines (CONGA): present status and recommendations

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Preface

The rising trend in allergic disease is a cause of great concern and is as yet unexplained. Pathomechanistic studies have

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indicated that release of histamine and its many effects on target cells contributes to the acute and chronic symptomatology characteristic of allergy disorders. Blockade of this receptor provides a highly successful approach to controlling allergic symptoms.

First-generation antihistamines were defined by their H1 receptor blocking power and despite pronounced unwanted side-effects they are still widely used, mainly in over-the-counter products, either alone or in combination with other drugs.

The second-generation antihistamines are said to be 'non-sedating H1 blockers' due to the lack of penetration through the blood–brain barrier. Compared to the first-generation products, they are known for their minimal central nervous system (CNS) effects.

Over the past 20 years, researchers have created new antihistamines with differing pharmacokinetics. Some of them undergo extensive metabolism, while others do not. Cardiotoxic effects – not related to H1 blockade emerged with some of the second-generation antihistamines and most of the new compounds exhibit properties on systems other than H1 receptors, i.e. anti-inflammatory properties. It is claimed that the second-generation compounds manifest a great diversity.

Most recently, the term 'third-generation antihistamines' has emerged spontaneously without a clear description of what this term means. Varying claims are being linked to this new group of drugs, which vary greatly and create confusion for both the health professionals and lay public. This is of considerable concern for the scientific community involved in allergy, mainly due to a lack of consensus.

To pave the way for the elaboration of a consensual definition, a *Consensus Group on New Generation Antihistamines (CONGA)* has been set up with the support of the *British Society for Allergy and Clinical Immunology (BSACI)* to address this important issue. The findings and recommendations of CONGA are reported in this issue of *Clinical and Experimental Allergy*.

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Executive summary and recommendations

H1 antihistamines are highly effective in controlling several allergic disorders. Following the introduction of the first antihistamines, problems with systemic side-effects, especially sedation and dry mouth, stimulated the pharmaceutical industry to develop a second generation of drugs that were ostensibly free of these properties and more efficacious. However, other side-effects became apparent, including cardiotoxicity and weight gain. Further refinement usually in the form of an active metabolite has led to the use of the term third-generation antihistamine to describe these, but this term has no universal definition and often results in confusion among patients and practitioners about the significance of this term and its practical implications. Because of this, an internal expert panel of scientists and clinicians outside the pharmaceutical industry convened to form a Consensus Group on New Generation Antihistamines (CONGA). CONGA identified a number of critical questions that it set about answering by consulting with further experts. From this, recommendations were drawn up that have received the endorsement of CONGA and the British Society of Allergy and Clinical Immunology. Recommendations were made in six areas as shown:

Anti-inflammatory properties:

Presently, it is not possible to establish what the real clinical relevance is, if any, of the anti-allergic/anti-inflammatory properties described in numerous experimental models. The anti-allergic properties should be demonstrable *in vivo*, in humans, at therapeutic doses and under natural exposure to the offending allergens. An antihistamine with anti-allergic properties should be demonstrated to be superior (in humans) to a comparator devoid of such properties. Since the major expression of chronic inflammation in the nose is nasal obstruction, anti-allergic/inflammatory properties should affect that symptom in a measurable way, especially in persistent rhinitis, where obstruction predominates over histamine-induced symptoms such as itching, sneezing and rhinorrhoea.

Potency, efficacy and effectiveness

The therapeutic index of an antihistamine, defined as its benefit-to-risk ratio, is a more important concept than either potency (as determined in preclinical studies) or efficacy (as determined in clinical trials). The second generation, relatively non-sedating H1 antihistamines, have a more favourable therapeutic index than the first-generation sedating H1 antihistamines; however, none of the second-generation medication justifies the designation 'third-generation' H1 antihistamine. A true third-generation H1 antihistamine will differ radically from existing compounds.

Lack of cardiotoxicity

Life-threatening adverse cardiac side-effects such as QT prolongation and torsades de pointes ventricular tachyarrhythmias have been described in association with the use of some second-generation antihistamines (astemizole and terfenadine). These effects are consequent to direct blockade of a

specific class of potassium channels controlling the repolarization phase of the cardiac action potential, and are not related to the blockade of the H1 receptor. Thus, cardiotoxicity by second-generation antihistamines is not a class effect. Preclinical and clinical studies of the predictive ability of such cardiotoxic effects must be performed before novel molecules enter the market. Several pharmacokinetic and pharmacodynamic factors can precipitate the occurrence of the arrhythmic episode; medical practitioners prescribing second-generation antihistamines must be aware of these factors in order to avoid exposing the patient to potential dangerous effects. The lack of cardiac toxicity, a feature already present in some second-generation antihistamines, must be retained in the development of novel congeners that enter the market in the future.

Drug interactions

A third-generation antihistamine ideally should not affect any cytochrome P450 (CYP) isoenzyme function, should not displace protein-bound medications and should not affect active transport mechanisms (i.e. P-glycoprotein) important in drug absorption and excretion to a clinically relevant degree.

Lack of CNS effects

Three factors, in particular (i) incidence of subjective sleepiness, (ii) objective cognitive and psychomotor functions, and (iii) positron emission tomography (PET) measurement of H1 receptor occupancy, specifically define criteria that allow an antihistamine to be classified as a 'non-sedative' drug. Although the latter two are particularly important in determining the non-sedative properties of drugs, all three criteria should be met as a 'minimum' acceptable, prior to classifying a new antihistamine as a 'non-sedative' drug.

Pharmacological approach

Although any new antihistamine, with clearly demonstrated clinical advantage/s over the existing drugs, has not been introduced over the last few years, the development of new class/es of antihistamines is nevertheless feasible. These drugs may either be identified by screening for common features, or specifically be designed on the basis of theoretical considerations. Thus, a new class of antihistamine may exhibit a combination of H1-receptor blockade and additional effect/s, but lack any side-effects, or be 'clean' blockers of H1 effects, offering a special feature (e.g. being a neutral antagonist). A new antihistamine based on improved theoretical properties should, however, be proven to have distinct clinical advantage/s over existing drugs.

Should the above-mentioned criteria be met, then the term third-generation antihistamine would be appropriate in representing a major breakthrough in the management of allergic disease.

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1 Introduction: the concept of third-generation antihistamines

The term 'third generation' or 'multifunctional' antihistamines was employed by Hanifin [1] in 1990 to refer to H1 antagonists, such as ketotifen, which exhibited additional activities such as inhibition of mediator release and interference with eosinophil migration. In 1992, Sabbah [2] reported that terfenadine was the first of the third-generation H1 antagonists, while Caproni et al. [3] described cetirizine as 'a highly selective, third-generation H1-receptor antagonist' in 1995. It has also been suggested that compounds, such as cetirizine, which exist as zwitterions and have pharmacological, pharmacokinetic and pharmacodynamic properties, which differ from those of 'first-' and 'second-generation' drugs in this class, could be considered as 'third-generation' antihistamines [4]. Although the term 'third-generation antihistamine' has frequently appeared in manuscripts, reviews, presentations and symposia, there is little or no consensus on the meaning of this term and which, if any, of the drugs currently available or in development would be classified as fulfilling the criteria for this appellation.

Second-generation antihistamines were differentiated from the first-generation antihistamines by their increased specificity with little or no affinity for muscarinic cholinergic receptors, and by their much lower penetration of the CNS and subsequently reduced sedative effects. While the second-generation antihistamines are generally better tolerated than their predecessors, there are still concerns particularly regarding the safety aspects of some of these compounds, which may lead to ventricular arrhythmias and can be life-threatening. Other unwanted side-effects include the somnolence caused by at least some of these drugs, particularly at high doses. On the positive side, however, many of the compounds have been reported to possess additional anti-inflammatory activities, and these may be of importance in differentiating between the efficacies of the drugs.

Recent advances following the cloning of the gene encoding for the H1 receptor have increased our understanding of ligand receptor interactions at the molecular level, thereby increasing the potential for the development of new antihistamines with greater potency, safety and selectivity than those currently available. It has been demonstrated that H1 antagonists may bind to the receptor differently at the third and fifth transmembrane domains depending on the specific amino acid residues [5–7] and whether a compound exists in a zwitterionic form [8]. In addition, differences in receptor expression or in the microenvironment surrounding the receptor may cause different signaling pathways to be activated following exposure to histamine [9]. Evidence has also emerged that all currently available antihistamines are inverse agonists, rather than (neutral) antagonists [10].

Similarly, it is likely that a better understanding of other aspects of antihistamine pharmacology, in particular the factors that influence the ability of these compounds to cross the blood–brain barrier may help in the design and selection of better new drugs. It is clear that, in order to minimize the CNS effects of a drug such as an antihistamine, there should be negligible brain penetration, primarily due to the absence of an active transport mechanism and secondly due to the specific pharmacological properties of the compound in

question. It is well known that compounds with high molecular weight do not readily pass into the brain because, unlike peripheral capillaries, the capillaries in the brain have tight junctions and no fenestrae. The lipophilicity or solubility of the compound in fatty solvents, compared to water, also determines the ability of a drug to penetrate the brain. For neutral compounds the lipophilicity/solubility is indicated by a log *P*-value, which is the logarithm of the distribution coefficient of a compound between an organic solvent and water. While uptake into the brain has been found to be maximal for compounds with a log *P*-value of around 2, compounds with either very low or very high log *P*-values have been shown to have relatively low brain penetration.

For non-neutral compounds, lipophilicity-solubility is indicated by a log *D* value (e.g. for the pair octanol–water), which corrects for the percentage of ionization and takes into account the pK_a of a compound at a given pH (e.g. pH 7.4). Thus, at pH 7.4 a compound with a log *D* < 0 has negligible brain penetration, while a compound with a log *D* > 3 has hindered brain penetration. A further calculation of Δ log *P*-value, which measures the difference in the solubility of a given compound between two solvents that are soluble in each other (e.g. the difference between log *P* in octanol–water compared to that in cyclohexane–water), is additionally used to assess the brain penetrating properties of compounds that cannot be explained solely on the basis of log *D* values between 0 and 3. A high Δ log *P* suggests that the compound may have high hydrogen-binding capacity and may thus bind to serum proteins and membrane-bound polarized phospholipid moieties, resulting in decreased brain penetration. A compound with a log *D* between 0 and 3 and a Δ log *P* < 2 has good brain penetration, while that with a Δ log *P* > 4 has hindered brain penetration [11]. Δ log *P*-values between these extremes are indicative of intermediate brain penetration capacity.

Additionally, the CNS concentrations of a drug may also be determined by the ability of the drug to cross the blood–brain barrier and interfere with transporters such as the P-glycoprotein transporter [8]. The volume of distribution, which varies greatly within this class of drugs (from 0.4 to 120), may also be important in determining the CNS concentrations of such drugs.

Although many of the studies on CNS effects of antihistamines have been performed in healthy volunteers, it may not be entirely appropriate to extrapolate their findings to patients with allergic disease because of the possibility that mediators released during an allergic reaction may affect the passage of compounds across the blood–brain barrier [12]. Consequently, there is an urgent need for similar studies in patients during an allergic episode. The methods used to assess CNS effects are also of great importance. While these have traditionally involved subjective tests, in which the patients self-rate symptoms of somnolence or drowsiness following ingestion of an antihistamine, more objective tests evaluating sensorimotor co-ordination speed, reaction time, information processing (mental arithmetic/verbal fluency) and a number of other parameters, all of which may be influenced by a subject's level of motivation, test familiarity or boredom have been increasingly used. Many of these objective tests can be performed under placebo- and verum-controlled conditions and cover a range of doses of

antihistamines [13]. The verum (positive control) should be a first-generation antihistamine known to have a sedative effect, given at the lowest dose, which causes a change in test scores [13]. More recently, PET has been shown to be a particularly useful tool to investigate the penetration of antihistamines into the brain and has shown a correlation between receptor occupancy and impaired cognitive performance [14].

The possibility that antihistamines may react with other drugs is also a cause for concern since antihistamines are often prescribed over relatively long periods. Unlike cetirizine and levocetirizine, which do not undergo metabolism in the liver and are excreted virtually unchanged in the urine, many of the second-generation antihistamines undergo extensive first-pass metabolism in the liver by the CYP enzymes. Drugs such as ketoconazole, erythromycin and azithromycin, which affect the activity of CYP, can lead to build up of the parent antihistamine compound, thus increasing the risk of side-effects [15]. It has also been demonstrated that grapefruit juice can inhibit isoenzyme CYP3A, probably via 6'7'-dihydroxybergamottin [16]. Thus there is a possibility that grapefruit juice even at normal strength or freshly squeezed may lead to increased concentrations of antihistamines [17, 18], such as terfenadine, loratadine and astemizole, which are metabolized by CYP3A4 to fexofenadine, desloratadine and norastemizole, respectively [19]. These metabolites are now being marketed as 'new antihistamines' and have sometimes been referred to as 'third-generation drugs'. Interestingly, while loratadine has no effect on the muscarinic receptors, desloratadine is a potent M1 and M3 antagonist [20]. However, as well as acting as a substrate, some antihistamines such as terfenadine, astemizole and a number of first-generation H1 antagonists can also modulate the activity of CYP enzymes [21–23], and therefore have the potential to lead to increased plasma levels of other concomitantly administered drugs, which are metabolized via these enzymes.

Cardiac arrhythmia, which may develop into the potentially fatal torsades de pointes, is a serious side-effect associated with antihistamines. It should, however, be borne in mind that the number of these severe reactions is very low compared to the number of patients treated with antihistamines; they are mainly restricted to astemizole and terfenadine and in most of these cases patients have either taken more than the recommended dose, taken CYP inhibitors concomitantly or have predisposing factors such as liver disease, pre-existing QT prolongation, heart disease or hypokalaemia [24]. Mechanistic studies have indicated that these effects are a consequence of interference of these drugs with cardiac K^+ channels involved in action potential repolarization. Terfenadine and astemizole increase the QT interval and block the delayed rectifier current IK_r generated by the K^+ channels encoded by the human ether-a-gogo-related gene (HERG), resulting in prolongation of the action potential and slowing of the repolarization [25, 26]. In contrast, cetirizine has no effect on the cardiac K^+ channels and so far there are no reports of any effects of fexofenadine or mizolastine on these channels. In the case of loratadine, several studies have reported an absence of cardiac effects at therapeutic doses [27], but recent publications suggest an effect of loratadine on HERG current amplitude at high concentrations [28]. Furthermore, loratadine and its metabo-

lite desloratadine have been shown to cause a concentration-, voltage- and time-dependent blockage of K^+ channels encoded by hKv1.5 gene [29]. Additionally, it has been shown that the pacing rate can also alter the effect of loratadine on the transient outward K^+ current, I_{to} , suggesting that extra care should be taken when administering antihistamines to patients with an existing tachyarrhythmia or in cases of stress or exercise when the heart rate may be increased [30].

Most antihistamines have been shown to have additional anti-inflammatory properties. However, Church [31] has suggested that many of the studies *in vitro* may be irrelevant since the concentrations required to produce an effect are much higher than those that would be expected *in vivo*. Although this may suggest that antihistamines are not sequestered preferentially in inflammatory cells *in vivo*, presently there is no evidence to indicate that this is indeed the case. Some studies have nevertheless demonstrated anti-inflammatory effects of some antihistamines at therapeutic concentrations, both *in vitro* and *in vivo*. For example, cetirizine has been shown to inhibit eosinophil chemotaxis at 100–1000 ng/mL *in vitro* and to inhibit pollen challenge-induced eosinophil accumulation in atopic skin at therapeutic dosage *in vivo* [32–35]. Similarly, both loratadine [36] and cetirizine [37] inhibit intercellular cell adhesion molecule (ICAM) expression after antigen conjunctival challenge. Recently, the Early Treatment of the Allergic Child (ETAC) study demonstrated that cetirizine can reduce the number of grass pollen- or house dust mite- sensitized young children with atopic dermatitis who go on to develop asthma [38], lending further support to the hypothesis that anti-inflammatory effects may indeed be an important mode of action of H1 antagonists. The usefulness of H1 antagonists in treating adult asthma, however, is less clear. Although a review of clinical studies has shown that antihistamines alone caused little improvement in airway calibre and resulted in increased undesirable side-effects [39], improvement of asthma symptoms as well as rhinitis symptoms have nevertheless been reported in patients with allergic rhinitis and mild seasonal asthma treated with antihistamines [40, 41]. It has been suggested that assessment of improvement in quality of life may also be useful in evaluating the efficacy of these drugs in lower and upper airways disease.

It appears that, although second-generation antihistamines represent a great advance over the first-generation antihistamines, there is still the possibility for improvement, especially in view of recent advances in molecular biology and related areas. In particular, effort should be directed at designing and selecting new compounds, which have increased specificity for the H1 receptor and are devoid of adverse cardiac, CNS and drug interaction effects, properties that cannot all be claimed for any of the currently available antihistamines. Adverse effects that could result from pre-existing medical conditions, the concomitant use of other medicines, diet and exercise should also be borne in mind when developing the new antihistamines.

Although some authors have defined the third generation of antihistamines on the basis of these being metabolites of some second-generation drugs (i.e. fexofenadine, norastemizole and desloratadine) [42–44], this definition is difficult to justify because these drugs do not have any significant advantages

over the parent compounds nor over other second-generation antihistamines. Indeed, the recommendations of the advisory panel, held after the 'Antihistamines: Back to the future' workshop, chaired by Professor S. T. Holgate and endorsed by the British Society for Allergy and Clinical Immunology, concluded that until new drugs are available that are completely free of sedative effects and adverse effects on the heart (even at high doses) as well as maintaining strong and selective H1 receptor binding, the term 'third-generation antihistamine' should not be used [45]. In a similar vein, a round table meeting held in Amsterdam in May 1998 concluded that '...it is too early to talk about a third-generation grouping of antihistamines; however, future membership of such a classification could be based on a low volume of distribution, coupled with a lack of sedation effects, drug interactions and cardiotoxicity'. More recently, Timmerman suggested that a third-generation H1 antagonist could be defined as one that 'shows appropriate H1 antagonism, does not reach the CNS, has no cardiac side effects and shows anti-inflammatory properties at relevant doses and dosing schemes' but that 'so far no such compound has been found or designed' [46].

These differing definitions for antihistamines are beginning to cause confusion and concern within the medical and scientific communities, mainly due to lack of consensus in use of the term 'third-generation antihistamine', and therefore it was felt appropriate to set up a Consensus Group in order to reappraise what is meant by this term, and to set the criteria for such drugs.

2. Methodology

The Consensus Group, chaired by Professor Stephen T. Holgate and consisting of six other expert members each

of whom heads a panel, proposes a rational approach to this problem. The purpose of the Consensus Group was to define the requirements to be fulfilled by a new generation of antihistamines, including proposals to help orientate basic and clinical research in specific fields. The Consensus Group made clear the necessity to clarify the theme and agreed on the following aims:

- (a) to review in depth the present status of the term 'third-generation antihistamines',
- (b) to analyse the potential criteria that drugs should fulfil to be labelled as third generation,
- (c) to find gaps and to bridge them with the back-up of expert networking, and
- (d) to issue a consensual statement endorsed by the scientific community.

A three-meeting structure over one year and a half was adopted and a consensus was agreed upon. To achieve this goal, the Consensus Group created a network with 17 internationally renowned experts from different fields concerning allergy (allergologists, chest specialists, paediatricians, dermatologists, immunologists, pharmacologists). Groups of these experts worked with different heads of panels to report on essential criteria including, (i) anti-inflammatory properties, (ii) potency, efficacy and effectiveness, (iii) lack of cardiotoxicity, (iv) drug-drug interactions, (v) lack of CNS effects and (iv) pharmacological approach. Each of these criteria was assessed on the basis of several key questions addressed by the panel members.

The Consensus Group is composed of Professor S. T. Holgate (UK; Chairman), Professor G. W. Canonica (Italy), Professor F. Estelle R. Simons (Canada), Professor M. Taglialatela (Italy), Professor M. Tharp (USA), Professor H. Timmerman (the Netherlands) and Professor K. Yanai (Japan). The other experts are as indicated for each panel.

3. Panel reports

3.1. Panel 1: anti-inflammatory properties

Chairperson: G. W. Canonica

Contributors: M. K. Church, E. Gelfand, D. Y. Wang

Introduction

Histamine, a primary amine synthesized and released predominantly by mast cells and basophils, plays a key role in the pathogenesis of allergic airway disease, as indicated by its contribution to bronchial obstruction in asthma, and to nasal blockage, sneezing and rhinorrhoea in rhinitis. At the cellular level, histamine causes smooth muscle contraction, increased vascular permeability and mucus secretion and parasympathetic nerve stimulation. Additionally, histamine may also act on inflammatory cells, leading to the release of pro-inflammatory mediators, such as leukotrienes and cytokines, and increased expression of class II antigens

(HLA-DR) and adhesion molecules. More recently, several studies have demonstrated that some of the non-sedating second-generation H1 receptor antagonists possess anti-inflammatory properties, suggesting that these may be potentially useful in the management of airway inflammation in allergic airway disease [47].

Questions addressed by the panel

1. What specific pathways should be inhibited to claim a relevant anti-inflammatory effect? Allergic inflammation is a highly complex network of cellular events with a high

degree of redundancy of mediators and signals [48]. Clinical symptoms are the result of the release of inflammatory mediators, which is in turn regulated by cytokines. Studies in humans have clearly shown that the blockage of single cytokines such as IL-4 [49] and IL-5 [50], and blockage of specific leukotriene activity with leukotriene receptor antagonists (LTRAs) has little or only partial effect on the clinical expression of the disease [51, 52]. The same is also true for circulating IgE, the triggering molecules for allergic reactions, because abating the serum levels of these molecules to over 90% results in a significant but far from complete control of clinical symptoms [53, 54]. These observations are explicable by the pleiotropic and overlapping action of cytokines and mediators themselves. On the other hand, histamine exerts numerous actions (on nervous, endocrine and immune systems) through interaction with histamine receptors. *In vitro* studies have shown that histamine released during the early-phase may act as a stimulatory signal for the production of cytokines (i.e. IL-6, IL-5, IL-8, IL-11 and GM-CSF (granulocyte macrophage-colony stimulating factor)) and the expression of endothelial adhesion molecules [55–57]. These effects are dose-dependant and can be affected by H1 and H2 receptor antagonists [55–58].

Thus, it is likely that the most quantitatively relevant effect on clinical symptoms is due to a concerted effect of several inflammatory mediators, the release of which represents the final stage of the inflammatory cascade. The inhibition of one or more cytokines may have an additional effect, but is unlikely to overcome the redundancy of the humoral signals. Consequently, a significant anti-allergic action should interfere with the actions of histamine and possibly other mediators at the therapeutic doses, a point of view that is sustained by the fact that only corticosteroids, which inhibit many steps of the allergic inflammatory cascade at the same time, have a significant effect on almost all symptoms of allergic rhinitis, and that a combination of antihistamines and antileukotrienes has a more than additive effect [59, 60].

2. In general most of the second-generation antihistamines have demonstrated multiple impacts on the inflammatory cascade at high doses. What should be the adequate methodology and standards to demonstrate anti-inflammatory properties? The multiple impacts of the second generation on the inflammatory cascade have been widely documented and generally been demonstrated at high doses [31]. These actions are independent of the 'inverse agonism' to the H1 receptor [61], of which the most frequently described additional properties are [62–64]:

- inhibition of leucocyte adhesion
- inhibition of mediators release (mainly LTs, TXs, PGs)
- inhibition of cytokine release (mainly IL-4, IL-6, IL-8, IL-13)
- down-regulation of the expression of adhesion molecules (mainly ICAM-1 and vascular cell adhesion molecule (VCAM)-1).

These anti-allergic properties have, however, been studied in a wide variety of models (Table 1), and there are several relevant conceptual problems with those studies:

- (a) Virtually each of the anti-allergic action described has been studied in a different model. This is comprehensible,

since each study group uses its own experimental model, which is supposed to be reliable and reproducible. However, differences in experimental conditions and provoking stimuli may influence the observed result. Nevertheless, a standardized procedure for studying each of these effects is presently lacking.

- (b) In the great majority of the studies, the anti-allergic effects can be detected at concentrations of the drug that are much higher (10 to 1000 times) than those achievable *in vivo* in humans.
- (c) the majority of the studies on anti-allergic activities have been performed *in vitro* (*in vitro ex vivo*) or in animal models
- (d) the inhibition of histamine release from mast cells and basophils has been shown to occur *in vitro*, but is of no relevance *in vivo* [65]
- (e) The allergic inflammation is a very complex network with a high degree of redundancy in mediators and signals. Indeed, the clinical relevance of the anti-allergic (anti-inflammatory) actions of antihistamines has not been clearly established to date.

Any demonstration of anti-allergic/anti-inflammatory property *in vitro* in cellular models is of speculative interest and adds something to our knowledge of the multi-faced allergic inflammation. Nevertheless, antihistamines are used to treat a disease with symptoms and signs, and therefore to be of relevance it is essential that anti-inflammatory actions have a clinical counterpart and that they can be demonstrated *in vivo*, in humans, at therapeutic doses, and under natural exposure to the offending allergens. Table 1

3. What should be the requirements to claim for anti-inflammatory properties? The crucial step in evaluating the anti-allergic activity is to extrapolate the *in vitro* results to humans. Ideally, two requisites should be satisfied:

the anti-allergic properties, described in *in vitro* models, are also present in humans *in vivo*, at the usual dosages and under natural exposure to the allergen or, at least, in controlled challenge procedures.

an antihistamine with ascertained anti-allergic properties is superior (concerning the clinical efficacy in humans) to a comparator devoid of such properties. This would allow demonstration of incontrovertibly that anti-allergic properties are clinically relevant.

At least the first requisite seems to be fulfilled by some of the new molecules. At present, there are some studies showing that the anti-allergic properties seen *in vitro* are also present *in vivo* at usual doses [66, 67]. On the other hand, there is no study showing that the anti-allergic activities have a direct additional clinical effect.

4. In this field, what should be considered as a breakthrough for a potential third-generation antihistamine? - When the analysis of effects of antihistamines is restricted to the clinical efficacy (independent of other aspects such as safety, pharmacokinetics and tolerability), it is clear that almost all antihistamines (first and second generation) are effective in counteracting the effects of histamine on tissues. In fact, they can effectively reduce the histamine-dependant

Table 1. Summary of the various experimental models used to study the anti-allergic effect of antihistamines; each effect can be studied *in vitro*, *ex vivo* or *in vivo*, in animals or humans, using a number of cell types and stimuli

Parameter		A/H	Source/model	Stimuli
Histamine and mediators release	<i>In vitro</i>	Animal	Rat mast cells	Anti-IgE
		Human	Lung, tonsils, skin and PBMC and basophils	Allergen
	<i>In vivo</i>	Human	Peripheral blood	Calcium ionophores
			Nasal lavage	P substance
			Skin microdialysis	40/80
Cell adhesion (neutrophils, eosinophils)	<i>In vitro</i>	Human	Skin chamber	
			HUVEC	
			Cell monolayers	
Migration/chemotaxis	<i>In vitro</i>	Human	Adhesion to glass plates	
			Monolayers, plates	PAF, fMLP IL-1, con A
CAMs expression	<i>In vivo</i>	Human	Skin chamber	
	<i>In vitro</i>	Human	Cultured epithelia	IFN- γ , IL-1
	<i>In vivo</i>	Human	Nasal scraping	Allergen challenge
			Conjunctival scraping	Natural exposure
Cytokine secretion	<i>In vitro</i>	Animal		PMA, IFN- γ , TNF- α
		Human	Mast cells, eosinophils	
	<i>In vivo</i>	Human	Nasal lavage	

CAM, cellular adhesion molecule; con A, concanavalin A; HUVEC, human umbilical vein endothelial cells; fMLP, fMet-leu-Phe; PAF, platelet-activating factor; PBMC, peripheral blood mononuclear cells; PMA, phorbol ester.

symptoms (mainly sneezing, itching and rhinorrhoea), as can easily be seen in the nasal challenge model, where a single allergenic stimulus provokes immediate histamine release and a rapidly subsiding late phase. When the stimulus is persistent, there is time enough for the inflammation to become chronic for cellular infiltration and therefore nasal obstruction to occur. At this stage, the effect of histamine becomes less relevant, as indicated by very poor efficacy of most of the antihistamines, compared with the efficacy of corticosteroids (and vasoconstrictors). Perennial (persistent) rhinitis is the naturally occurring experimental model.

Although histamine is able to induce nasal obstruction through its effect on increasing vasopermeability and oedema in the nasal mucosa, the pathogenic mechanism of nasal obstruction is much more complex than the experimental models, especially in patients with ongoing allergic inflammation during the natural allergen exposure. While one study has demonstrated that a single dose of levocetirizine (5 mg) and cetirizine (10 mg) significantly attenuated the histamine-induced increase in nasal airway resistance by nearly 50% at the maximal concentration of histamine investigated [68], another study of patients with ongoing seasonal allergic rhinitis demonstrated that nasal obstruction induced by nasal challenge with pollen mixtures was not attenuated by treatment with cetirizine (10 mg for 2 weeks) [69]. However, this latter study demonstrated that a significant reduction of nasal airway resistance was demonstrated only after a combined treatment with cetirizine (10 mg) and cimetidine (800 mg).

Since the anti-inflammatory action of antihistamines might contribute to relief of nasal obstruction, this effect should be evident in the case when nasal obstruction is sustained by chronic inflammation, and not only in the rapidly resolving

obstruction given by a single challenge or a short allergen exposure. In addition, the anti-inflammatory effects should be demonstrable by preventing the influx and activity of inflammatory cells (i.e. eosinophils, mast cells and lymphocytes) in the target organs during natural allergen exposure. This aspect is obviously subsidiary to the presence of other mandatory requisites including:

- prompt onset of action
- duration of action/single administration
- safety
- absence of drug-to-drug and drug-to-food interactions.

Recommendations and take-home message

- Presently, it is not possible to establish what, if any, is the real clinical relevance of the anti-allergic/anti-inflammatory properties described in numerous experimental models. The anti-allergic properties should be demonstrable *in vivo*, in humans, at therapeutic doses and under natural exposure to the offending allergens.
- An antihistamine with anti-allergic properties should be demonstrated to be superior (in humans) to a comparator devoid of such properties.
- Since the major clinical expression of chronic inflammation in the nose is nasal obstruction, anti-allergic/anti-inflammatory properties should affect that symptom in a measurable way. This should be demonstrable especially in persistent rhinitis, where obstruction predominates over histamine-induced symptoms.

3.2. Panel 2: potency, efficacy and effectiveness

Chairperson: Professor F. Estelle R. Simons

Contributors: Dr Carlos E. Baena-Cagnani
Professor Robert M. Naclerio
Dr Glenis K. Scadding

Introduction

Millions of people worldwide depend on H1 antihistamines for relief of symptoms of allergic rhinoconjunctivitis, urticaria and other allergic disorders [70–72]. Many claims are made for potency and added properties of individual H1 antihistamines [73–79]. At worst, some of these claims are misleading. At best, some of them confuse physicians, pharmacists and consumers of H1 antihistamines.

Definitions

Potency, efficacy and effectiveness describe different aspects of H1-antihistamine activity, that are more, or less, clinically relevant. Potency is H1-antihistamine activity expressed as the drug concentration required to produce an effect at the H1 receptor, and if the H1 antihistamine is not a selective medication, at muscarinic or other receptors. Efficacy is H1 antihistamine activity expressed as relief of symptoms and signs of allergic rhinoconjunctivitis, urticaria or other allergic disorder. Effectiveness is H1 antihistamine activity in the real world, as expressed by patients' willingness to use a specific H1 antihistamine based on their perception not only of its efficacy, but also of its safety, convenience, taste, etc.

Questions addressed by the panel

1. The concept of potency (binding to H1 receptors) is frequently extrapolated to induce clinical efficacy. What is the current opinion on this fact and how to set up a clear definition of potency, efficacy and effectiveness? Statements about potency are usually based on preclinical *in vitro*, *ex vivo* and *in vivo* studies, as described in the initial pharmacologic publications on new H1 antihistamines and promulgated in early reviews of new H1 antihistamines and investigators' brochures [73–79]. The relative potencies of H1 antihistamines at the H1 receptor reported in these publications may later be extrapolated to mean relative differences in clinical efficacy – a leap of faith that is usually unjustified.

2. There is no clear definition of clinical criteria related to efficacy in different pathological fields. What kind of guidelines can be defined to judge the clinical efficacy of an antihistamine according to the fields involved in allergy? It is possible for a potent H1 antihistamine to be ineffective or minimally effective. This occurs if the medication has one or more unsatisfactory clinical pharmacology properties, determined by objective measurement of drug concentrations in plasma (absorption, distribution, metabo-

lism and elimination), which interfere with getting it to the target organ and keeping it at the site of action for 12–24 h [80].

Most of the clinical criteria used to define the efficacy of an H1 antihistamine are subjective [73–79]. In allergic rhinoconjunctivitis, these subjective criteria include assessment of nasal itching, rhinorrhoea, sneezing and congestion, and also of eye itching and watering, palate itching and other non-nasal symptoms. Objective measures are generally limited to peak inspiratory nasal flow monitored on a twice-daily basis, but may include other tests such as rhinometry performed at intervals [71].

In urticaria, subjective assessment of efficacy is performed using patient assessment of itching, and number, size and duration of weals. In some types of physical urticarias, e.g. dermographism, cold-induced urticaria, cholinergic urticaria, solar urticaria and delayed pressure urticaria, objective tests are conducted to confirm the diagnosis; however, these objective measurements have been underutilized in monitoring patients with physical urticaria who are enrolled in clinical trials [72].

In asthma, in the few H1 antihistamine studies that have been performed, subjective symptoms of wheeze, cough and shortness of breath have been recorded and have been correlated with peak expiratory flows monitored regularly and other objective pulmonary function tests performed at intervals, including measurement of airways hyper-reactivity [70].

3. How could 'quality of life' (subjective feeling of relief perceived by the patient in their daily life) be integrated as a clinical criterion for efficiency? Validated generic and allergic disease-specific health-related quality-of-life questionnaires (HRQL) are readily available for use. Generic HRQL questionnaires are broad-based surveys that can be used in a variety of conditions and populations, irrespective of disease state. They include the Sickness Impact Profile, the Quality-of-Well-Being Scale and the Medical Outcomes Study Short Form 36 (SF-36) and 12 (SF-12). Specific instruments focused on specific allergic diseases include the Rhinitis Quality-of-Life Questionnaire, which has been standardized for use in patients with allergic rhinoconjunctivitis, and the Dermatology Life Index, which has been standardized for use in patients with urticaria. These instruments provide practical information about these two disorders, in which persistent or intermittent symptoms are associated with high morbidity. Moreover, in clinical trials, HRQL measurements correlate with other subjective outcomes such as symptom scores [81].

4. *Is the therapeutic index an important parameter to characterize antihistamines?* The therapeutic index (benefit-to-risk ratio), although often overlooked, is actually more important than potency, efficacy or effectiveness for characterization of H1 antihistamines. 'Second-generation' H1 antihistamines have different therapeutic indices, largely because of CNS safety issues that arise when the manufacturer's recommended doses are exceeded [82], rather than cardiac effects [61]. In adults, fexofenadine is considered to have the best therapeutic index, as, in subjective and objective tests, it does not cause CNS effects even when administered in three or four times the manufacturer's recommended dose, in contrast to other second-generation H1 antihistamines, which have dose-related CNS effects when the manufacturer's recommended dose is exceeded. In the paediatric population, however, cetirizine, the only H1 antihistamine that has been optimally studied with regard to its benefit-to-risk ratio in infants and children, is considered to have the best therapeutic index.

Two new H1 antihistamines, desloratadine [74] and levocetirizine [77], are also likely to have favourable therapeutic indices, although it is too soon to be absolutely certain of this, as, to date, most of the 'safety' publications on these two medications are in the form of product monographs and abstracts.

Additional new H1 antihistamines tecastemizole and epinastine are in even earlier stages of development. Interestingly, the manufacturer of tecastemizole is already promulgating the use of the term 'third-generation' for this H1 antihistamine.

5. *What could be considered as a 'breakthrough' for potential third generation antihistamines?* To date, most new H1 antihistamines have been derived from screening of

existing compounds and synthesis of additional metabolites and enantiomers of these compounds. Based on published information about these H1 antihistamines [73–79], it appears as if none of them is a true 'third-', 'next-' or 'new-generation' H1 antihistamine.

Potential breakthroughs are possible, however, and a genetically engineered designer H1 antihistamine might constitute such a breakthrough. Our understanding of the H₁-receptor, a G-protein-coupled receptor, is increasing. The gene encoding H1 receptors has been cloned and expressed as human cells. It has recently been demonstrated that these receptors demonstrate agonist-independent signal transduction and that H1 antihistamines inhibit this constitutive signalling, by stabilizing an inactive conformation of the H1 receptor and acting as inverse agonists. Thus, there is a sound rationale for expecting a true breakthrough during the next few years, leading to the development of true 'third-', 'next-' or 'new-generation' H1 antihistamines.

Recommendations and take-home message

- The therapeutic index of an antihistamine, defined as its benefit-to-risk ratio, is a more important concept than either potency (as determined in preclinical studies) or efficacy (as determined in clinical trials).
- The second generation, relatively non-sedating H1 antihistamines have a more favourable therapeutic index than the first generation, sedating H1 antihistamines; however, none of the second-generation medication justifies the designation 'third-generation' H1 antihistamine.
- A true third-generation H1 antihistamine will differ radically from existing compounds.

3.3. Panel 3: lack of cardiotoxicity

Chairperson: M. Taglialatela

Contributors: J. Morganroth, P. Potter

Introduction

The association of serious adverse cardiac side-effects such as QT prolongation and torsades de pointes ventricular tachyarrhythmias with the second-generation antihistamines astemizole and terfenadine resulted in these molecules being withdrawn from the market internationally. With subsequent extensive investigations into the adverse effects of the other currently available second-generation antihistamines, it should now be possible to either prevent the release of antihistamines with potentially harmful cardiac effects, or to avoid the use of drugs belonging to different therapeutic categories having potential cardiac side-effects, similar to

those exerted by antihistamines [83]. Since the potential for cardiotoxicity is now regarded as one of the main selection criteria for therapeutic selection among antihistamines [61], all newly introduced antihistaminic molecules as well as those congeners belonging to this therapeutic class already available on the market should now be extensively subjected to *in vitro*, *in vivo*, clinical and post-marketing assessments.

Questions addressed by the panel

1. *How can the lack of cardiotoxicity of a new antihistamine be assessed?* Guidelines recently issued by the European Agency for the Evaluation of Medicinal Products (EMEA) [84] have clearly pointed out that

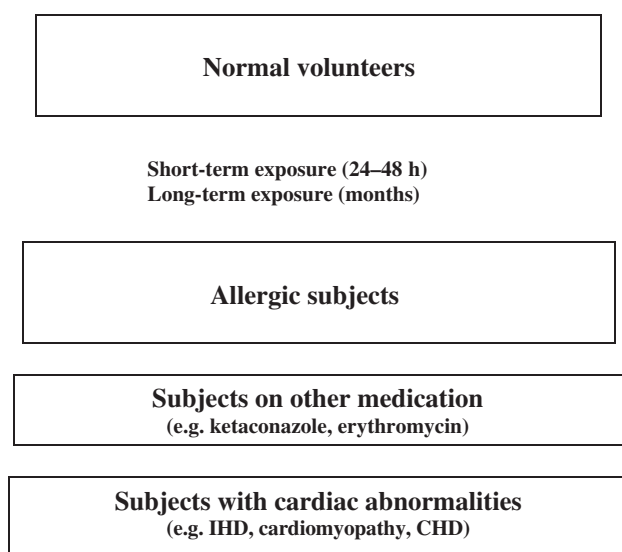


Fig. 1. Suggested clinical monitoring sequence for assessing cardiovascular safety.

evaluation of the safety of a novel antihistaminic compound, with regard to the lack of cardiotoxicity in humans, are of utmost importance and require a multidisciplinary approach integrating both basic and clinical expertise.

Non-clinical studies to screen for QT prolongation potential have employed both human and animal cardiac tissues *in vitro* and animal models *in vivo*. The *in vitro* studies have concentrated primarily on investigations of (i) ionic currents in animal or human cardiac myocytes or heterologous expression systems and (ii) action potential duration in isolated hearts or cardiac tissues from laboratory animals or dissociated cardiac myocytes from human or animal sources. Studies employing standard voltage or patch clamp techniques to measure the inhibitory effect of drugs on cardiac ionic currents such as I_{Na} , I_{Ca} , I_{to} , I_{K1} , I_{Kr} and I_{Ks} , which may prolong the QT interval when affected adversely, have indicated that I_{Kr} may be the most important site of action of QT-prolonging drugs (Fig. 1). Molecular biology studies have demonstrated that I_{Kr} is a heteromeric assembly of K^+ channels subunits encoded by the ether-a-gogo-related gene-1 (HERG1) and MiRP (or KCNE2) [85], and that this ionic channel can be investigated in mammalian cell lines or *Xenopus laevis* oocytes that have been manipulated genetically to express this channel specifically [86]. Several experimental models (human myocytes, or whole hearts, Purkinje fibres, papillary muscles and ventricular muscles from dog, rabbit and guinea-pig) are also currently available to measure the effect of drugs on cardiac action potential, and unlike the models measuring changes in cardiac ionic current, these models have the advantage of being able to detect the QT-prolonging effect of a drug, regardless of the ionic current affected. Other non-electrophysiological techniques include fluorescence assays with potential-sensitive dyes and competition binding against tritium-labelled dofetilide (a well-known I_{Kr} blocker). All these methods have specific advantages and drawbacks [87], and it should be underlined that the use of expression systems and cloned channels as the sole approach

to determining the ion channel-blocking profile of a drug is, therefore, undesirable.

The cardiotoxic effects of drugs *in vivo* have been investigated by changes elicited in the electrocardiograms of both conscious and anaesthetized animals, including dogs, monkeys, swine, rabbits and guinea-pigs, although studies in conscious and unrestrained (freely moving) animals are generally preferred. Although these *in vivo* models are not considered to have high predictive sensitivity for arrhythmogenic drugs, due to the interspecies differences in cardiac electrophysiology, these models do provide a unique advantage that it is possible to simulate pathological conditions (hypokalaemia, bradycardia, oxygen deprivation) or to use pharmacological models of arrhythmias (such as upon administration of the α_1 agonist methoxamine), which more closely simulate situations occurring in clinical settings.

Overall, it should be emphasized that no single standardized non-clinical test is considered to have sufficient predictive value to obviate the need of other screening methods. Although the *in vitro* models provide useful information on the potential for any cardiac effects of drugs, these have specific limitations, which prevent a direct extrapolation of the results to human subjects. Furthermore, any drug producing even a modest concentration-dependent prolongation of the action potential *in vitro* should be considered a candidate for QT prolongation in clinical settings [88]. False-negative results are possible, and, if action potential duration is studied in non-human experimental systems, multiple species, tissues or preparations should be tested before dismissing the possibility of a positive effect. In the case of *in vivo* studies, conditions that increase the heart rate should be avoided as lower heart rates enhance the likelihood of detecting QT prolongation and torsades de pointes. Escalating doses of the test drug over a period of successive days should be administered to the animals to characterize dose-effect curves, with dose ranges that include and surpass the anticipated human dose, unless this is precluded by effects that confound ECG interpretation (e.g. tremors, hyperactivity, seizures). Pharmacokinetic analysis of the drug should be simultaneously performed on blood samples taken from the animals and studies should include a positive control agent.

Placebo- and/or verum-controlled clinical trials, in both rigorously studied healthy volunteers and in the target population to investigate the influence of co-morbidities, concomitant medications and effects dependent on the duration of the exposure to the drug, are essential to define the effect of any new drugs (e.g. third-generation antihistamines) on cardiac repolarization. These trials should be performed in accordance with good clinical practice in well-established laboratories, and assessment of cardiac repolarization should be mandatory during phase I, II and III of the trial, whether or not positive findings were identified in non-clinical studies. This could readily be accomplished by defining the drug's effect on the QT_c interval duration on the standard 12-lead electrocardiogram (ECG). Since there is a large degree of spontaneous variability in the duration of the QT_c interval, even over a single day, attention must particularly be given to defining an adequate baseline, and taking into account the effects of age, sex, heart rate and

populations [89]. Preferably, digital ECGs should be obtained and cross-checked with manual accurate high-resolution analysis, and the QT interval should be expressed using Bazett's, Fridericia's or Lecocq's correction, depending on the heart rate effects of the compound under study.

For drugs showing some evidence of prolonged repolarization in non-clinical studies, initial phase I and early phase II trials should employ restrictive eligibility criteria to exclude patients and volunteers considered at risk. If QT_c prolongation is not observed in these initial studies, subsequent phase II and III trials may use expanded eligibility criteria; if QT_c prolongation is observed, higher risk patient groups should not be studied, unless the severity of the disease under treatment and the lack of therapeutic alternatives is considered to justify the risk of an arrhythmic event.

A suggested clinical monitoring sequence that advocates testing normal volunteers first and subsequent groups of individuals only if no effects of the drug are noticed in the preceding group is shown in Fig. 1.

Subjects who do not demonstrate any cardiac effects should continue to take the antihistamine for at least 6 months with further pharmaco-surveillance and ECG monitoring at periodic intervals.

2. What should be the approach towards identifying populations at specific risk (genetic factor, other pathology, potential interactions...)? Several groups of individuals, as shown, appear to be at risk from possible side-effects of antihistamines, with females being at slightly greater risk than males:

Patients and individual family members with genetic abnormalities of cardiac conduction defects.

Cardiac patients with bradycardia, ischaemic heart disease, African black patients who develop cardiomyopathy.

Patients on medications; especially cardiac medications, macrolides (erythromycin, azithromycin, clarithromycin), some fluoroquinolones, ketoconazole, cimetidine, anti-emetics, opioids, anti-migraine agents, anti-psychotics and anti-malarials; all of which may cause QT prolongation and torsades de pointes [90].

- Elderly patients (over 65 years).
- Patients with liver disease.
- Patients with hypo/hyperkalaemia.
- Patients undergoing anaesthesia.
- Patients on immunosuppressant therapy who develop drug allergies.
- Patients on certain diets (grapefruit diets).

Thus, it is possible to propose a *check list*, in which these high-risk groups are listed, that the practitioner should refer to before prescribing an antihistamine to any patient.

1. Is the patient a cardiac patient? Cardiotoxic antihistamines produce a more clinically significant QT_c increase in patients with cardiovascular disease than in normal subjects [91]. Furthermore, a drug-induced increase in QT_c ≥ 50 ms is likely to represent a drug effect at the 95% confidence interval.
2. Is the patient on any of the following medicines: macrolides, opioids, ketoconazole, anti-psychotics, anti-

malarials and anti-migraine medication? If so, the drug should be used with caution since these agents may also prolong cardiac repolarization.

3. Does the patient have any of the following high-risk factors, e.g. special diet (grapefruit), liver disease, elderly malignancy, immunosuppressant therapy anaesthesia?

However, it is critical to understand the metabolism of the antihistamine and to judge the potential of any metabolic interference on cardiac repolarization in these groups of individuals. Thus, if a drug were metabolized by CYP450-3A4, then interaction studies with ketoconazole would be essential to judge the potential impact of these drug-drug interactions. Pharmacogenetics is promising but as of today the characterization of the polymorphisms in a patient has not allowed for a clinically useful approach to determine risk of cardiac repolarization effect.

3. Is it possible to define a therapeutic margin? The safety of each antihistaminic molecule can be stated only after it has been evaluated comprehensively and critically by both preclinical and clinical studies, and therefore no general rule can be extrapolated. Although the therapeutic margin will be based on pharmacokinetic and dose-ranging studies for each antihistamine under consideration, the practical and/or ethical considerations often make it impossible to evaluate the effects of doses above the therapeutic range, particularly in any controlled clinical trials looking at effects on QT_c intervals in high-risk groups. Moreover, the benefit of antihistamine use is limited to symptomatic relief for a mostly non-life-threatening ailment and thus the potential to increase the risk of mortality via the development of torsades de pointes may be unacceptable. It is possible that a drug that affects the QT_c duration by even a small amount within a 10-fold concentration of its clinical dose may have an unacceptable margin of safety. Also, the lowest dose with a desired clinical effect should be chosen and drugs with a wide therapeutic margin without side-effects would be more advantageous.

4. How can the likelihood of cardiotoxic effects be determined? Both *in vitro* and *in vivo* tests should be conducted to determine any likelihood of cardiotoxic effects of a new antihistamine.

Well-controlled dose-, time-, and temperature-dependent I_{Kr} determination in cloned mammalian cells, coupled with assessment of action potential duration, is possibly the best form of *in vitro* testing. In contrast, evaluations of ECGs in live animal models are fraught with difficulties in interpretation of the ECG data due to very fast heart rates and lack of adequate sample sizes.

Clinical studies should be performed in normal adults and children, allergic adults and non-allergic patients with other diseases, and should evaluate both pharmacokinetic and potential drug interaction properties, in addition to specific ECG evaluations as shown:

Pharmacokinetic studies: volume of distribution; therapeutic margin; route of excretion, half-life; route of metabolism (e.g. first pass).

Drug interaction studies: dose ranging with ketaconazole; dose ranging with macrolides; dose ranging; combinations of ketaconazole plus macrolides.

Cardiac studies:

- ECG:*
- standardization of ECG
 - patient baseline variation
 - early effects (within 24 h)
 - later effects (after 24 h)
 - follow-up studies (weeks, months)
 - exercise/stress ECG
 - Holter 24-h monitoring
 - Surveillance studies (6 months to 1 year).

Depending on the results obtained in the above-mentioned tests, antihistamines may be classified into six groups as:

- Group 1: Free of any side-effects on the heart at any dose, from both clinical and preclinical studies.
- Group 2: Free of cardiac side-effects only when used within the therapeutic dose range, but clinical or pre-clinical studies documenting cardiac toxicity in doses above the therapeutic range.
- Group 3: No evidence of any human cardiac side-effects at any dose, but preclinical evidence of *in vitro* potential effects on the heart or from animal studies within the therapeutic range.
- Group 4: No evidence of any human cardiac side-effects at any dose, but preclinical evidence of *in vitro* potential effects on the heart or from animal studies above the therapeutic range.
- Group 5: Side-effects documented by clinical and preclinical studies at doses within the therapeutic range.
- Group 6: Side-effects only potentially present if used in high-risk patients, e.g. patients with cardiac disease,

patients on other medications, anaesthesia and metabolic disorders.

In all the above categories, side-effects on the heart may be classified either as minor or serious and potentially dangerous.

Recommendations and take-home message

- Life-threatening adverse cardiac side-effects such as QT prolongation and torsades de pointes ventricular tachyarrhythmias have been described in association with the use of some second-generation antihistamines (astemizole and terfenadine).
- These effects are consequent to direct blockade of a specific class of potassium channels controlling the repolarization phase of the cardiac action potential, and are not related to the blockade of the H1 receptor. Thus, cardiotoxicity by second-generation antihistamines is not a class effect.
- Preclinical and clinical studies of predictive ability of such cardiotoxic effects must be performed before novel molecules enter the market.
- Several pharmacokinetic and pharmacodynamic factors can precipitate the occurrence of the arrhythmic episode; medical practitioners prescribing second-generation antihistamines must be aware of these factors in order to avoid exposing the patient to potential dangerous effects.
- The lack of cardiac toxicity, a feature already present in some second-generation antihistamines, must be retained in the development of novel congeners that enter the market in the future.

3.4. Panel 4: lack of drug–drug interactions

Chairperson: M. Tharp

Contributors: R. E. O’Hehir, A. G. Renwick, B. Testa

Introduction

Many antihistamines are lipid-soluble molecules, which in common with other lipid soluble xenobiotic chemicals, undergo metabolism via the CYP system, comprising the CYP enzyme and isoenzyme families 1 (CYP1), 2 (CYP2) and 3 (CYP3). Some antihistamines have shown clinically significant interactions with inhibitors of the CYP enzymes, particularly the CYP3A4 isoenzyme that is present in large quantities in the liver and the intestinal wall. It is possible that interaction between inhibitors of CYP3A4 and an antihistamine may result in increased concentrations of the antihistamine through reduced first-pass metabolism and/or

increased uptake and decreased elimination via the GI tract, thus increasing the potential for adverse effects of the antihistamine.

Questions addressed by the panel

1. What pharmacokinetic criteria should a new antihistamine display to avoid drug-drug interactions? Since a large number of antihistamines are metabolized via the CYP enzymes, it is important that in order to avoid drug-drug interactions a new antihistamine should neither inhibit nor induce the CYP isoenzymes (including but not limited to CYP3A4, CYP2D6, CYP2C9). It is equally important that a third-generation antihistamine should not displace protein-

bound drugs or inhibit active transport mechanisms (i.e. P-glycoprotein) involved in the absorption or excretion of drugs through the GI tract or kidney, respectively [92].

2. Should the potential for drug interactions with antihistamines be defined on the basis of metabolic fate and biological activity of the antihistamine and its metabolites? It is clear that both the parent drug and its metabolite need to be examined for potential interactions with other medications. While drug metabolites are generally less likely to interfere with liver enzymes than the parent compound, there are other mechanisms involved in drug-drug interactions, for example displacement of protein-bound drug. Consequently, understanding the potential for interactions with other drugs is extremely important for both the parent drug and its metabolites, or further possible metabolites [93].

3. What is the relevance of the volume of distribution in the efficacy and safety of an antihistamine? It is probable that a low apparent volume of distribution would be a positive attribute for a third-generation antihistamine, based on the assumption that the drug is more likely to be localized (concentrated) at the receptor site on the cell membrane, rather than accumulating inside the cell. However, compounds with low apparent volumes of distribution are usually more water soluble and, unlike lipophilic drugs, cross cell membranes less readily, thereby possibly resulting in them being absorbed poorly from the GI tract. Thus, a potential solution to this problem might be the development of a more lipophilic 'pro-drug', which was metabolized to an active, hydrophilic compound by a widely distributed enzyme [94, 95].

4. What are the possible interactions between antihistamines and P-glycoprotein transporter in the GI tract, kidney and blood-brain barrier, and their consequences on efficacy and CNS effects? Antihistamines that inhibit P-glycoprotein transport proteins would be likely to affect both influx and/or efflux of drugs across the GI tract and the blood-brain barrier [96]. In the GI tract, inhibition of active transport (influx) could have a negative effect on drug absorption, whereas this same inhibitory process in the CNS could be advantageous (i.e. less sedation). However, inhibition of efflux from the CNS would also be expected to add to sedation. Ideally, little, if any, effect on transport proteins would be an appropriate characteristic for a third-generation antihistamine.

5. Should lack of drug-drug interactions be a requirement of third-generation antihistamines? While a lack of drug-drug interaction is clearly a highly desirable and essential characteristic of a third-generation antihistamine, especially if it affected drug safety and/or efficacy, this strict criterion may not necessarily be applicable because not all drug-drug interactions are noxious.

Recommendations and take-home message

With regard to drug-drug interactions, a third-generation antihistamine should ideally:

- not affect CYP isoenzyme function
- not displace protein-bound medication
- not affect active transportation mechanism (i.e. P-glycoprotein) important in drug absorption and excretion.

3.5. Panel 5: lack of CNS effects

Chairperson: K. Yanai

Contributors: I. Hindmarch, R. Ramaeker, D. F. Wong

Introduction

The lack of CNS effects for certain antihistamines at a recommended or commonly employed doses has been attributed to the apparent inability of these drugs to penetrate the blood-brain barrier, and has resulted in these drugs being labelled as 'non-sedative' [11, 14, 97]. More recently, however, it has become evident that all antihistamines have the potential to produce either or both subjective and objective sedation, dependent on the histaminergic mechanisms involved in the control of CNS arousal and the sensitivity of tests employed to detect the changes in CNS activity. Thus, it is vital that an apparent lack of any CNS effects for an antihistamine, demonstrable by clinically subjective and objective tests, can be related to appropriate biochemical tests.

Questions addressed by the panel

1. How can criteria for what is the 'minimum' acceptable for an antihistamine to be classified as a non-sedative drug

be defined? Criteria for what is a 'minimum' acceptable for an antihistamine to be classified as a 'non-sedative' drug depend specifically on three factors: (i) incidence of subjective sleepiness, (ii) objective cognitive and psychomotor functions and (iii) PET measurement of H1 receptor occupancy. Although the latter two are particularly important in determining the non-sedative properties of drugs, all three criteria should be met as a 'minimum' acceptable, prior to classifying an antihistamine as a 'non-sedative' drug.

2. What should be the appropriate methods and measures to discriminate and differentiate antihistamines on the criterion of 'sedation'? For incidence of subjective sleepiness, double-blind placebo-controlled studies should be performed in relatively large numbers of patients and statistically no significant difference should be observed between patients treated with placebo and patients treated with the 'non-sedative' third-generation antihistamine. It should be noted, however, that impairment in objective performance has

sometimes been established in the absence of subjective sleepiness, following treatment with an antihistamine, and therefore qualification of new-generation antihistamines should not be based on subjective evaluations alone [98, 99].

Cognitive studies should also be conducted in large numbers of patients, using a battery of tests to evaluate sensorimotor co-ordination speed, sensory skills and information processing (mental arithmetic/verbal fluency). Again, no significant differences should be observed between placebo and active treatment in multiple studies with *at least two different tasks*. Of the large number of cognitive tests applied to date, the critical flicker fusion (CFF) task, choice reaction time (CRT), Digital Symbol Substitution Test (DSST) and actual and simulated car driving have been found to be particularly sensitive to the effects of antihistamines [100, 101].

The new-generation antihistamines should also be examined for H1-receptor occupancy in order to validate their 'non-sedative' properties. The recommended criterion for H1-receptor occupancy is a maximum of around 20% receptor occupancy at the *highest recommended doses* [102, 103].

3. How should the results from multiple CNS tests performance be analysed and interpreted, and what should be the standards for assessment? In order to clarify the 'non-impairment' of cognition, the sensitivity of tasks should be evaluated using double-blind, placebo- and verum- (sedative antihistamines, or alcohol) controlled studies. The doses for the sedative antihistamines should be carefully chosen to establish the sensitivity of psychometric tests, and the doses for new-generation antihistamines are the *highest recommended doses*. Considering that sedative antihistamines cause a significant impairment in the quality of life of patients, practical tasks such as car driving and school performances are recommended as one of the multiple tasks. Interpretation of results from cognitive or psychomotor tests should start with an *a priori* definition of a clinically relevant change in performance.

4. What could be the input of brain imaging to clarify the notion of 'non-sedative'? The use of PET to examine antihistamine penetration into the human brain in relation to psychometric and other functional measures of CNS effects is a major breakthrough and provides a new standard by which the functional CNS effects of antihistamines can be related directly to H1-receptor occupancy [14]. While cognitive studies provide only relative information due to their sensitivities being determined by differences between the effects of placebo and selected sedative antihistamines, PET measurement can provide an absolute evaluation of blood-brain barrier penetration by an antihistamine. Consequently, this methodology should be applied, in addition to subjective and objective tests, to the assessment of all new H1 antihistamines, whereby binding of the drug to H1 receptors in the CNS *in vivo* in humans can be directly related to functional CNS data [104].

5. As lack of CNS effects is a clear cut-off between first- and second-generation antihistamines, should this parameter be recognized as a 'prerequisite' for potential new-

generation antihistamines? The first-generation antihistamines are profoundly sedative and impair CNS function at virtually all doses tested, due to their ease of passage across the blood-brain barrier. The second-generation antihistamines also cross the blood-brain barrier, but their effects on performance are much less marked than those of the first-generation drugs. There may be dose-related impairments of CNS function, since the effects are more pronounced with increasing doses of some second-generation antihistamines. Thus, when any impairment of CNS function is demonstrated for a particular second-generation drug, it can be classified only as a 'relatively non-sedative' or 'less sedative' drug, but not a 'non-sedative' drug. However, several second-generation drugs do not penetrate the blood-brain barrier, and are therefore truly non-sedative, i.e. not passing into the CNS and having no significant effects on CNS function in daily life, even when used at doses in excess of those commonly recommended or tested.

Although a lack of CNS effects is a clear cut-off between first- and second-generation antihistamines, the non-sedative properties of several second-generation antihistamines have not been carefully evaluated, possibly contributing to the notable variations in the findings for their sedative characteristics. Consequently, it is important that standardized test protocols (analogous to HRQL instruments currently available for assessment of quality of life of patients with allergic disease) are developed, possibly with the aid of but without any direct influence of the pharmaceutical industry, to assess the CNS effects of new antihistamines. Since the clinical use of antihistamines at higher than manufacturers' recommended doses is widespread, it is essential that standardized tests should be performed at supra-dose ranges, which demonstrate that drugs do not cross the blood-brain barrier and therefore, unequivocally, classify them as being non-sedative. Similarly, measuring H1-receptor occupancy at these supra-dose ranges and correlating the receptor occupancy with plasma concentrations may be useful in predicting the effects of supra-clinical doses. Nevertheless, the lack of CNS effects should be recognized as an important 'prerequisite' for new-generation antihistamines and 'non-sedative' properties should be certified only when the above-mentioned criteria have been fulfilled.

Recommendations and take-home message

- Three factors define the criteria for what is the 'minimum' acceptable for an antihistamine to be classified as a 'non-sedative' drug:
 - (i) incidence of subjective sleepiness,
 - (ii) objective and psychomotor functions
 - (iii) PET measurement of H1-receptor occupancy.
- Antihistamines should be classified as 'non-sedative' drugs when all the above criteria have been met, even though the latter two criteria are more important.

3.6. Panel 6: pharmacological approach

Chairperson: H. Timmerman

Contributors: R. Leurs, P. Panula, L. Hough

Introduction

Development of the non-sedating second-generation antihistamines marked a great advance over the 'old' first-generation antihistamines, which were of limited use due to their sedative effects. Some of the second-generation antihistamines, however, have been confounded by the finding that they impart adverse cardiac effects and have therefore been withdrawn from the market. Although there are some chemical and functional differences between the second-generation antihistamines, these are not great and clinically the drugs are thought to be highly comparable with one another. With the recent advances made in the understanding of histamine receptor structure and physiology, coupled with the development of highly specialized molecular biology techniques, it is likely that new classes of much safer and more potent antihistamines, with additional therapeutic properties, will be developed [105, 106].

Questions addressed by the panel

1. How have histamine H1 antagonists been classified so far? Biologically active compounds may be classified on the basis of several physico-chemical properties, for example similar chemical composition and mechanism of action, similar therapeutic effect, similar duration of action, lack of certain side-effects, availability by certain routes of administration, etc.

Histamine H1 antagonists have traditionally been grouped according to chemical classes (including benzhydryl ethers, ethene derivatives, piperazines, etc), which do not differ greatly with respect to pharmacological behaviour, therapeutic applicability or incidence of side-effects. Although there are some differences in the pharmacokinetic properties of the currently available antihistamines, these are not substantial and therefore do not presently merit separate definition or classification.

Historically, despite several failed attempts during the 1960s and 1970s to replace the earlier sedating antihistamines with newly developed non-sedating antihistamines, a new class of H1 antagonists emerged by chance with the development of terfenadine and a series of other compounds with a similar pharmacological profile (e.g. cetirizine, astemizole, loratadine), which was found to have both antihistaminic properties and additionally lacked CNS effects [107–109].

This new class of H1 antagonists (commonly referred to as second-generation antihistamines) are specific H1-receptor blockers with none or little penetrating capacity into the CNS, which results in the lack or low frequency of sedation demonstrated by these compounds. However, some members

of this class of agents, in particular terfenadine and astemizole, have been shown to cause adverse cardiac effects, and have therefore been removed from the market. More importantly, the demonstration of the potential for adverse cardiac effects by these second-generation antihistamines has led to a sub-classification for the other compounds in this class, defining them as 'second-generation antihistamines, which do not possess a cardiotoxic potential'.

2. Is it possible to define new classes of histamine H1 antagonists? Since the currently available H1 antagonists are not entirely ideal drugs in many functional and biochemical aspects, it is theoretically possible to develop new class/classes of drugs, which improve on these aspects and therefore confer a distinct clinical/therapeutic advantage over the existing drugs.

One such approach would be to develop a compound with an anti-H1 effect and an additional specific anti-inflammatory component in the same molecule. The potential anti-inflammatory component may be 'added' to the primary H₁ antagonistic property, on the basis of one of several potential mechanisms of action. For example, pro-inflammatory effects influenced by immunomodulatory mechanisms would be particularly important targets and could be mediated via receptors other than the H1 receptor. Another mechanism could be blockade of the newly defined histamine H4 receptor [110, 111], which has been shown to share some properties with the H3 receptor and suggested to play a role in regulating the pro-inflammatory features of histamine. However, the difference in the distribution of H4 and H3 receptors may be extremely important in defining a particular additional effect, since H4 receptors are present predominantly in leucocytes and in the GI tract, compared with H3 receptors, which are found predominantly in the CNS.

3. How can newly defined classes become reality? It is neither attractive nor productive to initiate a synthesis and screening programme at random. These drugs should be either identified by screening for common features, or specifically be designed on the basis of theoretical considerations. Although in principle it is possible to design new drugs, clarification of the desired additional anti-inflammatory effect and the mechanism/s by which this effect could be achieved should be made at the outset.

4. Can new classes be based on pharmacokinetic properties? 'A small volume of distribution', which has several potential advantages, could possibly be the basis for designing a new class of antihistamines. However, since some specific features observed for cetirizine cannot purely be explained on the basis of its low volume of distribution,

presently the real impact of this pharmacokinetic property for other antihistamines is not entirely clear. It is likely that a number of compounds with comparable volumes of distribution and similar additional specific pharmacologic or therapeutic properties would need to be developed and investigated before it can be determined whether or not such a property offers any real advantage over existing drugs.

5. Are there other ways to distinguish between histamine H1 blocking ligands? The understanding of the mechanisms underlying ligand-receptor interactions has increased greatly over the last few years and has raised the possibility of defining new classes of compounds on the basis of how they might interfere with a given receptor at the molecular level. It has been demonstrated that even chemically closely related ligands may bind/react with the same receptor via different amino acid residues at the receptor. Although this phenomenon has been observed for both H1 agonists and H1 antagonists [6, 7, 112, 113], the differences in the mechanisms underlying their binding may not necessarily lead to different effects. Furthermore, there is presently no indication that these differences will lead to new classes of drugs with distinct pharmacologic or therapeutic properties *in vivo*.

6. What could be the role of inverse agonism, compared with the classic neutral antagonists? Recently, a re-classification of receptor ligands has been introduced, leading to a subdivision of three groups instead of the classical two groups: the well-known agonists and competitive antagonists have been re-classified into agonists, inverse agonists and neutral antagonists [10, 114–116]. The re-classification was necessitated by the observation that many receptors have a basic constitutive activity (i.e. these receptors are constantly switched 'on' to a certain level without the presence of an agonist). On the basis of this finding, it has been hypothesized that interaction with an agonist would lead to an increase in basic activity, interaction with an inverse agonist to a reduction in basic activity, and interaction with a neutral antagonist in no change in basic activity of these receptors. A neutral antagonist has the same definition as the classic competitive antagonist, which binds to the receptor and in so doing blocks the actions of both an agonist and an inverse agonist, without perturbing its activation state. Under this new classification many compounds, including all the H1 antihistamines, which were presumed to be competitive antagonists for their respective receptor types, have actually been shown to be inverse agonists. However, no neutral H1 antagonists have been identified to date.

As inverse agonists and neutral antagonists may have different pharmacological properties it would be interesting to develop classes of both H1-inverse agonists and neutral H1 antagonists, which would have the potential to either induce or not induce rapid receptor sensitization, respectively. This difference in effects on H1-receptor activity may be of particular importance, for example in the case of an allergen-induced pathologic event that could potentially result from increased constitutive H1-receptor activity, similar to that shown for some mutated receptor types. Thus, it is likely that H1-inverse agonists would reduce the activity of constitutive H1 receptors and therefore be effective in treatment of patients with such receptors, whereas neutral

antagonists would probably not be effective, but block the effect of the agonist.

7. Currently, histamine H1 antagonists are distinguished in three classes: the 'old' antihistamines, the second-generation non-sedating antihistamines, and the second-generation non-sedating antihistamines without a potential for cardiotoxicity. Are there any examples of a new class, based on the considerations discussed in the preceding paragraphs? So far, no new class of antihistamines with properties that provide a clear clinical advantage over existing antihistamines has been introduced into the market, let alone be identified, on the basis of preceding discussion.

Recommendations and take-home message

- New classes of antihistamines are theoretically possible.
- Such classes may be identified by incidence: common features observed.
- Such classes may be defined on theoretical considerations as well.
- Theoretically new classes of compounds could become a reality either by screening (the old way) or by designing (the new way).
- There may be different kinds of new classes: e.g. H1 blockers with an extra effect (without being dirty drugs) or 'clean' blockers of the H1 effects, having a special feature (e.g. neutral antagonists).
- Theoretical classes having become a reality should in any case be proven to have clinical advantages.

4. General conclusions

Based on published information about H1 antihistamines, it appears that none of them is a true 'third-generation' antihistamine. There is a sound rationale for expecting a true breakthrough during the next few years leading to development of a new class. As highlighted in the pharmaceutical approach, new classes of antihistamines are possible in theory, but theoretical classes having become reality should still be proven to have distinct clinical/therapeutic advantages over the existing preparations.

Three 'prerequisites' seem necessary as primary components of a new class of antihistamines:

- lack of cardiotoxicity,
- lack of drug-drug interactions,
- lack of CNS effects. Although lack of CNS effects is a clear cut-off between the first and second generation, the non-sedative properties of several second-generation antihistamines were not carefully evaluated. Therefore their sedative characteristics varied considerably. The three criteria for what is the 'minimum' acceptable for an antihistamine to be classified as a 'non-sedative' drug are clearly expressed.

During the CONGA discussion, the approach 'anti H1 plus an extra' is explored and especially an anti-inflammatory component is considered as being attractive. Nevertheless, antihistamines are used to treat a disease with symptoms and signs, and it is therefore required that anti-inflammatory

actions have a clinical counterpart and that they can be demonstrated *in vivo*, in humans, at therapeutical doses and under natural exposure to the offending allergens.

One way to find a breakthrough could be the pharmacological approach. During recent years, the understanding of the mechanisms of ligand receptor interactions has increased enormously. It may now be possible to define classes of compounds on the basis of the way they interfere with a given receptor at a molecular basis. Nevertheless, it should be recalled that theoretical new pharmacologic classes having become a reality should still be proven to have advantages.

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The Consensus Group believes that readers need to be aware of any affiliation or financial relationship (employment, consultancies, stock ownership, honoraria, etc) between authors and any organization involved in the manufacture and/or distribution of H1 antihistamines. Each author has disclosed their potential conflict of interest as follows:

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References

- Hanifin JM. The role of antihistamines in atopic dermatitis. *J Allergy Clin Immunol* 1990; 86:666–9.
- Sabbah A. The rebirth of the H1-antagonists. *Allergie Immunol* 1992; 24:224–30.
- Caproni M, Palleschi GM, Falcos D, Papi C, Lotti T. Pharmacologic modulation by cetirizine of some adhesion molecules expression in psoriatic skin lesions. *Int J Dermatol* 1995; 34: 510–3.
- Pagliara A, Testa B, Carrupt P-A et al. Molecular properties and pharmacokinetic behaviour of cetirizine, a zwitterionic H1-receptor antagonist. *J Med Chem* 1998; 41: 853–63.
- Moguilovsky N, Varsalona F, Guillaume JP et al. Pharmacological and functional characterization of the wild-type and site directed mutants of the human H1 histamine receptor stably expressed in CHO cells. *J Receptor Signal Transduc Res* 1995; 15:91–102.
- Ohta K, Hayashi H, Mizuguchi H et al. Site-directed mutagenesis of the histamine H1 receptor: roles of aspartic acid¹⁰⁷, asparagine¹⁹⁸, and threonine¹⁹⁴. *Biochem Biophys Res Commun* 1994; 203:1096–101.
- Leurs R, Smit MJ, Tensen CP et al. Site-directed mutagenesis of the histamine H1 receptor reveals a selective interaction of asparagine207 with subclasses of H1 receptor agonists. *Biochem Biophys Res Commun* 1994; 201:295–301.
- Timmerman H. Why are non-sedating antihistamines non sedating? *Clin Exp Allergy* 1999; 29 (Suppl. 3):13–8.
- Smit MJ, Hoffman M, Timmerman H, Leurs R. Molecular properties and signalling pathways of the histamine H1 receptor. *Clin Exp Allergy* 1999; 29 (Suppl. 3):19–28.
- Bakker RA, Wieland K, Timmerman H, Leurs R. Constitutive activity of the histamine H1 receptor reveals inverse agonism of histamine H1 receptor antagonists. *Eur J Pharmacol* 2000; 387:R5–7.
- Timmerman H. Factors involved in the absence of sedative effects by the second-generation antihistamines. *Allergy* 2000; 55:1–6.
- Rapoport SI. Modulation of blood brain barrier permeability. *J Drug Target* 1996; 3:417–25.
- Hindmarch I, Shamsi Z. Antihistamines: Models to assess sedative properties, assessment of sedation, safety and side-effects. *Clin Exp Allergy* 1999; 29 (Suppl. 3):133–42.
- Yanai K, Okamura A, Tagawa M et al. New findings in pharmacological effects induced by antihistamines: from PET studies to knock out mice. *Clin Exp Allergy* 1999; 29 (Suppl. 3): 29–36.
- Zhang M-Q. Chemistry underlying the cardiotoxicity of antihistamines. *Curr Med Chem* 1997; 4:171–84.
- Edwards DJ, Bellevue II, Woster PM. Identification of 6′7′-dihydroxybergamottin, a cytochrome P450 inhibitor in grapefruit juice. *Drug Metab Dispos* 1996; 24:1287–90.
- Rau SE, Bend JR, Arnold JMO et al. Grapefruit juice-terfenadine single-dose interaction: magnitude, mechanism and relevance. *Clin Pharmacol Ther* 1997; 61:401–9.
- Clifford CP, Adams DA, Murray S et al. The cardiac effects of terfenadine after inhibition of its metabolism by grapefruit juice. *Eur J Clin Pharmacol* 1997; 52:311–5.
- Renwick AG. The metabolism of antihistamines and drug interactions: the role of cytochrome P450 enzymes. *Clin Exp Allergy* 1999; 29 (Suppl. 3):116–24.
- Handley DA, McCullough JR, Fang Y et al. Descarboethoxyloratadine is a superior antihistamine. *Ann Allergy Asthma Immunol* 1997; 78:143 (Abstract).
- Nicolas JM, Whomsley R, Collard P, Roba J. *In vitro* inhibition of human liver drug metabolizing enzymes by second generation antihistamines. *Chem-Biol Interact* 1999; 123:63–79.
- Hamelin BA, Bouayad A, Drolet B, Gravel A, Turgeon J. *In vitro* characterization of cytochrome P450 2D6 by classic histamine H receptor antagonists. *Drug Metab Dispos* 1998; 26:536–9.

- 23 Jones BC, Hyland R, Ackland M et al. Interaction of terfenadine and its primary metabolites with cytochrome P450 2D6. *Mol Pharmacol* 1998; 26:875–82.
- 24 Woolsey RL, Chen Y, Freiman JP, Gilles RA. Mechanism of the cardiotoxic actions of terfenadine. *J Am Med Assoc* 1993; 269:1532–6.
- 25 Salata JJ, Jurkiewicz NK, Wallace AA et al. Cardiac electrophysiological actions of the histamine H₁-receptor antagonists astemizole and terfenadine compared with chlorpheniramine and prilamine. *Circ Res* 1995; 76:110–9.
- 26 Suessbrich H, Waldegger S, Lang F, Busch AE. Blockade of HERG channels expressed in *Xenopus oocytes* by the histamine receptor antagonists terfenadine and astemizole. *FEBS Lett* 1996; 385:77–80.
- 27 De Buske LM. Second generation antihistamines: the risk of ventricular arrhythmias. *Clin Ther* 1999; 21:281–95.
- 28 Crumb W. Loratadine blockade of K⁺ channels in human heart: Comparison with terfenadine under physiological conditions. *J Pharmacol Exp Ther* 2000; 292:261–4.
- 29 Caballero R, Delpaon E, Valenzuela C et al. Effect of descarboethoxyloratadine, the major metabolite of loratadine on the human cardiac potassium channel kv1.5. *Br J Pharmacol* 1997; 122:796–8.
- 30 Crumb WJ. Rate dependent blockade of a potassium current in human atrium by the antihistamine loratadine. *Br J Pharmacol* 1999; 126:575–80.
- 31 Church MK. Non-H₁-receptor effects of antihistamines. *Clin Exp Allergy* 1999; 29 (Suppl. 3):39–48.
- 32 Sehmi R, Walsh G, Hartnell A et al. Modulation of human eosinophil chemotaxis and adhesion by anti-allergic drugs in vitro. *Pediatr Allergy Immunol* 1993; 4 (Suppl. 4):13–8.
- 33 Fadel R, Heprin-Richard N, Rihoux J-P, Henocq E. Inhibitory effect of oral cetirizine on eosinophil migration in vivo. *Clin Allergy* 1987; 17:373–9.
- 34 Charlesworth EN, Kagey-Sobotka A, Norman PS, Lichtenstein LM. Effect of cetirizine on mast cell mediator release and cellular traffic during the cutaneous late-phase reaction. *J Allergy Clin Immunol* 1989; 83:905–12.
- 35 Fadel R, Heprin-Richard N, Dufresne F, Rihoux J-P. Pharmacological modulation by cetirizine and loratadine of antigen- and histamine-induced skin wheals and flares and late accumulation of eosinophils. *J Int Med Res* 1990; 18: 366–71.
- 36 Ciprandi G, Pronzato C, Ricca V et al. Loratadine reduces ICAM-1 expression on conjunctiva after specific challenge in pollen allergic patients. *Allergy* 1998; 53:545–6.
- 37 Ciprandi G, Buscaglia S, Pesce G et al. Cetirizine reduces inflammatory cell recruitment and ICAM-1 (CD54) expression on conjunctival epithelium in both early and late phase reactions after allergen challenge. *J Allergy Clin Immunol* 1995; 95:612–21.
- 38 ETAC study group. Allergic factors associated with the development of asthma and the influence of cetirizine on this progression in a double-blind, randomised, placebo-controlled trial: first results of ETAC. *Ped Allergy Immunol* 1998; 9:116–24.
- 39 Van Ganse E, Kaufman L, Derde MP et al. Effects of antihistamines in adult asthma: a meta-analysis of clinical trials. *Eur Respir J* 1997; 10:2216–24.
- 40 Corren J, Harris AG, Aaronson D et al. Efficacy and safety of loratadine plus pseudoephedrine in patients with seasonal allergic rhinitis and mild asthma. *J Allergy Clin Immunol* 1997; 100: 781–8.
- 41 Grant JA, Nicodemus CF, Findlay SR et al. Cetirizine in patients with seasonal rhinitis and concomitant asthma: prospective, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 1995; 95:923–32.
- 42 Handley DA, Magnetti A, Higgins AJ. Therapeutic advantages of third generation antihistamines. *Exp Opin Invest Drugs* 1998; 7:1045–54.
- 43 Segura T, Casale P. Allergic rhinitis: basic pathophysiology and therapeutic strategies. *Can J Allergy Clin Immunol* 1999; 4:18–30.
- 44 McFadden ER. What new pharmacological trends could mean for allergy and asthma treatment. American College of Allergy Asthma & Immunology Annual Meeting 1999. Day 1, November 12.
- 45 Holgate ST, (Chair). Antihistamines: back to the future, summary of the conclusions. *Clin Exp Allergy* 1999; 29 (Suppl. 3):iv–vi.
- 46 Timmerman H. European Academy of Allergology and Clinical Immunology; requirements for anti-allergic treatment in the new millennium. Third Generation Antihistamines: Feasible or a Dream? Brussels, 3–7 July 1999.
- 47 Abdelaziz MM, Khair OA, Devalia JL. The potential of active metabolites in the management of allergic disease. *Allergy* 2000; 55:425–34.
- 48 Kay AB. Allergy and allergic diseases. Second of two parts. *N Engl J Med* 2001; 344:109–13.
- 49 Borish LC, Nelson H, Lanz MJ et al. Interleukin-4 receptor in moderate atopic asthma. A phase I/II randomized, placebo-controlled trial. *Am J Respir Crit Care Med* 1999; 160:1816–23.
- 50 Leckie MJ, ten Bincke A, Khan J et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000; 356:2144–8.
- 51 Donnelly AL, Glass M, Minkwitz T, Casale TB. The leukotriene D₄ receptor antagonist ICI204,219 relieves symptoms of acute seasonal allergic rhinitis. *Am J Respir Crit Care Med* 1995; 151:1734–9.
- 52 Pullerits T, Praks L, Skoog BE et al. Randomized placebo controlled study comparing a leukotriene receptor antagonist and a nasal glucocorticoid in seasonal allergic rhinitis. *Am J Respir Crit Care Med* 1999; 159:1814–8.
- 53 Casale TB, Bernstein IL, Busse WW et al. Use of an anti IgE humanized monoclonal antibody in ragweed induced allergic rhinitis. *J Allergy Clin Immunol* 1997; 100: 110–21.
- 54 Milgrom H, Fick RB, Su JQ et al. Treatment of allergic asthma with monoclonal anti IgE antibody. *N Engl J Med* 1999; 341:1966–73.
- 55 Mor S, Nagler A, Barak V, Handzel ZT, Geller-Bernstein C, Fabian I. Histamine enhances granulocyte-macrophage colony-stimulating factor and interleukin-6 production by human peripheral blood mononuclear cells. *J Leukoc Biol* 1995; 58: 445–50.
- 56 Schmidt J, Fleissner S, Heimann-Weitschat I, Lindstaedt R, Szelenyi I. Histamine increases anti-CD3 induced IL-5 production of TH₂-type T cells via histamine H₂-receptors. *Agents-Actions* 1994; 42:1–85.
- 57 Jeannin P, Delneste Y, Gosset P et al. Histamine induces interleukin-8 secretion by endothelial cells. *Blood* 1994; 84: 2229–33.
- 58 Delneste Y, Lassalle P, Jeannin P, Joseph M, Tonnel AB, Gosset P. Histamine induces IL-6 production by human endothelial cells. *Clin Exp Immunol* 1994; 98:44–9.
- 59 Meltzer EO. Concomitant montelukast and loratadine as treatment for seasonal allergic rhinitis: a randomized, placebo-controlled clinical trial. *J Allergy Clin Immunol* 2000; 105: 917–22.
- 60 Wilson AM, Orr LC, Sims EJ, Lipworth BJ. Effects of monotherapy with intra-nasal corticosteroid or combined oral histamine and leukotriene receptor antagonists in seasonal allergic rhinitis. *Clin Exp Allergy* 2001; 31:61–8.
- 61 Leurs R, Church MK, Taghialatela M. H₁ antihistamines: inverse agonism, antiinflammatory actions and cardiac effects. *Clin Exp Allergy* 2002; 489–98.
- 62 Baarody FM, Naclerio RM. Antiallergic effects of H₁-receptor antagonists. *Allergy* 2000; 55 (Suppl. 64):17–27.

- 63 Marshall GD Jr. Therapeutic options in allergic disease: antihistamines as systemic antiallergic agents. *J Allergy Clin Immunol* 2000; 106 (Suppl. 5):S303–9.
- 64 Ciprandi G, Passalacqua G, Canonica GW. Effect of H1 antihistamines on adhesion molecules: a possible rationale for long term treatment. *Clin Exp Allergy* 1999; 29:49–52.
- 65 Perzanowska M, Malhotra D, Skinner SP et al. The effect of cetirizine and loratadine on codeine induced histamine release in human skin assessed by microdialysis. *Inflam Res* 1996; 45: 486–90.
- 66 Ciprandi G, Tosca M, Passalacqua G et al. Continuous versus on demand treatment with cetirizine for allergic rhinitis. *Ann Allergy Asthma Immunol* 1997; 79:507–11.
- 67 Ciprandi G, Pronzato C, Ricca V et al. Loratadine treatment of rhinitis due to pollen allergy reduces epithelial ICAM-1 expression. *Clin Exp Allergy* 1997; 27:1175–83.
- 68 Wang DY, Hanotte F, De Vos C, Clement P. Effect of cetirizine, levocetirizine and dextrocetirizine on histamine-induced nasal response in healthy adult volunteers. *Allergy* 2001; 56: 339–43.
- 69 Wang D, Clement P, Smits J. Effect of H1 and H2 antagonists on nasal symptoms and mediator release in atopic patients after nasal allergen challenge during the pollen season. *Acta Otolaryngol (Stockh)* 1996; 116:91–6.
- 70 Simons FER. H₁-antihistamines: more relevant than ever in the treatment of allergic disorders. *J Allergy Clin Immunol* 2003; 112, (in press).
- 71 Bousquet J, Van Cauwenberge PB et al. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001; 108 (part 2): S147–S334.
- 72 Kaplan AP. Clinical practice. Chronic urticaria and angioedema. *N Engl J Med* 2002; 346:175–9.
- 73 Spencer CM, Faulds D, Peters DH. Cetirizine. A reappraisal of its pharmacological properties and therapeutic use in selected allergic disorders. *Drugs* 1993; 46:1055–80.
- 74 Geha RS, Meltzer EO. Desloratadine: a new, non-sedating, oral antihistamine. *J Allergy Clin Immunol* 2001; 107:752–62.
- 75 Hurst M, Spencer CM. Ebastine. An update of its use in allergic disorders. *Drugs* 2000; 59:981–1006.
- 76 Simons FER. Fexofenadine. *J Drug Eval* 2002; 1:13–33.
- 77 Xyzal product monograph. UCB Pharma, 2001.
- 78 Haria M, Fitton A, Peters DH. Loratadine. A reappraisal of its pharmacological properties and therapeutic use in allergic disorders. *Drugs* 1994; 48:617–37.
- 79 Simons FER. Mizolastine: antihistaminic activity from preclinical data to clinical evaluation. *Clin Exp Allergy* 1999; 29 (Suppl. 1): 3–8.
- 80 Simons FER, Simons KJ. Clinical pharmacology of new histamine H₁-receptor antagonists. *Clin Pharmacokinet* 1999; 36:329–52.
- 81 Juniper EF. Quality of life in adults and children with asthma and rhinitis. *Allergy* 1997; 52:971–7.
- 82 Welch MJ, Meltzer EO, Simons FER. H₁-antihistamines and the central nervous system. In: Simons FER, ed. *Histamine and H₁ – antihistamines in allergic disease*, 2nd Edn. New York: Marcel Dekker, Inc., 2002:337–88.
- 83 Yap YG, Camm J. Risk of torsades de pointes with non-cardiac drugs. Doctors need to be aware that many drugs can cause QT prolongation. *Br Med J* 2000; 320:1158–9.
- 84 Committee for Proprietary Medicinal Products (CPMP/ICH/423/02). Safety pharmacology studies for assessing the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals. EMEA ICH S7B, February 2002.
- 85 Shieh C-C, Coghlan M, Sullivan JP, Gopalakrishnan M. Potassium channels: molecular defects, diseases, and therapeutic opportunities. *Pharmacol Rev* 2000; 52:557–93.
- 86 Taglialatela M, Timmerman H, Annunziato L. Cardiotoxic potential and CNS effects of first-generation antihistamines. *Trends in Pharmacol Sci* 2000; 21:52–6.
- 87 Taglialatela M. Heterologous expression systems and screening technologies in ion channel drug discovery. In: Pugsley MK, ed. *Cardiac drug development guide*. Totowa, NJ: Humana Press, 2002, (in press).
- 88 Woosley RL. Cardiac actions of antihistamines. *Annu Rev Pharmacol Toxicol* 1996; 36:233–52.
- 89 Morganroth J, Brozovich FV, McDonald JT, Jacobs RA. Variability of the QT measurement in healthy men, with implications for selection of an abnormal QT value to predict drug toxicity and proarrhythmia. *Am J Cardiol* 1991; 67:774–6.
- 90 <http://www.dml.georgetown.edu/depts/pharmacology/torsades.html>
- 91 Pratt CM, Ruberg S, Morganroth J et al. The dose response relationship between terfenadine (Seldane) and the QTc interval on the scalar electrocardiogram in normals and patients with cardiovascular diseases and the QTc interval variability. *Am Heart J* 1996; 131:472–80.
- 92 Gonzalez MA, Estes KS. Pharmacokinetic overview of oral second-generation H1 antihistamines. *Int J Clin Pharmacol Ther* 1998; 36:292–300.
- 93 Tozer TN. Concepts basic to pharmacokinetics. *Pharmacol Ther* 1981; 12:109–31.
- 94 Tillement J-P. A low distribution, as a determinant of efficacy and safety for histamine (H1) antagonists. *Allergy* 1995; 50:12–16.
- 95 Tillement J-P. The apparent volumes of distribution of H1 receptor antagonists. *Dermatologic Ther* 2000; 13:337–43.
- 96 Testa JB, Pagliara A, Carrupt P-A. The molecular behavior of cetirizine. *Clin Exp Allergy* 1997; 27:13–8.
- 97 Simons FER. H1- receptors antagonists: safety issues. *Ann Allergy Asthma Immunol* 1999; 83:481–8.
- 98 Mann RD, Pearce GL, Dunn N, Shakir S. Sedation with 'non-sedating' antihistamines: four prescription-event studies in general practice. *BMJ* 2000; 320:1184–7.
- 99 Ramaekers JG, Vermeeren A. All antihistamines cross blood–brain barrier. *BMJ* 2000; 321:572.
- 100 Hindmarch I, Shamsi Z. Sedation and antihistamines: a review of inter-drug differences using proportional impairment ratios. *Hum Psychopharmacol Clin Exp* 2000; 15:S3–30.
- 101 O'Hanlon JF, Ramaekers JG. Antihistamine effects on actual driving performance in a standard test: a summary of Dutch experience, 1989–94. *Allergy* 1995; 50:234–42.
- 102 Yanai K, Ryu JH, Watanabe T et al. Histamine H1 receptor occupancy in human brains after single oral doses of histamine H1 antagonists measured by positron emission tomography. *Br J Pharmacol* 1995; 116:1649–55.
- 103 Okamura N, Yanai K, Higuchi M et al. Functional neuroimaging of cognition impaired by a classical antihistamine, d-chlorpheniramine. *Br J Pharmacol* 2000; 129:115–23.
- 104 Tagawa M, Kano M, Okamura N et al. Neuroimaging of histamine H1-receptor occupancy in human brain by positron emission tomography (PET): a comparative study of ebastine, a second-generation antihistamine, and d-chlorpheniramine, a classical antihistamine. *Br J Clin Pharmacol* 2001; 52:501–509.
- 105 Hill SJ, Ganellin CR, Timmerman H et al. International union of pharmacology. XIII. Classification of histamine receptors. *Pharmacol Rev* 1997; 49:253–78.
- 106 De Backer MD, Gommeren W, Moereels H et al. Genomic cloning, heterologous expression and pharmacological characterisation of a human histamine H₁-receptor. *Biochem Biophys Res Commun* 1993; 197:1601–1608.
- 107 Ter Laak AM, Tsai RS, Donné-Op den Kerlder GM, Carrupt PA, Testa B, Timmerman H. Lipophilicity and hydrogen-bonding capacity of H₁-antihistaminic agents in relation to their central sedative side-effects. *Eur J Pharm Sci* 1994; 2:373–84.

- 108 Yanai K, Son LZ, Endou M et al. Behavioural characterisation and amounts of brain monoamines and their metabolites in mice lacking histamine H₁-receptors. *Neuroscience* 1998; 312:479–87.
- 109 Quach T, Duchemin AM, Rose C, Schwartz JC. *In vivo* occupation of cerebral histamine H₁-receptors evaluated with 3H-merypiramine may predict sedative properties of psychotropic drugs. *Eur J Pharmacol* 1979; 60:391–2.
- 110 Morse KL, Behan J, Laz TM et al. Cloning and characterisation of a novel human histamine receptor. *J Pharmacol Exp Ther* 2001; 296:1058–66.
- 111 Liu C, Ma XJ, Jiang X et al. Cloning and pharmacological characterisation of a fourth histamine receptor (H₄) expressed in bone marrow. *Mol Pharmacol* 2001; 59:420–6.
- 112 Ter Laak AM, Timmerman H, Leurs R, Nederkoorn PHJ, Smit MJ, Donné-Op den Kelder GM. Modelling and mutation studies on the histamine H₁ receptor agonist binding site reveal different binding modes for H₁-agonists: Asp¹¹⁶ (TM3) has a constitutive role in receptor stimulation. *J Computer Aided Mol Design* 1995; 9:319–30.
- 113 Leurs R, Smit MJ, Meeder R, Ter Laak AM, Timmerman H. Lysine²⁰⁰ located in the fifth transmembrane domain of the histamine H₁ – receptor interacts with histamine but not with all H₁-antagonists. *Biochem Biophys Res Commun* 1995; 214:110–7.
- 114 Zhang MQ, Van de Stolpe A, Zuiderveld OP, Timmerman H. Synthesis and pharmacological evaluation of some amino – acid – containing cyproheptadine derivatives as dual antagonists of histamine H₁ – leukotriene D₄ – receptors. *Eur J Med Chem* 1997; 32:95–102.
- 115 Alewijnse AE, Smit MJ, Hoffmann M, Verzijl D, Timmerman H, Leurs R. Constitutive activity and structural instability of the wild-type human H₂ receptor. *J Neurochem* 1998; 71: 799–807.
- 116 Smit MJ, Leurs R, Alewijnse AE et al. Inverse agonism of histamine H₂-antagonist accounts for upregulation of spontaneously active histamine H₂-receptors. *Proc Natl Acad Sci USA* 1996; 93:6802–7.