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Anticholinergic effects of desloratadine, the major metabolite of loratadine, in rabbit and guinea-pig iris smooth muscle

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Abstract

Allergic conjunctivitis is the most common ocular allergic disease. Although very symptomatic it does not endanger vision, and topical antihistamines or chromones are the first choice treatment in clinical practice. Recently, equivalent nanomolar affinities for histamine H_1 and muscarinic M_1 and M_3 cloned human receptors have been reported for desloratadine, the active metabolite of loratadine, a widely prescribed antihistamine. This property might enhance its utility in the treatment of asthma, but could induce adverse anticholinergic effects after topical administration. In the present study, we compare the anticholinergic activity of desloratadine with other known muscarinic antagonists and antihistamines on rabbit and guinea-pig iris smooth muscle. Desloratadine was found to be a competitive antagonist ($pA_2 = 6.67 \pm 0.09$) of carbachol-induced contractions in isolated rabbit iris smooth muscle. Atropine ($pA_2 = 9.44 \pm 0.02$) and NPC-14695 ($pA_2 = 9.18 \pm 0.03$) also behaved as competitive antagonists, whereas tiotropium bromide ($pD'_2 = 9.06 \pm 0.02$) exhibited a non-competitive behaviour in this tissue. Carebastine ($pA_2 = 5.64 \pm 0.04$) and fexofenadine ($pA_2 < 4.0$) were also studied. After topical administration on the guinea-pig eye conjunctiva, desloratadine produced a potent ($ED_{50} = 2.3$ mg/ml) and long lasting mydriasis (> 120 min at the ED_{50}) in conscious animals. Fexofenadine and carebastine were inactive even at the highest concentration tested (10 mg/ml). Atropine ($ED_{50} = 30$ μg/ml) and tiotropium bromide ($ED_{50} = 10$ μg/ml) were much more potent than desloratadine or pirenzepine ($ED_{50} = 3$ mg/ml) in this model. The competitive muscarinic antagonism of desloratadine in vitro, and its potency and duration of action in vivo, suggest that topical treatment of allergic conjunctivitis and rhinitis with desloratadine could produce undesirable peripheral anticholinergic side effects such as mydriasis and xerostomia.

Keywords: Loratadine; Desloratadine; Muscarinic receptor; Mydriasis; Smooth muscle, iris

1. Introduction

Allergic conjunctivitis is often treated with topical antihistamines rather than systemic antihistamines as a way to improve onset of action and safety (Davies et al., 1996). For this reason, active metabolites of several widely used antihistamines are currently being investigated as novel antihistamines and antiallergic drugs (Handley et al., 1998). Desloratadine, the major metabolite of loratadine (Yumibe et al., 1996), has shown not only antihistaminic activity (Clissold et al., 1989), but also some additional antiallergic properties in vitro (Weyer et al., 1992; Vignola et al., 1995; Genovese et al., 1997). Compared with loratadine, desloratadine shows a higher affinity for histamine H_1 human receptors, $IC_{50} = 721$ nM versus $IC_{50} = 51$ nM, (Handley et al., 1997). Furthermore, in vivo desloratadine exhibits 2–3 fold greater oral potency over loratadine in inhibiting the histamine-induced wheal and flare skin response in the guinea-pig, (Handley et al., 1997). However, in the same study it was shown that desloratadine also has nanomolar affinities for cloned human muscarinic M_1 and M_3 receptors ($IC_{50} = 48$ and 125 nM respectively), but not for muscarinic M_2 receptors; Loratadine itself did not exhibit affinity for muscarinic receptors (Handley et al., 1997). In addition, peripheral anticholinergic effects such as inhibition of pilocarpine-induced salivation, degranulation of submandibular acinar cells and mydriasis have been described in rodents (Cardelús et al., 1998).

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The predominant muscarinic receptor subtype in human and guinea-pig iris sphincter muscles has been pharmacologically characterized as the M_3 type by the use of selective antagonists (for review, see Eglen et al., 1996). Rabbit iris muscarinic receptors are less well defined, but prejunctional muscarinic M_2 receptors are present (Bognar et al., 1989) and postjunctional receptors appear to be similar to or identical with the muscarinic M_3 subtype (Choppin et al., 1998). The purpose of the present study was to compare the in vitro inhibitory effects of desloratadine to muscarinic M_3 receptor antagonists such as NPC-14695 (Kaiser et al., 1993; Howell et al., 1994) and tiotropium bromide (Maesen et al., 1995), on carbachol-induced contractions of isolated rabbit iris smooth muscle. Other third generation antihistamines were also studied for comparison. The in vivo anticholinergic activity of these compounds was also studied in conscious guinea-pigs after ocular topical administration in order to evaluate their mydriatic effect.

2. Materials and methods

2.1. Rabbit isolated iris muscle

Female New Zealand white rabbits (Biocentre, Spain) of weight range 3–4 kg, were maintained without food but with free access to water for 16 h before experimentation. The animals were killed by cranial percussion, the whole eye dissected free and placed into Tyrode solution at 20°C. The ocular sphere was cut, the lens was removed and the underlying iris muscle was carefully excised. Each iris was suspended in a 30 ml organ bath, containing Tyrode solution with indomethacin (3 μM), at 37°C and gassed with 5% CO_2 in O_2. Tension was recorded with isometric transducers connected onto a polygraph. After an equilibration period of 1 h, during which the resting tension was maintained at 500 mg, at least two control responses to carbachol (10^{-5} M) were obtained at 30 min intervals. After washing twice to restore basal tension, a carbachol

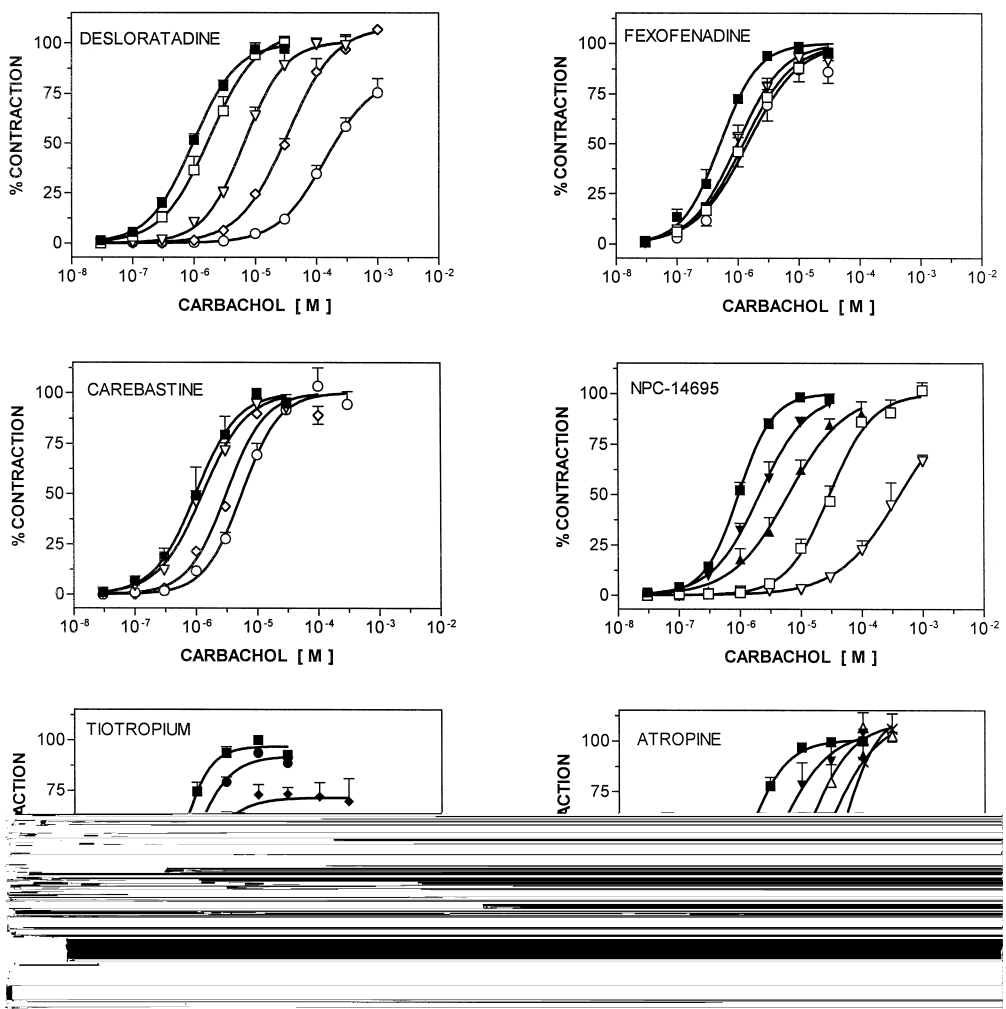

Fig. 1. Effects of desloratadine (□ 10^{-7}, ▽ 10^{-6}, ◇ 3×10^{-6} and ○ 10^{-5} M), fexofenadine (□ 10^{-7}, ▽ 10^{-6} and ○ 10^{-5} M), carebastine (▽ 10^{-6} and ◇ 3×10^{-6} and ○ 10^{-5} M), NPC-14695 (▼ 10^{-9}, ▲ 10^{-8}, □ 10^{-7}, ▽ 10^{-6} M), tiotropium bromide (● 10^{-11}, ◆ 10^{-10}, ▼ 10^{-9}, ▲ 10^{-8} M) and atropine (▼ 10^{-9}, △ 3×10^{-9}, ▲ 10^{-8} and X 3×10^{-8} M), on carbachol-induced contractions (■, vehicle), on isolated rabbit iris smooth muscle. Results are expressed as mean ± S.E.M., $n = 7–11$.

concentration–response curve (3×10^{-8} to 3×10^{-5} M) was constructed. After a washing period, a single concentration of each drug under study was added to the preparation and 30 min later a new concentration–response curve with carbachol was performed.

2.2. Guinea-pig iris relaxation

Groups of 10 male guinea-pigs were administered with increasing concentrations of each drug under study in a 20 μl drop volume and under constant and standardized illumination. The measurement of pupil diameter was adapted from Norohna-Blob and Kachur (1991). Briefly, by using a graduate scale (pupilometer), pupillary responses to drugs were measured to the nearest 0.01 mm at the point of greatest diameter. Responses were measured 5, 10, 20, 30, 60 and 120 min after drug administration. The area under the curve between time 0 and 120 min after drug administration for the stated drug and dose ($AUC_{0-120\ min}$) was calculated. ED_{50} values, defined as the dose that elicited 50% of $AUC_{0-120\ min}$ maximum dilation, were calculated from dose–response relationships by linear regression.

2.3. Materials

Desloratadine, fexofenadine, carebastine, tiotropium bromide and NPC-14695 were synthesized by the Department of Medicinal Chemistry, Almirall Prodesfarma. Indomethacin, carbachol and atropine sulphate were obtained from Sigma-Aldrich Química (Madrid, Spain). Pirenzepine was purchased from RBC (London, UK). For the in vitro study, carbachol (at 10^{-3} M stock solution) was dissolved in distilled water containing 0.5 mM ascorbic acid. Stock solutions of desloratadine, fexofenadine, carebastine and

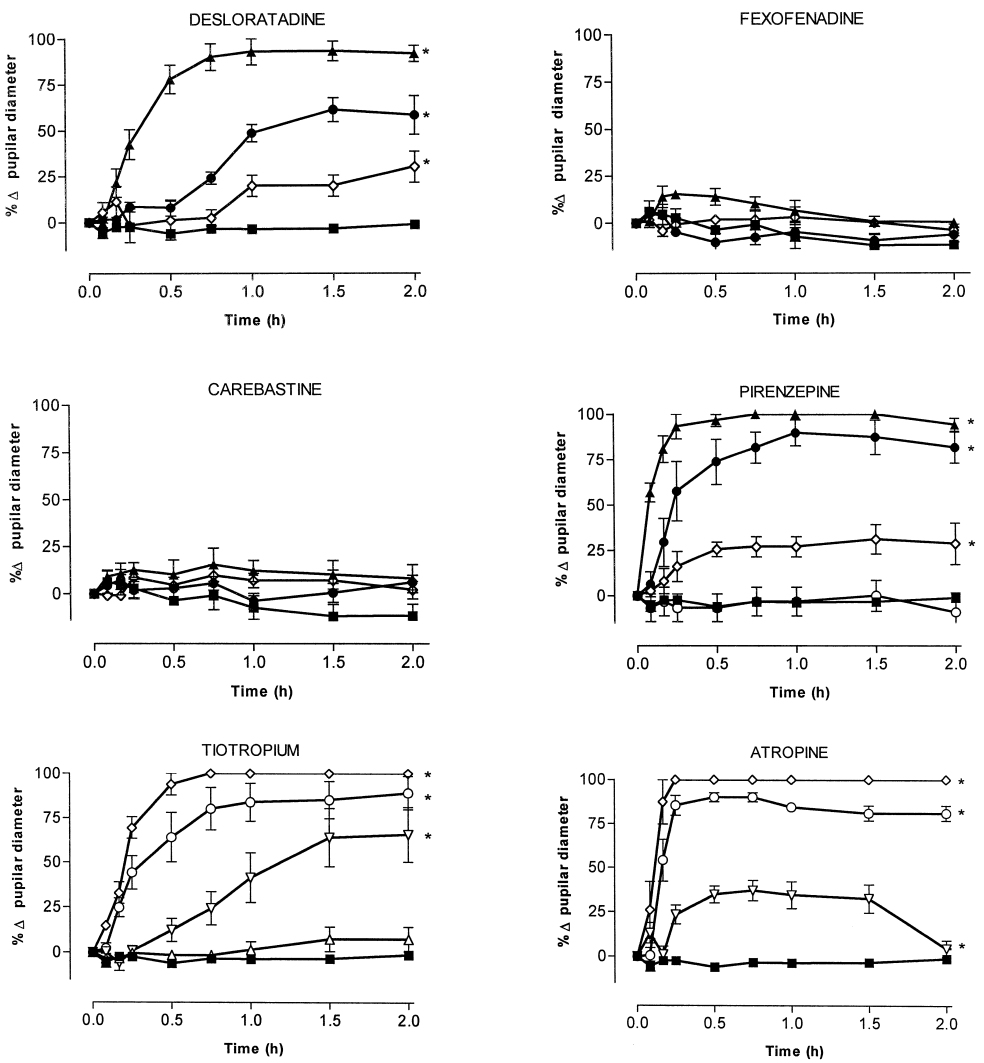

Fig. 2. Time course of pupil diameter increase (mydriasis) after ocular administration of desloratadine, fexofenadine, carebastine, pirenzepine, tiotropium bromide and atropine in the conscious guinea-pig. Vehicle (■) or 1 (△), 10 (▽), 100 (○), 1000 (◇), 3000 (●) and 10,000 (▲) μg/ml of the corresponding compound. Volume drops of 20 μl were administered into the low conjunctiva sac. Results are expressed as mean ± S.E.M., $n = 8$. * $p < 0.01$ Student's t-test considering the $AUC_{0-120\ min}$.

NPC-14695 were prepared in 20% polyethylene glycol-300 at 10^{-3} M. Atropine and indomethacin were dissolved in distilled water (at 10^{-2} M stock solution). Subsequent dilutions of drugs were made in Tyrode solution which had the following composition (in mM): NaCl 137.0, KCl 2.7, $CaCl_2$ 1.8, $MgCl \cdot 6H_20$ 1.0, KH_2PO_4 0.4, $NaHCO_3$ 11.9 and glucose 5.6. For the in vivo studies all drugs tested were prepared daily and dissolved in a innocuous vehicle (polyethylenglycol-300 solution). All experimental procedures described in this paper were previously notified to the regulatory authorities, and the guidelines approved by the Catalonia Parliament were strictly followed.

2.4. Data analysis

Values are given as mean ± S.E.M. Statistical analysis of results was carried out using Student's *t*-test for unpaired data. In vitro data was analysed according to Arunlakshana and Schild (1959). When the slopes of the Schild plots did not differ significantly from the unit, plots with their slopes constrained to unity were used to calculate the pA_2 values for the antagonists, (Tallarida and Jacobs, 1979). Analysis of the data was performed with SAS software (SAS, NC, USA).

3. Results

3.1. Isolated rabbit iris smooth muscle

Carbachol induced concentration-dependent contractions in this tissue ($EC_{50} = 9.96 \times 10^{-7}$ M; 95% confidence interval: 9.287×10^{-7} M to 1.069×10^{-6} M; $E_{max} = 289 \pm 13$ mg; $n = 77$). Fig. 1 shows the parallel rightward displacement of cumulative carbachol concentration–response curves by desloratadine in comparison with other antihistamines such as fexofenadine and carebastine on rabbit isolated iris smooth muscle. Additionally, atropine, NPC-14695 and tiotropium were also tested. The order of potency found for such drugs was as follows (pA_2): Atropine (9.44 ± 0.02) > NPC-14695 (9.18 ± 0.03) > desloratadine (6.67 ± 0.09), carebastine (5.64 ± 0.04) and fexofenadine (< 4.0). Tiotropium bromide behaved as a non-competitive antagonist with a pD'_2 value of 9.06 ± 0.02. None of the compounds tested affected the basal tension of the preparation (data not shown).

3.2. Mydriasis in conscious guinea-pig

Topical ocular application of increasing concentrations of desloratadine (1, 3 and 10 mg/ml) induced a dose-dependent and long lasting mydriasis in the guinea-pig in vivo (Fig. 2). Mydriasis became significant ($p < 0.05$, Student's *t*-test, $AUC_{0-20\ min}$ versus vehicle) 20 min after desloratadine application (1 mg/ml). The maximum effect was reached at 60 min and lasted with the same intensity for at least the next 60 min. Atropine ($ED_{50} = 30$ μg/ml), pirenzepine ($ED_{50} = 3$ mg/ml) and tiotropium ($ED_{50} = 10$ μg/ml), also induced a potent and long lasting mydriasis in this model. Conversely, neither fexofenadine nor carebastine, (1–10 mg/ml) showed any significant effect in this model.

4. Discussion

Acetylcholine regulates pupil diameter via activation of pre- and post-junctional receptors in most species. Human (Woldemussie et al., 1993; Gil et al., 1997) and guinea-pig (Bognar et al., 1990) iris sphincter muscles are known to contain predominantly functional muscarinic M_3 receptors that mediate contraction, and muscarinic M_2 receptors that mediate autoinhibition of acetylcholine release, (Fuder, 1994). Bognar et al. (1990) have shown that selective muscarinic M_2 receptor antagonists enhance the in vitro evoked acetylcholine release and thereby increase the iris sphincter tone. Thus, in contrast to muscarinic M_3 receptor antagonists, selective muscarinic M_2 receptor antagonists would be expected to reduce pupil size (miosis). Considering the low affinity (250–1000 nM) of desloratadine for human cloned muscarinic M_2 receptors reported by Handley et al. (1997), it seems to be unlikely that desloratadine could induce an increase of the iris tone. Based on the fact that the affinity values for the available antagonists were inconsistent with the blockade of muscarinic M_1–M_4 receptors, a novel type of muscarinic receptor in the rabbit iris was initially suggested (Bognar et al., 1992). More recently, with the aid of more selective antagonists, the muscarinic receptor that mediates contraction of the rabbit isolated iris muscle has been pharmacologically characterized as being also of the M_3 type (Choppin et al., 1998). However, since the available ligands have still a poor discriminative power between the muscarinic M_3 and M_5 receptors, the presence of M_5 receptors in the rabbit iris cannot be ruled out definitively. The identification of M_5 subtype receptors in the rabbit iris will be of particular interest, since the human iris is one of the few known localizations outside the central nervous system (CNS) where the M_5 receptor has been described (Gil et al., 1997; Ishizaka et al., 1998).

NPC-14695 has previously been shown to poses an in vitro muscarinic receptor antagonist selectivity rank of $M_3 > M_1 > M_2$, based on K_D values of 15, 25 and 60 nM in guinea pig ileum, rabbit vas deferens and guinea pig atria, respectively (Kaiser et al., 1993). In contrast, the apparent K_D-values of tiotropium bromide reported for the human muscarinic receptor subtypes are very similar: 0.33, 0.27, and 0.12 nM for M_3, M_1 and M_2 receptors respectively (Disse et al., 1993). However, tiotropium bromide has been considered a selective M_3 receptor antagonist due to its receptor subtype kinetic dissociation (Disse et al., 1999). In the present study, NPC-14695, tiotropium bro-

mide and pirenzepine behaved as potent muscarinic antagonists both in vitro and in vivo. Of the antihistamines tested, only desloratadine inhibited in a concentration-dependent fashion, the responses of isolated iris to carbachol, and produced a potent mydriasis in vivo.

The peak plasma concentration of desloratadine found in healthy volunteers after loratadine administration at the therapeutic dose is about 28 ng/ml (Haria et al., 1994). This low concentration explains the lack of anticholinergic side effects reported when loratadine, the parent compound, is administered orally. However, it is well known that ocular antihistamines achieve higher local concentrations (Hingorani, 1998). In fact, other potent antihistamines such as ketotifen, levocabastine or azelastine are currently given at a concentration range of 0.5–1 mg/ml. Taking into account the human eye surface and the drop volume (50 μl aprox.) used in clinical practice, and accepting the fact that the pharmacokinetic properties of compounds may influence their dilution factor, the concentrations of desloratadine used in the present study (1–10 mg/ml) could be considered of clinical relevance.

The lack of functional histamine receptors on rabbit iris smooth muscle (Yoshitomi et al., 1995) does not allow a direct comparison of desloratadine as an antagonist of histamine and carbachol-induced contractions in the same tissue. The potency of desloratadine as an antagonist of histamine-induced contractions on guinea-pig isolated ileum is around 100-fold higher than that obtained in the present study as an antagonist of the carbachol-induced contractions of rabbit iris sphincter. However, the in vitro antiallergic properties reported for desloratadine (Weyer et al., 1992; Vignola et al., 1995; Genovese et al., 1997) have only been shown at concentrations equivalent to or higher than those affecting the muscarinic receptor system, as reported in the present paper.

Desloratadine is known to have very low CNS penetration in humans (Clissold et al., 1989). This property could explain the absence of any central anti-muscarinic side effects reported with this drug, as well as the absence of CNS anticholinergic activity in rodents after systemic treatment with desloratadine (Cardelús et al., 1998).

In conclusion, taking into account the reported similar affinity of desloratadine for human histamine and muscarinic cloned receptors, and the functional study reported here, dry mouth (xerostomia) and blurred vision (mydriasis) could be two adverse effects of nasal and ocular desloratadine formulations respectively, due to its antagonism of muscarinic M_1 and M_3 receptors.

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