Bulletin of Okayama Prefectural Junior College Vol.36 60~66 (1991)

Antiallergic effects of terfenadine and its isomers

Reiko Akagi and Masaaki Akagi*

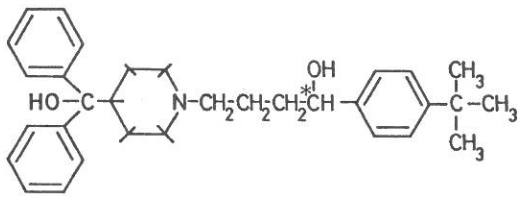
Abstract

d, dl, l-Terfenadine dose-dependently inhibited histamine release induced by compound 48/80 from rat peritoneal mast cells. IC_{50} values were 7.1,7.6 and 7.1 μM, respectively. No significant difference between three optical isomers was observed. Three optical isomers also inhibited A23187-induced histamine release, and the profile of inhibitory effects were similar to that of 48/80-induced release. Three optical isomers elicited the similar antagonistic effects to histamine, LTD_4 and PAF on isolated guinea pig ileum. These isomers also inhibited the increase of airway resistance in a dose-dependent fashion, and ID_{50} values were 1.9,2.2 and 2.8 mg/kg, respectively.

Introduction

Terfenadine, α-[4-(1,1-dimethylethyl) phenyl]-4-(hydroxydiphenylmethyl)-1-piperidine-butanol, is a selective histamine H_1-receptor antagonist. Unlike common H_1-receptor antagonists, it is a safe compound with no sedative, anticholinergic, antiserotonergic or antiadrenergic effects.[1)2)] It has been reported that terfenadine inhibits rat homologous passive cutaneous anaphylaxis (PCA) , and experimentally-induced asthma in guinea pigs, dose-dependently.[3)] In ex vivo, terfenadine also inhibits the antigen-induced release of both histamine and SRS-A from sensitized guinea pig lungs.[3)]

It has been known that terfenadine contains the assymmetric carbon in the chemical structure (Fig.1) , and that d (+)-and l (−)-terfenadine have similar antihistaminic potency and profile of action as rasemic isomer.[4)] The present experiments were performed to study if the optical isomers of terenadine exhibit antiallergic activities, such as histamine release inhibition, antagonistic activities to leukotriene (LT) D_4- and PAF-induced contraction of guinea pig ileum, and prevention of experimentally-induced asthma in guinea pigs.



Terfenadine

Fig. 1: Structure of terfenadine. * indicates an assymmetric carbon in chemical structure.

Methods

Histamine release from isolated rat peritoneal mast cells

Rat peritoneal mast cells were collected and purified to a level higher than 95% by means of Percoll density gradient centrifugation.[5)] The viable cells were counted using the dye (toluidine blue) exclusion test. Having prewarmed 1.6 ml of mast cell suspension (10^4 cells/ml) at 37℃ for 5 min, 0.2 ml of each test compound suspended in 0.25% sodium carboxymethylcellulose (CMC-Na) were added and the incubation was continued for 15 min. Thereafter,0.2 ml of compound 48/80 (48/80) or A23187 was

*Tokushima Bunri University

added to make a final concentration of 0.5 μg/ml or 0.5 μM, respectively, and the incubation was continued for another 10 min. The histamine releasing process was stopped by chilling the test tube in an ice bath, afterwhich the histamine released in supernatant and the residual histamine were determined by means of fluorometric assay.[6] Histamine release was expressed as the percentage of total histamine in mast cells.

Antagonistic effects against histamine, LTD_4 and PAF in isolated guinea pig ileum

Male Hartley strain guinea pigs, weighing about 400g, were exsanguinated. Ileal strips, 2 cm in length, were removed, tied at one end, connected the other end to a force displacement transducer and mounted in an organ bath containing 10 ml of Tyrode's solution. Tyrode's solution aerated with 95% O_2-5% CO_2 at 32℃ had the following composition (mM) : NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.1; NaH_2PO_4 0.42; $NaHCO_3$ 11.9; glucose 5.6. The ileal strips were then allowed to equilibrate for half an hour before starting the experiment.

Cumulative dose-response curves for histamine or LTD_4 were obtained according to the method of Van Rossum[7] by cumulatively adding geometrically equal spaced doses (0.5 log unit) of agonist to the organ bath until the contractile response of the tissue reached a maximum or began to decline. Contractile response were expressed as the percentage of the maximal contraction attainable. An initial challenge dose-response curve experiment was followed by a second one which was then considered as the control curve. Following washout of the agonist, the ileal strips were then incubated with terfenadine for 30 seconds and in the same manner, the cumulative dose-response curves for agonists were obtained. Each muscle strips was treated with progressively increasing concentration of terfenadine. Dose-response curves were constructed and pA_2 values calculated.[8]

Terfenadine (10 mM) was dissolved the equivalent mixture of 1% lactic acid and 0.1NHCl, and pH was adjusted to 7.0 with 0.1NNaOH.

Experimentally-induced asthma in actively sensitized guinea pigs

Male Hartley strain guinea pigs, weighing 400-450g, were sensitized according to the method of Mota et al.[9] Ovalbumin was used as an antigen. Two weeks later, the animals were anesthetized by i.p. injection of pentobarbital sodium (30 mg/kg) and a cannula was inserted into the trachea. The animals were then paralyzed by intravenous injection of gallamine triethiodide (1 mg/kg) and were artificially ventilated by means of a respiratory pump (Narishige, TW-65AS) connected to the tracheal cannula (stroke volume 10 ml, 72 strokes/min) . The airway resistance was measured according to the method of Konzett-Rössler[10] and the impedance method.[11] Terfenadine was suspended in 0.25% sodium carboxymethylcellulose, and given p.o. 1 h before antigen challenge.

Results

Effects on histamine release from rat peritoneal mast cells

When rat peritoneal mast cells were exposed to 48/80 (0.5 μg/ml) or A23187 (0.5 μM) , histamine was released at 23.6± 0.9 and 73.4 ± 2.5% of the total histamine content, respectively, As shown in Fig. 2, the pretreatment with d (+)-, l (−)-or dl (±)-terfenadine remarkably inhibited histamine release induced by these compounds in a dose-dependent fashion. The profile of inhibitory effects by dl (±) -terfenadine was similar to the previous report.[3] d-and l-terfenadine also inhibited histamine release induced by 48/80 and A23187. No significant difference in the potency of inhibitory effects between three optical isomers was observed (Fig. 2) . IC_{50} values of inhibitory effects by three optical isomers were summarized in Table 1.

Antagonistic effects against histamine, LTD_4 or PAF

Dose-response curves of histamine and LTD_4 in the absence of antagonists were showed in Fig. 3.

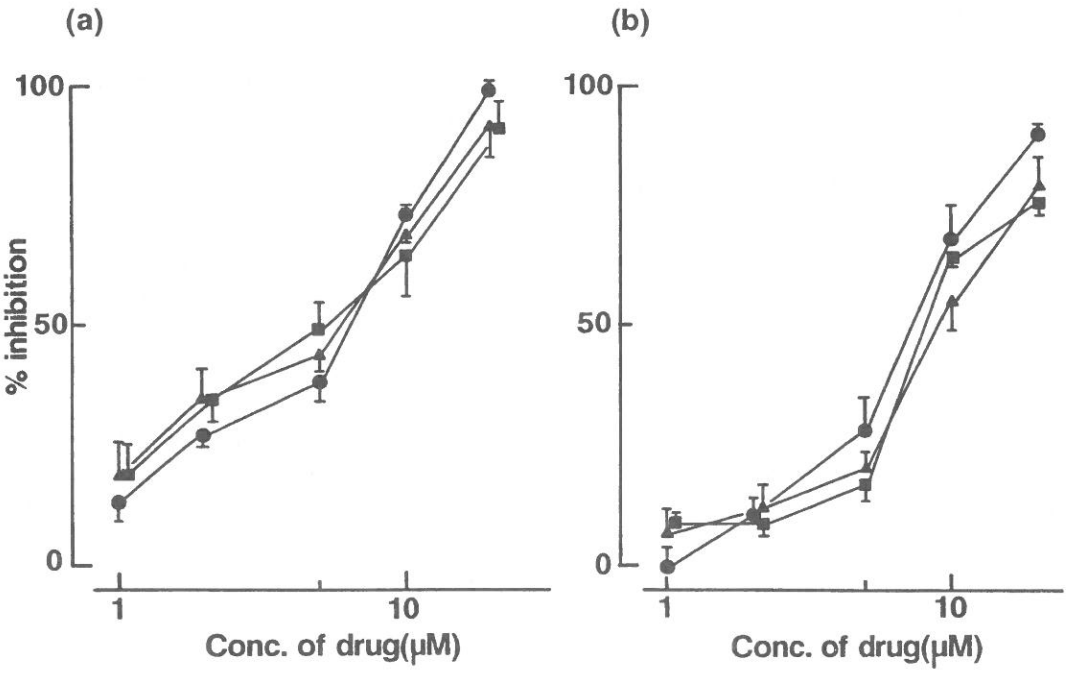


Fig. 2: Inhibitory effects of dl (●) -, d (▲) -and l (■) -terfenadine on histamine release induced by compound 48/80 (0.5 μg/ml) (a) and A23187 (0.5 μM) (b) from rat peritoneal mast cells. Each value indicates means ±S.E. of % inhibition of histamine release from 5 experiments.

Drugs	IC_{50} (μM)	
	48/80	A 23187
d-Terfenadine	7.1	11.5
l-Terfenadine	7.1	11.4
dl-Terfenadine	7.6	9.8

In each experiments, at least n = 3 were performed.

Table 1. Kinetic Data of the Inhibitory Effects of Terfenadine and Its Optical Isomers on Histamine Release from Rat Peritoneal Mast Calls Induced by Compound 48/80(0.5μg/ml) or A 23187(0.5 μM).

The pD_2 values of both compounds calculated from the dose-response curves were 7.63 and 7.53, respectively. In the presence of dl-terfenadine, the dose-response curves of histamine and LTD_4 were shifted to the right demonstrating a progressively increasing antagonistic activities with increasing concentrations of terfenadine. In the presence of d- or l-terfenadine, the dose-response curves of histamine and LTD_4 were also shifted to the right, and in the profiles of antagonistic activities, a little or no difference between three optical isomers was observed (Fig. 4,5) . pA_2 values of the antagonistic effects on histamine- or LTD_4 -induced contraction in guinea pig ileum was summarized in Table 2.

Guinea pig ileum contracted slowly in the presence of 50 nM PAF and reached a maximum response about 5 min later. In the presence of d-,l- or dl-terfenadine, the contractions induced by PAF were inhibited in a concentration-dependent fashion (Fig.6) . The IC_{50} values of antagonistic effects were

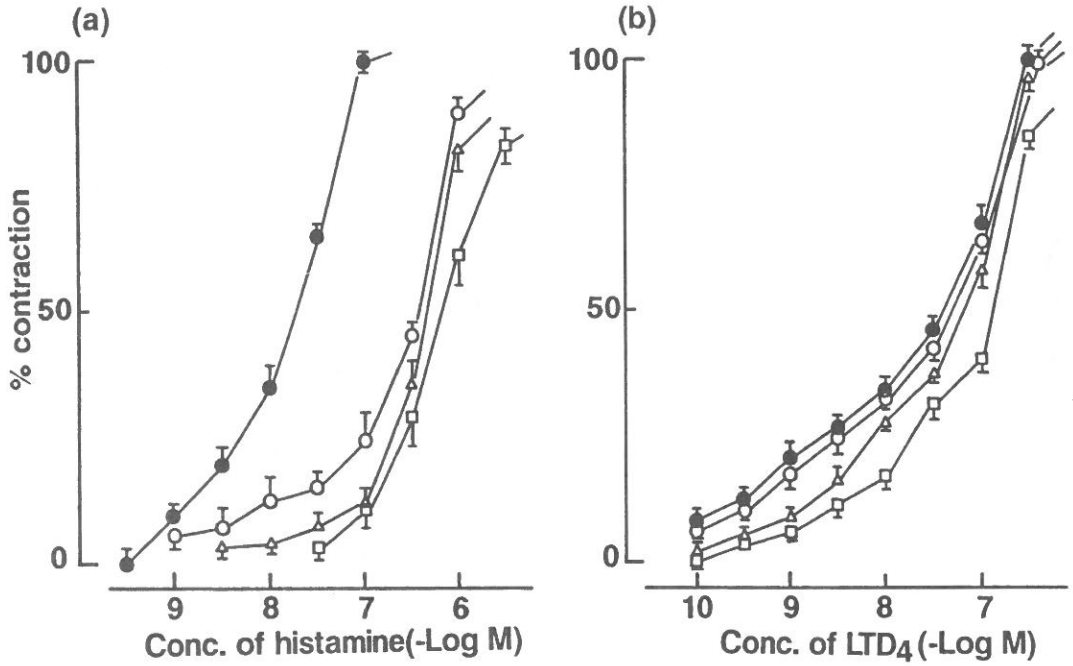


Fig. 3: Inhibitory effects of dl-terfenadine on histamine (a) -and LTD_4 (b) -induced contraction of isolated guinea pig ileum. ● shows dose-response curves of histamine and LTD_4 in the absence of dl-terfenadine. The pD_2 values were 7.63 and 7.53, respectively. In the presence of dl-terfenadine, the dose-response curves are shifted to the right with increasing concentrations of terfenadine. (○) 10 nM, (△) 100 nM, (□) 1000 nM. Each value indicates means ± S.E. of % contraction from 5 experiments.

Drugs	pA_2	
	Histamine	LTD_4
d-Terfenadine	7.55	7.00
l-Terfenadine	7.62	6.88
dl-Terfenadine	7.59	6.81

In each experiments, at least n = 3 were performed.

Table 2. kinetic Data of the Antagonistic Effects of Terfenadine and Its Optical Isomere on Histamine- and Leukotriene(LT)D_4-Induced Coutractions in Guinea-Pig Ileum

0.69, 0.88 and 0.75 μM, respectively, and the significant difference between three optical isomers was not observed.

Effects on experimentally-induced asthma in actively sensitized guinea pigs

The intravenous injection of ovalbumin (0.3 mg/kg) to actively sensitized guinea pigs elicited the remarkable increase of airway resistance. The pretreatment of dl-, d- or l-terfenadine 1 h before antigen challenge inhibited the increase of airway resistance in a dose-dependent fashion (Fig. 7). The ID_{50} values were 2.2, 1.9 and 2.8 mg/kg, respectively, and the difference in the three values could not be regarded as significant.

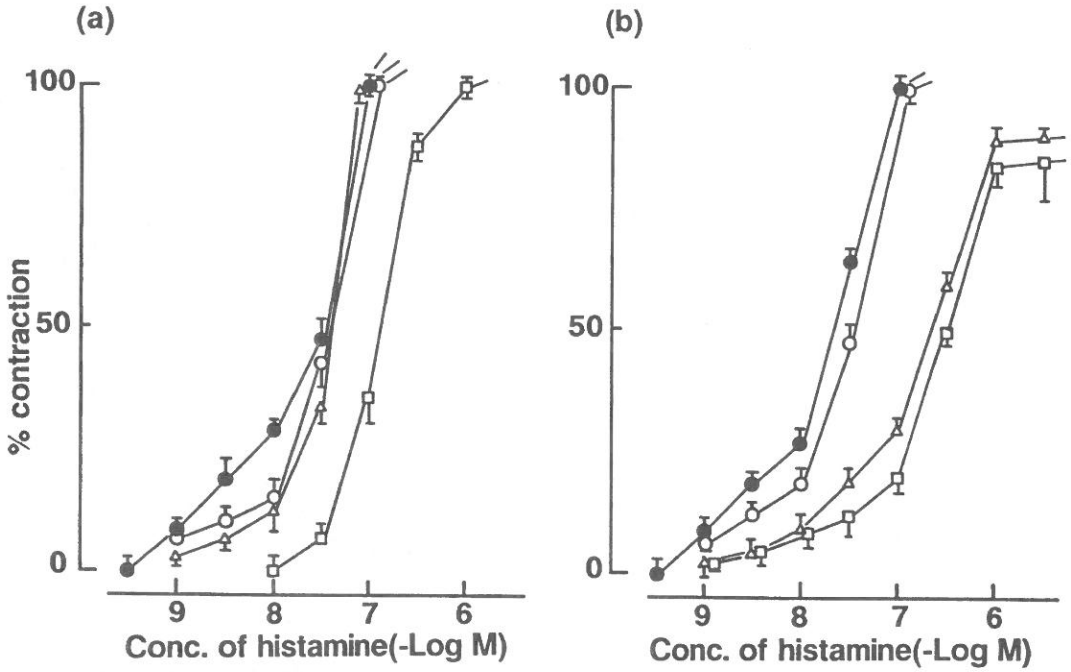


Fig. 4: Inhibitory effects of d (a) - and l (b) -terfenadine on histamine-induced contraction of isolated guinea pig ileum. ● shows dose-response curves in the absence of terfenadine. In the presence of terfenadine, the dose-response curves are shifted to the right with increasing concentrations of terfenadine. (○) 10 nM, (△) 100 nM, (□) 1000 nM. Each value indicates means ± S.E. of % contraction from 5 experiments.

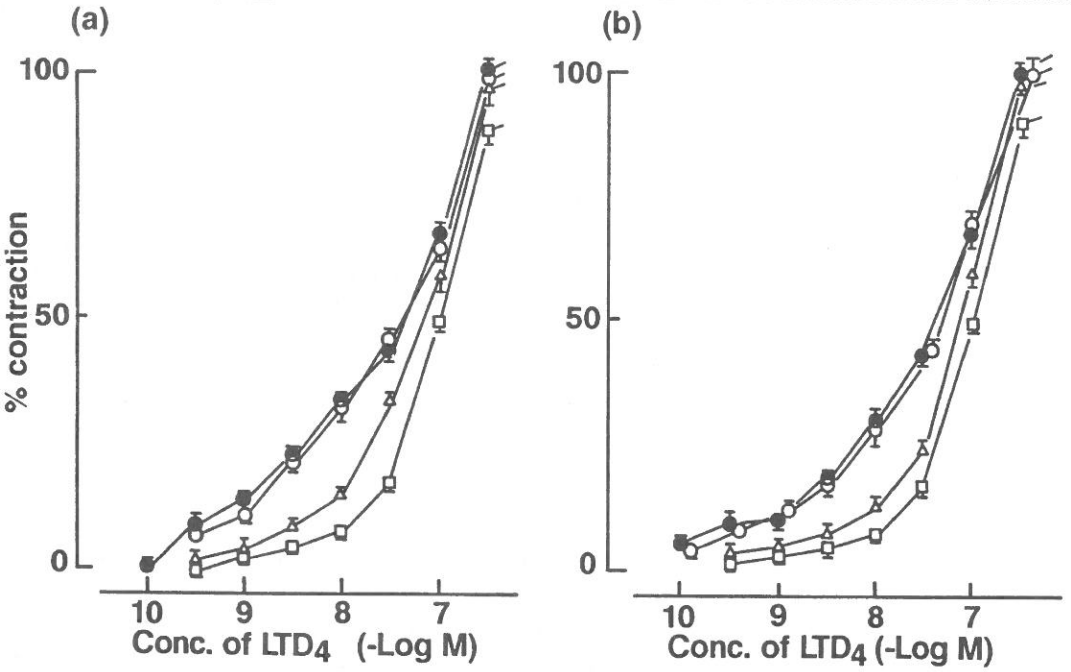


Fig. 5: Inhibitory effects of d (a) - and l (b) -terfenadine on LTD_4-induced contraction of isolated guinea pig ileum. ● shows dose-response curves in the absence of terfenadine. In the presence of tefenadine, the dose-response curves are shifted to the right with increasing concentrations of terfenadine. (○) 10 nM, (△) 100 nM, (□) 1000 nM. Each value indicates means ± S.E. of % contraction from 5 experiments.

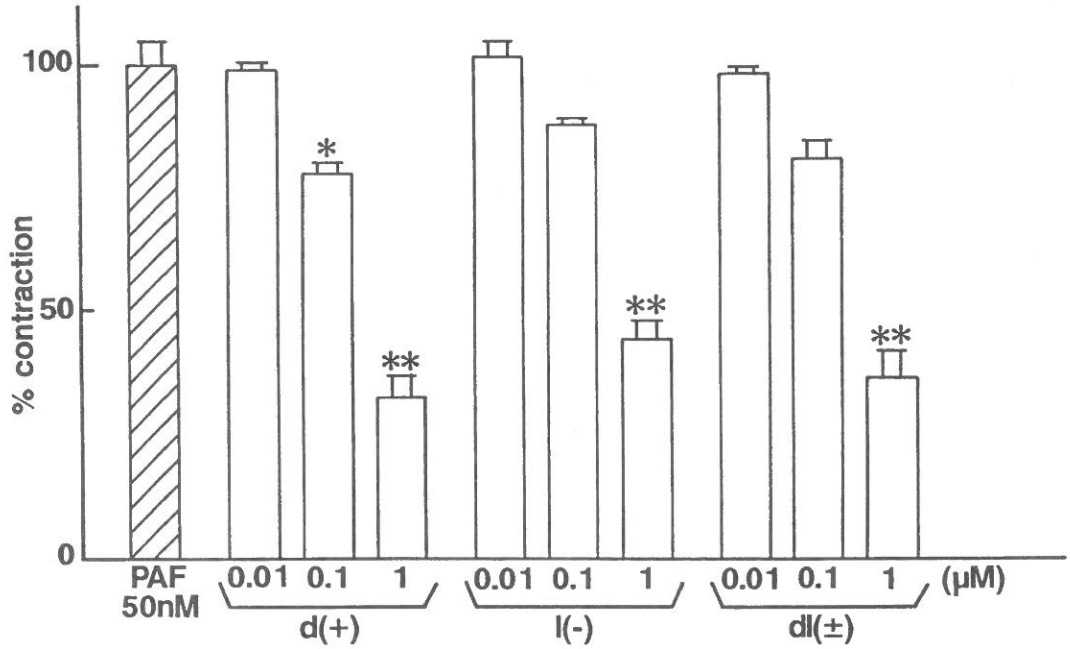


Fig. 6: Inhibitory effects of three optical isomers of terfenadine on PAF-induced contraction of isolated guinea pig ileum. Hatched column shows PAF-induced contraction in the absence of antagonist. In the presence of d-, l- and dl-terfenadine, the contractions were inhibited in a concentration-dependent fashion. IC_{50}values were 0.69, 0.88 and 0.75 μM, respectively. Each value indicates means ± S.E. of % contraction from 5 experiments. * $P < 0.05$, ** $P < 0.01$.

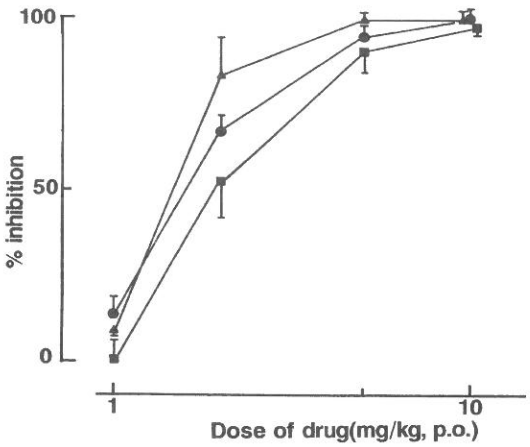


Fig. 7: Inhibitory effects of dl (●) -, d (▲) - and l (■) -terfenadine on the increase of airway resistance in actively sensitized guinea pigs. ID_{50} values were 2.2, 1.9 and 2.8 mg/kg, respectively. Each value indicates means ± S.E. of % inhibition from 3 experiments.

Discussion

Some histamine H_1-antagonists contain the assymmetric carbon in the those chemical structures and show stereoselective activity. The d-form of chlorpheniramine is much more potent than its l-enantiomer. d-Chlorpheniramine has also been shown to be some 200 times more effective than its enantiomer in vivo in protecting guinea pigs against histamine.[12] Similarly, d-chlorpheniramine is 240 times more potent as an inhibition of [^{3}H]-mepyramine binding than l-chlorpheniramine.[13]

Terfenadine also contain the assymmetric carbon in the molecule. In the present study, a comparison of the activities of antipodes was done in the preventive effects on histamine release from rat peritoneal mast cells, the antagonistic activities to histamine-, LTD_4-or PAF-induced contraction of guinea pig ileum, and the inhibitory effect on experimentally-induced asthma in actively sensitized guinea pigs. As shown in Fig. 2-7, both optical isomers of terfenadine had almost similar potency and profile of action as the racemic isomer, unlike chlorpheniramine.

The results of chlorpheniramine shown that the drug activities are very sensitive to the precise stereochemistry clearly indicate that specific molecular interactions must occur between drug and receptor. Therefore, the above results of terfenadine indicate that the portion of assymmetric center may be of minor importance for stereoselectivity. In fact, it has been reported that as in promethazine and clemastine, an assymmetric center close to the sidechain nitrogen is of minor importance for sidechain of minor importance for stereoselectivity.[14]

References

1) Cheng, H.C. and Woodward, J.K.: A kinetic study of the antihistaminic effect of terfenadine. Arzneim.-Forsch., 32, 1160-1166, (1982)
2) Weiner, M.:Sedation and antihistaminics. Arzneim.-Forsch., 32,1193-1195, (1982)
3) Akagi, M., Mio, M., Miyoshi, K. and Tasaka, K.:Antiallergic effects of terfenadine on immediate type hypersensitivity reactions. Immunopharmacol. Immunotoxicol., 9,257-279, (1987)
4) Project Report P-75-05:Kinetic study of the antihistaminic activity of terfenadine and chlorpheniramine on the isolate guinea pig ileum. 247-261.
5) Nemesh, A. and Röhlich, P.:Rapid separation of rat mast cells with Percoll. Eur.J. Cell Biol., 20, 272-275, (1980)
6) Shore, P.A.:Fluorometric assay histamine. in Methods in Enzymology, Vol. 17/b, edited by H. Tabor and C.W. Tabor, P.842-845, Academic Press, New York, (1971)
7) Van Rossum, J.M.: Cumulative dose-response curves, II. Techniques for the making of dose-response curves in isolated organs and the evaluation of drug parameters. Arch. Int. Pharmacodyn., 142, 299-330, (1963)
8) Arunlakshana, O. and Schild, H.O.:Some quantitative uses of drug antagonists. Br.J. Pharmacol., 14, 48-58, (1959)
9) Mota, I. and Da Silva, W.D.:Antigen-induced damage to isolated sensitized mast cells. Nature, 186, 245-246, (1960)
10) Konzett, H. and Rössler, R.: Versuchsanordnung zu intersuchunge an der bronchial-muskulatur. Arch. Exp. Path. Pharmakol., 195, 71-74, (1940)
11) Akagi, M. and Tasaka, K.:Analysis of bronchomotor tone in anesthetized guinea pigs by impedance plethysmography: A simple method for the evaluation of bronchodilator action. Meth. and Find. Exptl. Clin. Pharmacol., 10, 143-150, (1988)
12) Roth, F.E. and Govier, W.M.: Comparative pharmacology of chlorpheniramine (chlor-trimeton) and its optical isomers. J. Pharmacol. exp. Ther., 124, 347-349, (1958)
13) Hill, S.J., Emson, P.C. and Young, J.M.: The binding of [^{3}H] mepyramin to histamine H_1 receptors in guinea-pig brain. J. Neurochem., 31, 997-1004, (1978)
14) Witiak, D.T.: Antiallergic agents in medicinal chemistry. in Medicinal Chemistry, 3rd edition, edited by A. Burgen, Wiley-Interscience, New York, (1970)

Recceived September,11,1991
Accepted September,17,1991