

Inhibition of IgE- and non-IgE-mediated histamine release from human basophil leukocytes in vitro by a histamine H₁-antagonist, desethoxycarbonyl-loratadine

Jörg Kleine-Tebbe, MD,^a Christa Josties,^a Gabriel Frank,^a
Dirk Stalleicken, MD,^b Armin Buschauer, PhD,^c Walter Schunack, MD, PhD,^c
Gert Kunkel, MD,^a and Beate Czarnetzki, MD^a
Berlin and München, Germany

Loratadine, a new nonsedating histamine H₁-antagonist, has been shown to inhibit immunologic release of inflammatory mediators in addition to its H₁-receptor blocking properties. After oral administration, the agent is metabolized primarily to desethoxycarbonyl-loratadine (DCL). The basic piperidine, DCL, is readily soluble in water, whereas the nonbasic urethane, loratadine, is insufficiently soluble in water for some in vitro investigations. Therefore we used the metabolite, DCL, to study its influence on in vitro leukocyte histamine release (LHR) in 24 allergic and 22 nonallergic subjects. IgE-mediated and calcium ionophore A23187-induced LHR were inhibited by DCL in a dose-dependent fashion (values of drug concentration to induce 30% inhibition after stimulation with inhalant antigen, anti-IgE, concanavalin A, and calcium ionophore A23187 were 6, 8, 5, and 11 µmol/L, respectively). Higher concentrations of DCL caused mediator release in all subjects (n = 45, 30 µmol/L DC: 11% ± 2% LHR, 100 µmol/L DCL: 35% ± 1% LHR), abolishing any inhibitory effect of the drug. Rapid onset of inhibition by 10 µmol/L DCL was found in kinetic studies (n = 10). The inhibition of anti-IgE-induced histamine secretion was synergistically increased by simultaneous preincubation of DCL with the potent histamine H₂-agonist, FRA-19. Additional data indicate that the inhibition of LHR by DCL might involve biochemical events that occur after cellular Ca⁺⁺ influx because LHR induced by N-formyl-methionyl-leucyl-phenylalanine or the phorbol ester, 12-O-tetradecanoyl phorbol-12-acetate, was not significantly affected by DCL. (J ALLERGY CLIN IMMUNOL 1994;93:494-500.)

Key words: Antihistamines, basophils, histamine release, loratadine

Loratadine, a recently introduced nonsedating histamine H₁-receptor antagonist for use in the symptomatic therapy of allergic disorders such as allergic rhinoconjunctivitis^{1, 2} and urticaria,^{3, 4} has been shown to inhibit immunologic and nonim-

munologic release of inflammatory mediators in vitro and in vivo.⁵⁻⁸ In addition to its histamine H₁-receptor blocking capabilities, antiallergic properties of loratadine occurred in rat peritoneal mast cells and in a murine mast cell line stimulated by concanavalin A (Con A) and calcium ionophore A23187 (A23187).⁵ Inhibition of allergic bronchospasm and anaphylaxis in guinea pigs and rats by loratadine was observed in the same studies.⁵ It has also been shown that the enhanced uptake of ⁴⁵Ca⁺⁺ by MC 9 cells stimulated with Con A was significantly inhibited by loratadine (drug concentration to induce 50% inhibition [IC₅₀] = 3 µmol/L) at concentrations similar to those that inhibited histamine and leukotriene C₄ release from these cells. Inhibition of Ca⁺⁺ uptake was recently confirmed by other groups in IgE-sensitized rat basophilic leukemia cells

From ^aClinics of Dermatology, Clinical Immunology and Asthma, University Hospital Rudolf Virchow, Freie Universität Berlin; ^bDepartment of Pharmaceutical Research, Essex Pharma, München; and ^cInstitute of Pharmacy, Freie Universität Berlin.

Received for publication Feb. 2, 1993; revised June 15, 1993; accepted for publication June 25, 1993.

Reprint requests: Jörg Kleine-Tebbe, MD, The Johns Hopkins Asthma and Allergy Center, Allergy and Clinical Immunology Division, Unit Office 2, 5501 Hopkins Bayview Circle, Baltimore, MD 21224-6801.

Copyright © 1994 by Mosby-Year Book, Inc.
0091-6749/94 \$3.00 + 0 1/1/49761

Abbreviations used

A23187:	Calcium ionophore A23187
Ag:	Timothy grass pollen allergen extract
A-IgE:	Anti-IgE antiserum
Con A:	Concanavalin A
DCL:	Desethoxycarbonyl-loratadine
FMLP:	<i>N</i> -formyl-methionyl-leucyl-phenylalanine
FRA-19:	Histamine H ₂ agonist: N ¹ -[3,3-bis(4-fluorophenyl) propyl]-N ² -3-[(1H imidazole-4-yl)propyl]guanidine-trihydrochloride
IC ₃₀ /IC ₅₀ :	Drug concentration required to induce 30%/50% inhibition
LHR:	Leukocyte histamine release
PAG:	PIPES-albumin-glucose buffer
PAG-CM:	PAG-buffer supplemented with Ca ⁺⁺ and Mg ⁺⁺
PIPES:	Piperazine-N,N'-bis(2-ethane sulfonic acid)
TPA:	12-O-tetradecanoyl phorbol-12-acetate

(RBL-2H3)⁶ and on platelet activating factor-induced Ca⁺⁺ increase in rat adherent peritoneal macrophages.⁷ Additional effects of loratadine on the release of leukotrienes and histamine from human lung fragments have been studied with A23187 and *Dermatophagoides pteronyssinus* antigens.⁸

After oral intake, loratadine is metabolized primarily to the water-soluble compound, desethoxycarbonyl-loratadine (DCL),⁹ reported to be four times more active in binding to the histamine H₁-receptor than the parent drug.¹⁰ Because of the very poor solubility of the urethane, loratadine, in water, which hampers in vitro studies, we investigated the potential inhibitory effects of its metabolite, DCL, on human leukocyte histamine release (LHR) in vitro.

METHODS

The following materials were purchased: piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) (Serva, Feinbiochemica GmbH & Co., Heidelberg, Germany), human serum albumin (Red Cross Blood Donation Service, Niedersachsen, Springe, Germany), 6% wt/vol dextran in physiologic saline solution (Knoll AG, Ludwigshafen, Germany), 50% wt/vol glucose (Braun Melsungen AG, Melsungen, Germany), anti-IgE antiserum (A-IgE) (Behringwerke AG, Marburg, Germany), timothy grass pollen allergen extract SQ aquagen (Ag) (ALK Laboratories, Hørsholm, Denmark), Con A (Sigma Chemie GmbH, Deisenhofen, Germany), *N*-formyl-methio-

nyl-leucyl-phenylalanine (FMLP) (Sigma, Germany), A23187 (Sigma, Germany), and 12-O-tetradecanoyl phorbol-12-acetate (TPA) (Sigma, Germany).

PIPES-albumin-glucose (PAG)-buffer contained 25 mmol/L PIPES, 110 mmol/L NaCl, 5 mmol/L KCl, 0.003% wt/vol human serum albumin, and 0.1% wt/vol glucose. PAG supplemented with 1 mmol/L CaCl₂ and 1 mmol/L MgCl₂ (PAG-CM) was used during the release experiments. Dilutions of all pharmacologically active reagents were prepared fresh every day in PAG-CM.

Several attempts were made to dissolve loratadine in a water-based buffer: stock solutions of 10 or 100 mmol/L loratadine in 100% wt/vol dimethylsulfoxide, methanol, or ethanol before dilution in PAG; dissolving after decreasing pH with 0.1 N HCl; dilution in a 20% wt/vol human serum albumin-containing buffer. All attempts to dissolve loratadine in water failed. Therefore the experiments were performed with the main metabolite, desethoxycarbonyl-loratadine (DCL). The histamine H₂-agonist, FRA-19, was synthesized as described recently.¹¹ Its relative activity on the histamine H₂-receptor of the guinea pig atrium is approximately 50 times higher than the activity of histamine.¹¹ Both compounds were dissolved and diluted in PAG-CM.

Cell isolation

Venous blood was collected from normal and grass pollen-allergic volunteers who had given informed consent. Grass pollen allergy was defined by a positive skin prick test result (allergen-induced wheal > positive control: wheal induced by 1 mg/ml histamine × 2 HCl) with a concordant case history to one or more common inhalant allergens, including timothy grass. Mixed leukocyte preparations were obtained by means of dextran sedimentation, as described elsewhere.¹² Briefly, 20 ml of blood was mixed with a solution containing 5 ml 6% wt/vol dextran, 2 ml 0.1 mmol/L ethylenediaminetetraacetic acid and 0.8 ml 50% wt/vol glucose. After sedimentation of red cells (90 to 120 minutes), the basophil-containing supernatant was carefully removed and centrifuged for 8 minutes at 400 g at room temperature. The cell pellet was washed twice with PAG by centrifugation (8 minutes, 400 g, 4° C). Finally, the cells were resuspended in PAG-CM to a volume that was required for the subsequent experiments.

Challenge

The cells were preincubated with either the drug or PAG-CM as control and then challenged with the appropriate stimulus for 40 minutes at 37° C. The release process was stopped by centrifugation (800 g, 15 minutes, 4° C), and supernatants were removed for histamine analysis. Total histamine content was determined by lysing aliquots of the cells with 2% wt/vol HClO₄. Cells incubated in PAG-CM alone served as an index of spontaneous histamine release (<5% of the total histamine content). Histamine release was expressed as percentage of the total histamine content

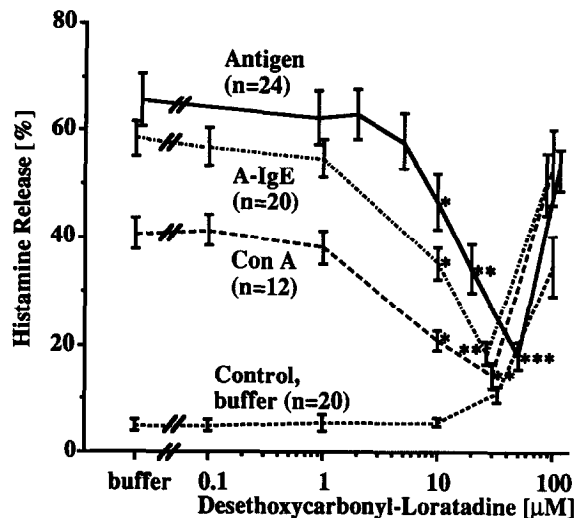


FIG. 1. Influence of preincubation with DCL on IgE-dependent histamine release (means $\pm$ SD) from human peripheral blood leukocytes. Mediator release was triggered by 100 SQ/ml Ag, 100 IU/ml A-IgE, or 10 μ mol/L Con A. Asterisks denote a significant difference (* p < 0.05, ** p < 0.01, *** p < 0.001) from control release, represented by samples preincubated with buffer instead of the drug before stimulation. In addition, the effect of DCL on spontaneous release of histamine was determined (control, buffer). Maximum inhibition and concentrations for 30% inhibition (IC_{30}) are listed in Table I.

after subtracting the spontaneous release. All experiments were performed in duplicate. When control LHR (without inhibition) reached greater than or equal to 30% of the total histamine content, maximal percent inhibition (means $\pm$ SEM) and the dose of DCL causing 30% inhibition (IC_{30}) (geometric means and standard errors of the mean after log transformation) were calculated.

Histamine assay

Histamine release was measured by means of an automated fluorometric method.¹²

Experimental protocol

First, cells of nonallergic donors were preincubated for 10 minutes with DCL at different concentrations (0.1, 1, 10, 30, and 100 μ mol/L) before 40-minute stimulation with 100 IU/ml A-IgE, 10 μ g/ml Con A, 1 μ mol/L FMLP, 10 ng/ml TPA, or 100 ng/ml A23187 in parallel to investigate dose-related inhibitory effects. Second, cells of allergic donors were stimulated for 40 minutes with 100 SQ/ml Ag after 10-minute preincubation with DCL (1, 2, 5, 10, 20, 50, and 100 μ mol/L). Third, for time course experiments, the time of preincubation with 30 μ mol/L DCL before stimulation with 100 IU/ml A-IgE varied: 0, 2, 5, 10, 20, or 40 minutes. Fourth, cells were preincubated with DCL (0.1, 1, 10, 30 μ mol/L) in combination with the specific histamine

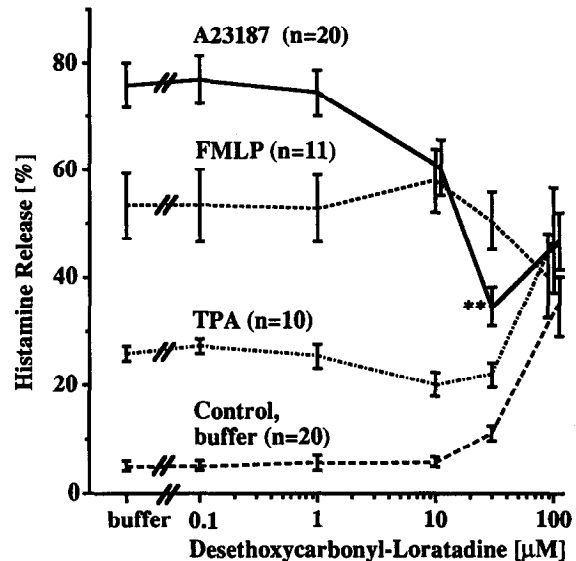


FIG. 2. Influence of preincubation with DCL on non-IgE-dependent histamine release (means $\pm$ SD) from human peripheral blood leukocytes. Mediator release was triggered by 1 μ mol/L FMLP, 10 ng/ml TPA, or 100 ng/ml A23187. Asterisks denote a significant difference (* p < 0.05, ** p < 0.01, *** p < 0.001) from control release, represented by samples preincubated with buffer instead of the drug before stimulation. In addition, the effect of DCL on spontaneous release of histamine was determined (control, buffer). Maximum inhibition and concentrations for 30% inhibition (IC_{30}) are listed in Table I.

H_2 agonist, FRA-19,^{11, 13} at different concentrations (0.01, 0.1, 1, μ mol/L).

Statistical analysis

The statistical significance of drug-related effects was analyzed for control and treated cells with the Wilcoxon signed-rank test for paired data. Differences in the IC_{30} values for DCL against various stimuli were tested for significance by the Mann-Whitney U test. Values were considered significant at p < 0.05.

RESULTS

LHR induced by IgE-dependent stimuli was inhibited by DCL in a dose-dependent fashion (Fig. 1). Inhibition occurred at concentrations greater than 1 μ mol/L DCL, reaching a maximum at 30 μ mol/L DCL for Con A and A-IgE and at 50 μ mol/L DCL for Ag-induced LHR. Dose-dependent inhibition was also obtained after basophil activation with A23187, whereas FMLP- and TPA-induced LHR were not significantly inhibited by DCL (Fig. 2). High concentrations of DCL (100 μ mol/L) also caused LHR in all subjects, abolishing the inhibitory effect on LHR (Figs. 1 and 2). On the basis of individual LHR curves,

IC₃₀ doses were calculated and are shown in Fig. 3. The geometric means and standard errors of the mean after log transformation of the IC₃₀ doses and the maximum inhibition (means $\pm$ SEM) are given in Table I. Probability (*p*) values of the different IC₃₀ values for DCL against various stimuli are listed in Table II.

Kinetic studies with DCL demonstrate a fast onset of its inhibitory effect (Fig. 4), which occurs without preincubation (>50% inhibition of LHR by 30 μ mol DCL), as well as after extended periods of preincubation. A further decrease in A-IgE-induced LHR with prolonged preincubation was not only observed in DCL-pretreated cells but also in buffer-preincubated control cells (Fig. 4).

In additional experiments the effects of DCL were studied during combined incubation with a specific histamine H₂-agonist. The inhibitory effect of DCL at micromolar concentrations was significantly enhanced by increasing doses of the histamine H₂-agonist (Fig. 5). The combined inhibition was more pronounced than the effect of either compound after preincubation alone, indicating synergism in the modulation of IgE-mediated histamine release.

DISCUSSION

On the basis of animal studies and clinical observations, inhibition of mediator release by loratadine has been thought to provide an additional action, which contributes to the therapeutic effectiveness of this H₁-antagonist.⁵ The present findings with peripheral blood basophils confirm these results and show differential effects of loratadine's main metabolite, DCL, on the release of the preformed mediator histamine induced by IgE-dependent and non-IgE-mediated stimuli.

IgE-dependent and A23187-induced LHR were significantly inhibited by DCL in the micromolar range. The differences found in the modulation of the various IgE-dependent stimuli (Ag, A-IgE, and Con A) might be due to the strength of the signal, leading to different amounts of histamine release. In fact, the dose-related inhibitory effect of a specific histamine H₂-agonist was shown to be more pronounced when histamine release was triggered by a suboptimal stimulus for IgE-mediated activation of basophil leukocytes.^{13, 14}

It is well accepted that IgE-dependent activation of human basophils leads to Ca⁺⁺ influx,¹⁵ an event that can be mimicked by nonphysiologic stimuli such as A23187. Because inhibition of LHR by DCL was demonstrated under both con-

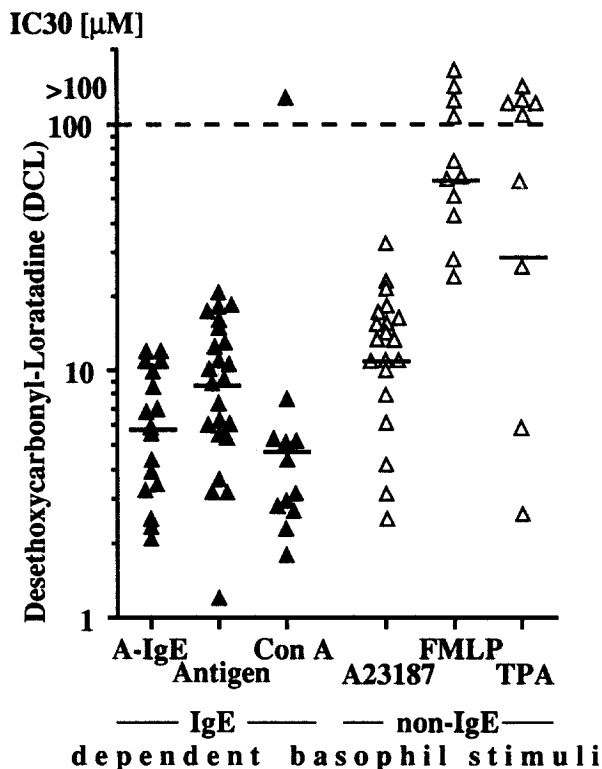


FIG. 3. Inhibition of IgE- and non-IgE-mediated LHR by DCL. With the individual histamine release curves, IC₃₀ levels were calculated and are shown beside the geometric means (—). Standard errors of the mean were calculated after log transformation and are listed in Table I. Differences in the IC₃₀ values were tested for significance; *p* values are given in Table II.

ditions, its action might be on biochemical events occurring after cellular Ca⁺⁺ influx. Interestingly, inhibition of A-IgE- and A23187-induced LHR has been demonstrated after pretreatment of peripheral blood basophils with another nonsedating antihistamine, terfenadine, at similar concentrations (maximal inhibition at 10 μ mol/L and 20 μ mol/L of the drug).¹⁶

Mediator release induced by TPA, known to be an activator of protein kinase C,¹⁷ was not strongly affected by DCL; therefore, the influence of DCL on LHR is unlikely to involve modulation of basophil protein kinase C. FMLP-induced LHR was also essentially unaffected by DCL; however, the clinical relevance of this receptor-mediated stimulus, a chemotactic peptide, and its pathway of basophil activation remains unclear, but marked differences between release induced by FMLP and IgE-mediated stimuli have been shown.¹⁸

The onset of inhibition by DCL did not require preincubation with the cells; this phenomenon,

TABLE I. Inhibition of IgE- and non-IgE-mediated LHR from allergic ($n = 24$) and nonallergic donors ($n = 22$) by DCL

	Stimuli					
	Ag	A-IgE	ConA	FMLP	TPA	A23187
n^*	24/24	18/22	12/22	11/22	9/22	22/22
$I_{\max}$ (%)	69 ± 5	68 ± 3	62 ± 6	22 ± 7	21 ± 9	60 ± 4
IC_{30} ($\mu\text{mol/L}$)†	5.5 ± 1.1	8.2 ± 1.2	4.8 ± 1.4	60.2 ± 1.2	27.6 ± 1.7	11.1 ± 1.2

$I_{\max}$, Maximum inhibition (means $\pm$ SEM).

*Proportion of the experiments in which the stimulus led to significant release of histamine ($>30\%$), the requirement for calculating inhibition by DCL.

†Geometric means $\pm$ SEM; SEM calculated after log transformation.

TABLE II. Probability (p) values* of the differences in the IC_{30} values for DCL inhibiting IgE- and non-IgE-mediated LHR

	Stimuli				
	Ag	A-IgE	ConA	FMLP	TPA
A-IgE	=0.05				
ConA	<0.01	NS			
FMLP	<0.001	<0.001	<0.001		
TPA	<0.05	<0.05	<0.05	NS	
A23187	NS	<0.01	<0.01	<0.001	=0.06

NS, Not significant.

*Significance was determined by the Mann-Whitney U test.

the modulation of LHR even during activation, has also been shown for other agents such as phosphodiesterase inhibitors and H_2 -agonists.^{13, 19} DCL and the histamine H_2 -agonist, FRA-19, caused synergistic effects on LHR, indicating different modes of action and distinct mechanisms of both drugs in modulating mediator release. Also, inhibition of LHR by a new class of histamine H_2 -agonists has been found to be effective in the nanomolar range, showing an increase of 100 to 1000 times in potency in modulating LHR compared with DCL.¹³ Thus the inhibitory effects of DCL on LHR are unlikely to be H_1 -receptor-related; neither specific H_1 -antagonists without antiinflammatory effects nor recently developed specific H_3 -agonists or antagonists were able to modulate IgE-mediated or FMLP-induced LHR in the micromolar range,²⁰⁻²² indicating no evidence for a functional role for H_1 - and H_3 -receptors on basophil leukocytes. In rat peritoneal mast cells, morphologic observations demonstrated that histamine release induced by another new nonsedating antihistamine, loderix, at high concentrations is due to cytotoxic effects.²³ In an

early study classical H_1 -antihistamines, such as phenothiazines, have been shown not only to inhibit IgE-mediated LHR at doses of 0.1 mmol/L, but also to cause histamine release at higher drug concentrations of about 1 mmol/L. It was assumed to be a toxic effect, although overt lysis of the leukocytes was not observed.²⁴ Because the inhibitory effect of DCL occurs only in cells after appropriate stimulation, whereas the increase in LHR appears even in unstimulated cells (after incubation with buffer), it might be speculated that these effects are unrelated to each other, affecting the cells via different mechanisms. The increase in spontaneously released histamine, which occurs after incubation with unphysiologically high doses of DCL ($>30 \mu\text{mol/L}$) probably has little clinical relevance, because the serum concentrations obtained after oral administration of 20 mg loratadine peaked at 28 nmol/L (loratadine) and 32 nmol/L (DCL).⁹

Antiallergic properties of loratadine have been demonstrated with different in vitro models, showing effects on mediator release equipotent to those we obtained with DCL.^{1, 2} Additional find-

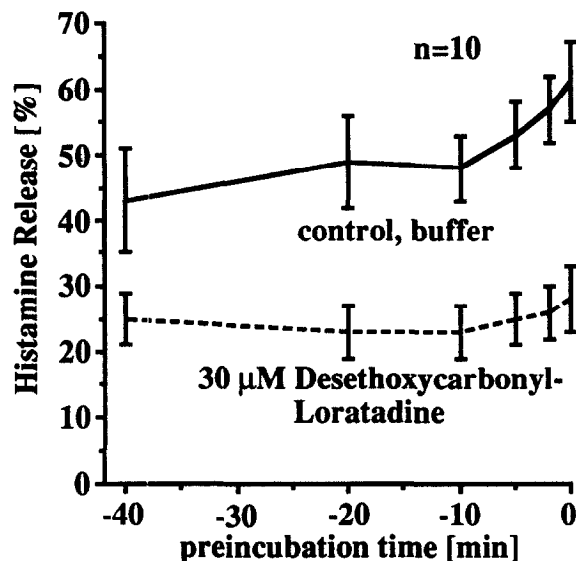


FIG. 4. Kinetics of DCL's effect on IgE-mediated histamine release. After different times of preincubation, the cells were stimulated with 100 IU/ml anti-IgE for 40 minutes.

ings in several in vivo studies indicate antiallergic activities of loratadine, which occur with pharmacologic doses of this agent.²⁵⁻²⁷ In agreement with these findings, another nonclassical antihistamine, terfenadine, was shown not only to antagonize the H_1 activity of released histamine during the immediate allergic reaction but also to inhibit the release of histamine at currently prescribed doses.²⁸ Other nonsedating antihistamines, (e.g., azelastine, cetirizine, and ketotifen) inhibited LHR²⁹⁻³¹; however, in some of these in vitro studies the concentrations of the antihistamines used to inhibit mediator release were higher than concentrations achieved in vivo after the usual doses of H_1 -receptor antagonists.³² Thus the exact mechanisms by which these several compounds exert their effects remain unclear.

Several in vivo models have been successfully used to demonstrate an inhibitory effect of the second-generation H_1 antihistamines during the early and late reactions to antigen.^{33, 34} Despite the promising outcome of many of these studies, the question of whether the statistically significant changes found in these various models translate into an effect under physiologic conditions and into a benefit for the patient under clinical conditions has not yet been answered.³⁵

In summary, we demonstrated dose-dependent inhibition of IgE-mediated and A23187-induced histamine release from human peripheral leukocytes in vitro by DCL in the micromolar range.

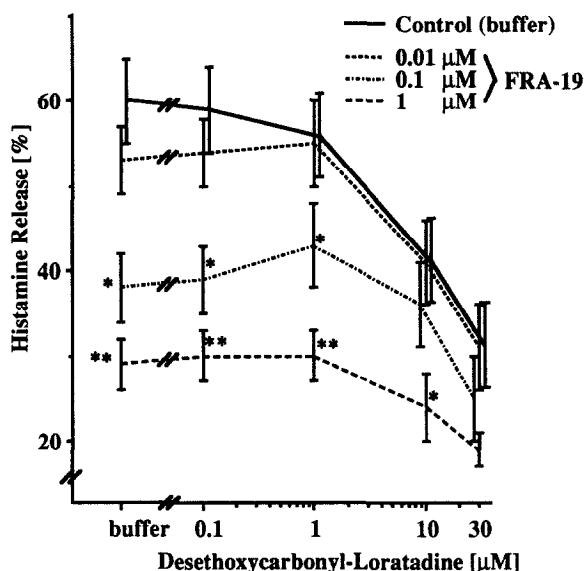


FIG. 5. Inhibition of IgE-mediated histamine release after preincubation of cells with DCL and the histamine H_2 -agonist FRA-19 for 10 minutes alone or in combination. The release process was initiated by 100 IU/ml anti-IgE. Asterisks denote a significant difference (* $p < 0.05$, ** $p < 0.01$) from control release, represented by samples preincubated without the histamine H_2 -agonist before stimulation.

Whether this antiinflammatory property of DCL has relevance in vivo remains to be determined.

We thank Dr. Anne Kagey-Sobotka (The Johns Hopkins Asthma and Allergy Center, Allergy and Clinical Immunology Division, Baltimore, Maryland) for editorial assistance.

REFERENCES

1. Kemp JP, Bahna SL, Chervinsky P, et al. A comparison of loratadine, a new nonsedating antihistamine, with clemastine and placebo in patients with fall seasonal allergic rhinitis. *Am J Rhinol* 1987;1:151-4.
2. Skassa-Brociek W, Bousquet J, Montes F, et al. Double-blind placebo-controlled study of loratadine, mequitazine, and placebo in the symptomatic treatment of seasonal allergic rhinitis. *J ALLERGY CLIN IMMUNOL* 1988;81:725-30.
3. Paul D. Loratadine and terfenadine in the treatment of chronic urticaria [Abstract]. *Allergy* 1988;43(suppl 7):122.
4. Bernstein IL, Bernstein DI. The efficacy and safety of loratadine in the management of chronic idiopathic urticaria [Abstract]. *J ALLERGY CLIN IMMUNOL* 1988;81:211.
5. Kreutner W, Chapman RW, Gulbenkian A, Siegel MI. Antiallergic activity of loratadine, a non-sedating antihistamine. *Allergy* 1987;42:57-63.
6. Berthon B, Cobettes L, Carmi-Leroy A, Czarlewski W, Taudou G, Weyer A. In vitro inhibition by loratadine and its active metabolite of anti-IgE induced histamine release from human basophils and of intracellular calcium fluxes

- on IgE sensitized rat basophilic leukemia cells (RBL-2H3) [Abstract]. *Allergy* 1992;47(suppl):46.
7. Letari O, Miozzo A, Sala A, et al. Loratadine inhibits calcium increase in peritoneal macrophages and leukotriene release in human bronchial strips [Abstract]. *Allergy* 1992;47(suppl):237.
 8. Temple DM, McCluskey M. Loratadine, an antihistamine, blocks antigen- and ionophore-induced leukotriene release from human lung in vitro. *Prostaglandins* 1988;35:549-54.
 9. Hilbert J, Radwanski E, Weglein R, et al. Pharmacokinetics and dose proportionality of loratadine. *J Clin Pharmacol* 1987;27:694-8.
 10. Barenholtz HA, McLeod DC. Loratadine: a nonsedating antihistamine with once-daily dosing. *DICP* 1989;23:445-50.
 11. Buschauer A, Friese-Kimmel A, Baumann G, Schunack W. Synthesis and histamine H₂ agonistic activity of arpromidine analogues: replacement of the pheniramine-like moiety by non-heterocyclic groups. *Eur J Med Chem* 1992;27:321-30.
 12. Siraganian RP, Hook WA. Histamine release and assay methods for the study of human allergy. In: Rose NR, Friedman H, ed. *Manual of clinical laboratory immunology*. 2nd ed. Washington, DC: American Society for Microbiology, 1980:808-21.
 13. Kleine-Tebbe J, Buschauer A, Friese A, Schunack W, Kunkel G. Modulation of IgE-mediated histamine release from human leukocytes by a new class of histamine H₂-agonists. *Agents Actions* 1992;35:185-91.
 14. Tung RS, Lichtenstein LM. Cyclic AMP agonist inhibition increases at low levels of histamine release from human basophils. *J Pharmacol Exp Ther* 1981;218:642-6.
 15. Galli SJ, Lichtenstein LM. Biology of mast cells and basophils. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, eds. *Allergy: principles and practice*. 3rd ed. St. Louis: CV Mosby, 1988:106-34.
 16. Nabe M, Agrawal DK, Sarmiento EU, Townley RG. Inhibitory effect of terfenadine on mediator release from human blood basophils and eosinophils. *Clin Exp Allergy* 1989;19:512-20.
 17. Castagna M, Takei Y, Kaibuchi K, Sano K, Kikkawa U, Nishizuka Y. Direct activation of calcium activated, phospholipid dependent protein kinase by tumor-promoting phorbol esters. *J Biol Chem* 1982;257:7847-51.
 18. Knol EF, Koendermann L, Mul FPJ, Verhoeven AJ, Roos D. Differential activation of human basophils by anti-IgE and formyl-methionyl-leucyl-phenylalanine: indications for protein kinase C-dependent and independent activation pathways. *Eur J Immunol* 1991;21:881-5.
 19. Kleine-Tebbe J, Wicht L, Gagné H, et al. Inhibition of IgE-mediated histamine release from human peripheral leukocytes by selective phosphodiesterase inhibitors. *Agents Actions* 1992;35:185-91.
 20. Kleine-Tebbe J, Schramm J, Bolz M, et al. Influence of histamine H₁, H₂, and H₃ agonists and antagonists on IgE mediated histamine release from human basophils. In: Ring J, Przybilla B, eds. *New trends in allergy III*. Heidelberg, Germany: Springer-Verlag, 1991:152-7.
 21. Kleine-Tebbe J, Schramm J, Bolz M, Lipp R, Schunack W, Kunkel G. Influence of histamine H₃-antagonists on human leukocytes. *Agents Actions* 1990;30:137-9.
 22. Tedeschi A, Lorini M, Arquati M, Miadonna A. Regulation of histamine release from human basophil leukocytes: role of H₁, H₂ and H₃ receptors. *Allergy* 1991;46:626-31.
 23. Nemeth A, Huszti Z. The effect of a new antihistamine drug, Loderix (EGIS-2062), on stimulus-evoked histamine release from rat peritoneal mast cells. *Agents Actions* 1988;23:194-7.
 24. Lichtenstein LM, Gillespie E. The effects of the H₁ and H₂ antihistamines on "allergic" histamine release and its inhibition by histamine. *J Pharmacol Exp Ther* 1975;192:441-50.
 25. Bousquet J, Lebel B, Chanal I, Morel A, Michel FB. Antiallergic activity of H₁-receptor antagonists assessed by nasal challenge. *J ALLERGY CLIN IMMUNOL* 1988;82:881-7.
 26. Skassa-Brociek W, O'Quigley J, Blizard R, Cougnard J, Michel FB, Bousquet J. Differentiation between the anti-allergic and anti-histaminic effects of loratadine (L) and astemizole (A) by skin tests [Abstract]. *J ALLERGY CLIN IMMUNOL* 1988;81:175.
 27. Andersson M, Nolte H, Baumgarten C, Pipkorn U. Suppressive effect of loratadine on allergen-induced histamine release in the nose. *Allergy* 1991;46:540-6.
 28. Naclerio RM, Kagey-Sobotka A, Lichtenstein LM, Friedhoff L, Proud D. Terfenadine, an H₁ antihistamine, inhibits histamine release in vivo in the human. *Am Rev Respir Dis* 1990;142:167-71.
 29. Little MM, Casale TB. Azelastine inhibits IgE-mediated human basophil histamine release. *J ALLERGY CLIN IMMUNOL* 1989;83:862-5.
 30. De Vos C, Joseph M, Leprevost C, et al. Inhibition of human eosinophil chemotaxis of the IgE-dependent stimulation of human blood platelets by cetirizine. *Int Arch Allergy Appl Immunol* 1989;83:862-5.
 31. Tomioka H, Yoshida S, Tanaka M, Kumagai A. Inhibition of chemical mediator release from human leukocytes by a new antiasthma drug HC20-511 (ketotifen). *Monogr Allergy* 1979;14:313-7.
 32. Simons FER. The antiallergic effects of antihistamines (H₁-receptor antagonists). *J ALLERGY CLIN IMMUNOL* 1992;90:705-15.
 33. Naclerio RM. Inhibition of mediator release during the early reaction to antigen. *J ALLERGY CLIN IMMUNOL* 1992;90:715-9.
 34. Antiallergic properties of the second-generation H₁ antihistamines during the early and late reactions to antigen. *J ALLERGY CLIN IMMUNOL* 1992;90:720-5.
 35. Kaliner MA, Naclerio RM, Simons FER, Townley RG. Commentary and discussion: inhibition of mediator release. *J ALLERGY CLIN IMMUNOL* 1992;90:725-8.