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Abstract

The effects of levocabastine, a novel histamine H1-receptor antagonist, on lipid mediator release induced by antigen-antibody reaction from actively sensitized guinea pig lung fragments were studied. Levocabastine dose-dependently inhibited the release of leukotriene C4 from guinea pig lung fragments induced by antigen. A significant effect was observed with levocabastine at a concentration of 10(-4) M. On the other hand, levocabastine produced no effect on the release of leukotriene E4 or thromoboxane B2. From these findings, it was concluded that levocabastine may be useful for relieving the nasal obstruction in allergic rhinitis caused by inhibition of leukotriene C4 release.

KEYWORDS: levocabastine, guinea pig, lung fragment, lipid mediator

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Brief Note

Effects of Levocabastine on Lipid Mediator Release from Guinea Pig Lung Fragments

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The effects of levocabastine, a novel histamine H_1-receptor antagonist, on lipid mediator release induced by antigen-antibody reaction from actively sensitized guinea pig lung fragments were studied. Levocabastine dose-dependently inhibited the release of leukotriene C_4 from guinea pig lung fragments induced by antigen. A significant effect was observed with levocabastine at a concentration of 10^{-4} M. On the other hand, levocabastine produced no effect on the release of leukotriene E_4 or thromoboxane B_2. From these findings, it was concluded that levocabastine may be useful for relieving the nasal obstruction in allergic rhinitis caused by inhibition of leukotriene C_4 release.

Key words: levocabastine, guinea pig, lung fragment, lipid mediator

Levocabastine ((-)-trans-1-[cis-4-cyano-4-(p-fluorophenyl)cyclohexyl]-3-methyl-4-phenylisonipecotic acid monohydrochloride, R 50547, CAS 79516-68-0) is a potent histamine H_1-receptor antagonist with a distinctive chemical structure (1), and it has been reported that the local administration of levocabastine is effective in the treatment of allergic conjunctivitis and rhinitis (2, 3). Levocabastine has been reported to effectively relieve the nasal obstruction in allergic rhinitis in clinical use (4). Allergic rhinitis, characterized by the 3 major symptoms of sneezing, rhinorrhea and nasal obstruction, is presumed to be triggered by the release of various mediators from mast cells and other inflammatory cells responsible for IgE-mediated allergic reactions. The symptoms of sneezing and rhinorrhea, but not that of nasal obstruction, can be attenuated with histamine H_1-receptor antagonists (5, 6). Therefore, mediators other than histamine seem to be involved in the development of nasal obstruction.

It was recently demonstrated that, in the late phase of the nasal reaction induced by nasal allergen challenge in patients with allergic rhinitis, increases in leukotriene (LT) C_4 level were well correlated with increases in nasal airway resistance (7). These findings suggest that LTC_4 plays an important role in the nasal obstruction characteristic of allergic rhinitis. In addition, it has been reported that selective thromboxane (TX) B_2 receptor antagonists (Bay u 3405 and S-1452) relieved intranasal pressure, as an index of nasal obstruction, induced by a topical antigen challenge in actively sensitized guinea pigs (8, 9). Furthermore, the level of TXB_2, a stable TXA_2 metabolite, was increased in the nasal lavage fluid of the late phase nasal reaction, and U-44619, a TXA_2 mimetic, increased nasal airway resistance in guinea pig models (9). Therefore, these lipid mediators appear to contribute to the nasal obstruction in the late phase reaction of allergic rhinitis.

On the other hand, we have shown that levocabastine inhibits histamine release from lung fragments of guinea pigs actively sensitized by antigen challenge (10). Moreover, several studies have indicated that many lipid mediators are released from actively sensitized guinea pig lung fragments (11, 12). This study was performed to clarify the effects of levocabastine on the release of lipid mediators (LTC_4, LTE_4 and TXB_2) from lung fragments of guinea pigs actively sensitized by antigen challenge.

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Materials and Methods

Six-week-old male Hartley guinea pigs, weighing 250 -300 g (Shimizu Laboratory Supplies, Kyoto, Japan), were used. The animals were housed in an air-conditioned room at 22-26°C with 40-70% humidity, and given food and water *ad libitum*. The following chemicals were used: Levocabastine hydrochloride (Janssen Kyowa, Tokyo), egg albumin (Sigma, St. Louis, MO, USA) and *Bordetella pertussis* (Kitasato Institute Research Center for Biologicals, Saitama, Japan). All other reagents used in the study were of the highest grade available commercially. Guinea pigs were sensitized with egg albumin as an antigen according to the method of Mota (13). The animals were injected intramuscularly with 1 mg of egg albumin in saline solution (10 mg/ml) together with 1 ml of *Bordetella pertussis* (2×10^{10} organisms/ml) intraperitoneally. After 2 weeks, animals were exsanguinated by decapitation and the lungs were excised. The large airways, blood vessels and lung fat were resected, and the lung parenchyma cut into fragments of approximately 1 mm^3. The lung fragments were transferred onto a piece of gauze and washed with 100 ml/lung of Tyrode's solution of the following composition (in mM): NaCl, 137; KCl, 3.3; $CaCl_2$, 2; $MgCl_2$, 0.1; NaH_2PO_4, 0.4; $NaHCO_3$, 12; and glucose, 5. Fragments of 200 ± 5 mg (wet weight) were put into test tubes and 1.9 ml of Tyrode's solution was added. Experimental compounds were dissolved in dimethylsulfoxide and diluted to an appropriate final concentration with Tyrode's solution. Cosolvent did not exceed 0.2% (v/v). Samples of lung tissue were preincubated with compounds at 37°C for 15 min. Incubation with antigen was started by adding 0.1 ml of egg albumin solution (50 μg/ml) and continued for 20 min. The reaction was terminated by placing the test tubes into an ice bath, after which they were centrifuged at $400 \times g$ for 10 min at 4°C. Aliquots of 1 ml of supernatant were stored at −80°C until the assay was performed. LTC_4, LTE_4 and TXB_2 contents were determined using enzyme immunoassay kits (Cayman Chemical Co., Ann Arbor, MI, USA). All values are expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance with Dunnett's test.

Results and Discussion

The antigen-induced amounts of LTC_4, LTE_4 and TXB_2 released from actively sensitized guinea pig lung fragments in the control group were 7.6 ± 0.8, 37.8 ± 6.1 and 4925.2 ± 442.9 ng/g wet tissue, respectively. The effect of levocabastine on antigen-induced LTC_4 release from lung fragments is shown in Fig. 1. As can be seen, levocabastine of 10-100 μM inhibited the release in a dose-dependent manner. A significant effect was observed at the concentration of 100 μM levocabastine. On the other hand, even at 100 μM levocabastine had no significant effects on the release of LTE_4 or TXB_2 from actively sensitized guinea pig lung fragments (Figs. 2 and 3).

Levocabastine has attracted attention for its potential use in the treatment of allergic rhinitis and conjunctivitis due to its histamine H_1-receptor antagonistic activity and inhibitory effects on mediator release. However, there have been few studies of the effects of levocabastine on lipid mediator release from not only the tissues but also the inflammatory cells in animals. It is well known that allergic rhinitis is induced by activation of nasal mucosal mast cells. However, because nasal mucosal mast cells cannot be obtained from experimental animals in sufficient quantities for their study, lung tissue mast cells have been generally used for the reason that lung tissues include mucosal mast cells abundantly. Histamine and lipid mediators have been shown to be released from tissue fragments of the guinea pig lung by an antigen-antibody reaction due to IgE antibodies (10-12). We have also

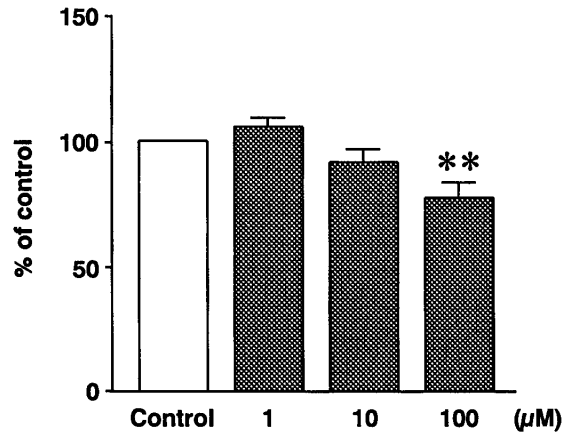

Fig. 1 Effects of levocabastine on leukotriene C_4 release from actively sensitized guinea pig lung fragments induced by antigen challenge. Each column and vertical bar represents the mean ± SEM of 5 experiments. **Significantly different from control group with $P < 0.01$.

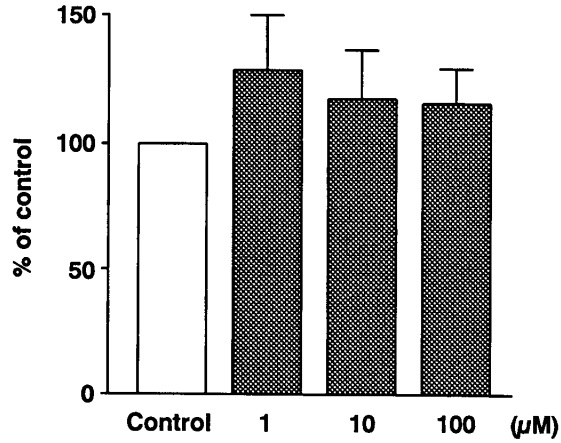

Fig. 2 Effects of levocabastine on leukotriene E_4 release from actively sensitized guinea pig lung fragments induced by antigen challenge. Each column and vertical bar represents the mean ± SEM of 6 experiments.

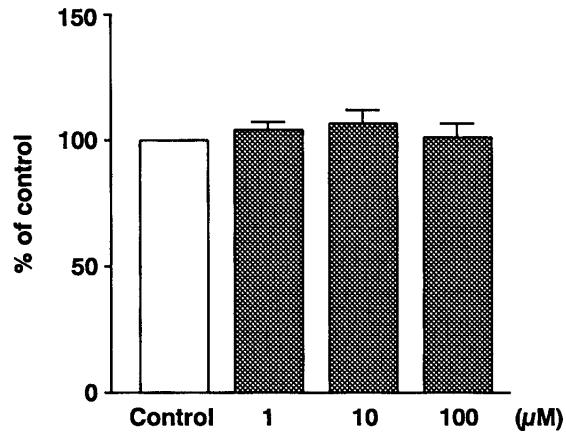

Fig. 3 Effects of levocabastine on thromboxane B_2 release from actively sensitized guinea pig lung fragments induced by antigen challenge. Each column and vertical bar represents the mean ± SEM of 8 experiments.

reported that levocabastine inhibited histamine release from the lung fragments of actively sensitized guinea pigs (10). In the present study, levocabastine at the concentration of 100 μM (45.7 μg/ml) was shown to inhibit antigen-induced LTC_4 release from guinea pig lung fragments. Okuda *et al.* (4) reported that 0.025% (250 μg/ml) levocabastine nasal spray significantly inhibited nasal symptoms in case of perennial allergic rhinitis. Among the 3 major symptoms of allergic rhinitis, *i.e.*, sneezing, rhinorrhea and nasal obstruction, nasal obstruction was most strongly inhibited by levocabastine. Therefore, our present finding is essentially in agreement with the clinical data showing that levocabastine is effective in improving nasal obstruction in case of allergic rhinitis.

By contrast, the drug had no effect on the release of LTE_4 or TXB_2 from lung fragments. These results suggested that this drug does not affect the 5-lipoxygenase or cyclooxygenase activities in the arachidonic acid cascade. Histamine H_1-receptor antagonists have been reported to inhibit release of chemical mediators that contain leukotrienes (14, 15). This suggests that anti-allergic drugs, including levocabastine, inhibit the synthesis of leukotrienes by blocking one or more of the enzymatic steps. However, the detailed mechanisms by which levocabastine inhibits the enzymes responsible for synthesis of leukotrienes remain to be elucidated. Further animal experiments in models that approximate the clinical symptoms of allergic rhinitis will be needed.

On the other hand, levocabastine has been shown to bind to neurotensin NT_2 receptors (16, 17). Carraway *et al.* (18) reported that neurotensin is a very potent and specific stimulator of leukotriene release from inflammatory cells. Therefore, the anti-NT_2 receptor effect of levocabastine may also be influenced by the inhibition of leukotriene release induced by antigen-antibody reaction.

In conclusion, levocabastine may be useful for treatment of the nasal obstruction in allergic rhinitis due to not only its anti-NT_2 receptor effect but also its inhibitory effect on LTC_4 release from mucosal mast cells.

References

1. Stokbroekx RA, Luyckx MGM, Willems JJM, Janssen M, Bracke JOMM, Joosen RLP and Van Wauwe JP: Levocabastine (R 50547): the prototype of a chemical series of compounds with specific H_1-antihistaminic activity. Drug Dev Res (1986) **8**, 87-93.
2. Pipkorn U, Bende M, Hedner J and Hedner T: A double-blind evaluation of topical levocabastine, a new specific H_1 antagonist in patients with allergic conjunctivitis. Allergy (1985) **40**, 491-496.
3. Kolly M and Pécoud A: Comparison of levocabastine, a new selective H_1-receptor antagonist, and disodium cromoglycate, in a nasal provocation test with allergen. Br J Clin Pharmacol (1986) **22**, 389-394.
4. Okuda M: Clinical investigation of R 50547 (Levocabastine hydrochloride) nasal spray in perennial allergic rhinitis — phase III comparative trial with sodium cromoglicate —. Otologia Fukuoka (1995) **41**, Suppl. 1: 381-403.
5. Skoner DP, Asman B and Fireman P: Effect of chlorpheniramine on airway physiology and symptoms during natural pollen exposure. Am J Rhinol (1994) **8**, 43-48.
6. Wagenmann M, Baroody FM, Kagey-Sobotka A, Lichtenstein LM and Naclerio RM: The effect of terfenadine on unilateral nasal challenge

with allergen. J Allergy Clin Immunol (1994) **93**, 594-605.
7. Wang D, Clement P, Smitz J and Derde MP: Concentrations of chemical mediators in nasal secretions after nasal allergen challenges in atopic patients. Eur Arch Otorhinolaryngol (1995) **252**, S40-S43.
8. Narita S, Asakura K and Kataura A: Effects of thromboxane A_2 receptor antagonist (Bay u 3405) on nasal symptoms after antigen challenge in sensitized guinea pigs. Int Arch Allergy Immunol (1996) **109**, 161-166.
9. Yasui K, Asanuma F, Furue Y and Arimura A: Involvement of thromboxane A_2 in antigen-induced nasal blockage in guinea pigs. Int Arch Allergy Immunol (1997) **112**, 400-405.
10. Tasaka K, Kamei C, Akagi M, Mio M, Shirasaka T and Chokki M: Antiallergic profile of the novel H_1-antihistaminic compound levocabastine. Arzneim-Forsch (1993) **43**, 1331-1337.
11. Kusner EJ, Buckner CK, Dea DM, DeHaas CJ, Marks RL and Krell RD: The 5-lipoxygenase inhibitors ZD2138 and ZM230487 are potent and selective inhibitors of several antigen-induced guinea-pig pulmonary responses. Eur J Pharmacol (1994) **257**, 285-292.
12. Akagi M, Fukuishi N, Kan T, Sagesaka YM and Akagi R: Anti-allergic effect of tea-leaf saponin (TLS) from tea leaves (*Camellia sinensis* var. *sinensis*). Biol Pharm Bull (1997) **20**, 565-567.
13. Mota I: The mechanism of anaphylaxis; I. Production and biological properties of mast cell sensitizing' antibody. Immunology (1964) **7**, 681-699.
14. Kalayci Ö, Saraclar Y, Adalioglu G, Sekerel B and Tuncer A: The effect of cetirizine on sulfidoleukotriene production by blood leukocytes in children with allergic rhinitis. Allergy (1995) **50**, 964-969.
15. Hamasaki Y, Shafigeh M, Yamamoto S, Sato R, Zaitu M, Muro E, Kobayashi I, Ichimaru T, Tasaki H and Miyazaki S: Inhibition of leukotriene synthesis by azelastine. Ann Allergy Asthma Immunol (1996) **76**, 469-475.
16. Kitabgi P, Rostène W, Dussaillant M, Schotte A, Laduron PM and Vincent JP: Two populations of neurotensin binding sites in murine brain: Discrimination by the antihistamine levocabastine reveals markedly different radioautographic distribution. Eur J Pharmacol (1987) **140**, 285-293.
17. Vincent JP: Neurotensin receptors. Binding properties, transduction mechanisms, and purification. Ann N Y Acad Sci (1992) **668**, 90-100.
18. Carraway RE, Cochrane DE, Salmonsen R, Muraki K and Boucher W: Neurotensin elevates hematocrit and plasma levels of the leukotrienes, LTB4, LTC4, LTD4 and LTE4, in anesthetized rats. Peptides (1991) **12**, 1105-1111.

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