MCI-826 Is a Potent and Selective Antagonist of Peptide Leukotrienes (p-LTs) and Has Characteristics Distinctive from Those of FPL 55712

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Received January 25, 1992 Accepted June 2, 1992

ABSTRACT — Antagonistic effects of a newly synthesized compound, (E)-2,2-diethyl-3'-[2-[2-(4-isopropyl)thiazolyl]ethenyl]succinanilic acid sodium salt (MCI-826) on the contraction of the isolated guinea pig trachea and human bronchus induced by various agonists including peptide leukotrienes (p-LTs), histamine, acetylcholine (ACh), prostaglandin (PG) D2 and others were investigated and compared with the effects of a p-LT antagonist, FPL 55712, in some experiments. MCI-826 potently antagonized LTD4 and LTE4-induced contractions at extremely low concentrations in the isolated guinea pig trachea with pA2 values of 8.3 and 8.9, respectively, on a molar basis. These values indicated that MCI-826 is over 100 times stronger than FPL 55712. Similarly, MCI-826 at 10^{-8} g/ml (2.4 X 10^{-8} M) markedly antagonized LTD4-induced contractions of the isolated human bronchus. Although FPL 55712 fairly inhibited the 10^{-9} g/ml LTC4-induced contraction of the isolated guinea pig trachea, MCI-826 had little effect on the contraction at high concentrations like 3 X 10^{-6} g/ml (7.1 X 10^{-6} M). MCI-826 modestly affected the other agonist-induced contractions and the resting tonus of the isolated guinea pig trachea at 10^{-6} g/ml (2.4 X 10^{-6} M) or higher concentrations, but FPL 55712 caused fair inhibition of some of those contractions and gradually lowered the resting tonus with time. These results indicate that MCI-826 is a highly potent and selective antagonist of LTD4 and LTE4 and can be a useful tool for biological and pharmacological experiments on p-LTs.

Keywords: MCI-826, FPL 55712, Leukotriene, Trachea

Peptide leukotrienes (p-LTs) were formerly designated the slow reacting substance of anaphylaxis (SRS-A) and are well-known to be a family of arachidonate 5-lipoxygenase pathway products consisting of LTC4, LTD4 and LTE4. Since SRS-A, or p-LTs, induce potent airway smooth muscle contractions and airway mucus secretion in some species, especially humans (1–6) and guinea pigs (7), it has been proposed that these arachidonate metabolites are important chemical mediators in airway dysfunctions including bronchial asthma.

In 1973, the Fisons group reported that a chromone derivative, FPL 55712, shows a potent and competitive antagonistic effect on SRS-A-induced contraction of the isolated guinea pig ileum (8). Unfortunately, this compound has an extremely short half life when administered parenterally and there is little absorption after oral administration (9), but still it has served as a worldwide and standard p-LT antagonist in in vitro experiments.

In the latest decade, many workers have intensively searched for orally active and more potent antagonists of p-LTs than FPL 55712. MCI-826 has been newly synthesized with modification of the LTD4 structure by a computer analysis. The chemical structure of MCI-826 is completely different from that of FPL 55712 as shown in Fig. 1.

In this paper, we report that MCI-826 is a potent and selective antagonist of p-LTs, which has characteristics distinct from those of FPL 55712, using isolated guinea pig tracheas and human bronchi.

MATERIALS AND METHODS

Reagents

(E)-2,2-Diethyl-3'-[2-(4-isopropyl)thiazolyl]ethenyl]succinanilic acid sodium salt (MCI-826, supplied from
Mitsubishi Kasei, Tokyo); FPL 55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate, supplied by Mr. P. Sheard of Fisons, Loughborough); leukotriene (LT) C₄, LTD₄ and LTE₄ (supplied from Mitsubishi Kasei, Tokyo); acetylcholine chloride (ACh) and histamine dihydrochloride (Nacalai Tesque, Kyoto); prostaglandin (PG) D₂ (Wako Pure Chem., Osaka); 9,11-dideoxy-9α,11α-methanoepoxy-PGF₂α (U-46619, Cayman Chem., Ann Arbor); L-serine, sodium borate and L-cysteine • HCl • H₂O (Wako Pure Chem., Osaka); and L-isoproterenol bitartrate and mepyramine maleate (Sigma Chem., St. Louis); and bradykinin • 2CH₃000H • 3H₂O (Peptide Inst., Minoh). Other reagents used were the highest grade commercially available.

MCI-826 and FPL 55712 were dissolved in distilled water; LTC₄, LTD₄, LTE₄, PGD₂ and U-46619, in 50% methanol; and ACh and histamine, in 0.9% saline.

**Physiological solution**

Modified Tyrode's solution was used throughout the isolated guinea pig tracheal and human bronchial experiments. The composition of modified Tyrode's solution was: 8.0 g/l NaCl, 0.20 g/l KCl, 0.058 g/l NaH₂PO₄•2H₂O, 0.10 g/l MgCl₂•6H₂O, 0.264 g/l CaCl₂•2H₂O, 1.0 g/l NaHCO₃, and 1.0 g/l glucose.

**Preparation of the isolated human bronchi and guinea pig tracheas**

Macroscopically normal human lung portions were obtained at the time of resection for carcinoma and used as soon as possible. Bronchi, with an inner diameter of 2 mm or less, were carefully removed from the parenchyma with fine scissors for the opthalmological surgery in Ca²⁺-free Tyrode's solution and spirally cut 1 to 1.5 mm in width. The spiral strip, 2 cm in length, was suspended in a Magnus bath.

Five to nine-week-old Hartley male guinea pigs weighing 350–800 g (Japan SLC, Hamamatsu) were killed by bleeding from the femoral artery following a blow on the head. The trachea isolated was split longitudinally through the ventral cartilage. Segments (either one- or two-cartilage-rings wide) were cut and ligatures were tied to the cartilage end. The isolated tracheal preparation consisting of 3 segments was suspended in a Magnus bath. Twelve and six preparations from one animal were made for experiments on the antagonistic effect on p-LTs and other agonists, respectively.

**Measurement of movement of the isolated trachea and bronchus**

The isolated tracheal chain or the bronchial strip was suspended in a 5-ml Magnus bath. Conditions of the experiments were: temperature 37 ± 0.1°C and loading weight of 300 mg. The movement of the smooth muscle was isotonically (isotonic transducer: TD-112S, Nihon Kohden, Tokyo) recorded (recorder: RJK-4124, Nihon Kohden, Tokyo) through amplification (amplifier: AA-601H + isotonic coupler: EG-650H, Nihon Kohden, Tokyo). Prior to beginning the experiments, 10⁻⁵ g/ml ACh was repeatedly applied to the isolated tracheal strips until almost equal contractions were observed as an indication of the sensitivity having become stable.

**Contractions induced by p-LTs**

The contraction of the isolated guinea pig trachea induced by the singular or cumulative method to apply LTC₄ at 10⁻⁹ g/ml, LTD₄ at 10⁻¹⁰ – 10⁻⁹ g/ml or LTE₄ at 10⁻¹⁰ – 3 × 10⁻⁹ g/ml was observed for 15 min at each concentration with or without MCI-826 or FPL 55712 (5 min treatment before p-LT addition). In the human bronchial experiments, MCI-826 or FPL 55712 was added 5 or 2 min before LTC₄ or LTD₄ application or when the contraction by LTD₄ had reached a plateau level. Respective contractions induced by LTC₄ and LTD₄ were measured in the presence of 10 mM l-serine + 20 mM sodium borate (10 mM serine borate complex) (10, 11) and 10 mM l-cysteine (12), respectively, to prevent the enzymatic conversion to LTD₄ and LTE₄.

To determine pA₂ values for MCI-826 and FPL 55712 against LTD₄ and LTE₄ in the isolated guinea pig trachea, log (dose ratio − 1) values were plotted against the negative log of the molar concentrations of the drugs.

**Contractions induced by other agonists**

To the guinea pig tracheas various agonists including...
histamine, ACh, U-46619, PGD₂ and bradykinin were added, and their contractions were observed for 5 to 10 min. MCI-826 and FPL 55712 at final concentrations of $10^{-6}$ and $10^{-5}$ g/ml were administered 5 min before administration of the agonists. It was also determined if MCI-826 influences contractions induced by histamine and Ba²⁺ in the isolated human bronchus.

Resting tonus

Effect of MCI-826 and FPL 55712 on the resting tonus of the isolated guinea pig trachea was examined at the final concentrations of $10^{-6}$ g/ml and observed for 20 min.

RESULTS

Effect on the contractions induced by p-LTs of the isolated guinea pig trachea and human bronchus

Respective sigmoidal concentration-contraction curves of LTD₄ in the presence of cysteine and LTE₄ using the isolated guinea pig trachea appeared to be parallel shifted to the right when the preparation was treated by MCI-826 or FPL 55712.

Table 1 shows the pA₂ values and slopes of the Schild plot for MCI-826 and FPL 55712 against the LTD₄- and LTE₄-induced contractions. The slope for MCI-826 versus either LTD₄ or LTE₄ was approximately 1, whereas that for FPL 55712 was slightly larger than 1, but not significantly different from MCI-826. pA₂ values for MCI-826 versus LTD₄ and LTE₄ were 8.3 and 8.9, respectively, indicating that the drug is over 100 times as potent as FPL 55712 in either case. While FPL 55712 concentration-dependently antagonized the contraction induced by $10^{-9}$ g/ml LTC₄, MCI-826 did not affect the contractions up to $3 \times 10^{-6}$ g/ml (7.1 $\times 10^{-6}$ M) (IC₅₀ = 42 $\pm$ 14.8 μg/ml, N = 6) (Fig. 2).

As shown in Fig. 3, similar to the results in the guinea pig experiments, pretreatment with $10^{-8}$ g/ml (2.4 $\times 10^{-8}$ M) MCI-826 strongly antagonized the contractions induced by LTD₄ in isolated human bronchi. The contraction induced by $3 \times 10^{-10}$ g/ml LTD₄ of the bronchus was gradually but completely reversed by the addition of $10^{-8}$ g/ml at the final concentration of the compound as well (Fig. 4). The compound at $10^{-8}$ g/ml also completely antagonized the contraction induced by LTE₄ of up to $10^{-7}$ g/ml (data not shown). On the other hand, on the contrary to the result in guinea pig trachea, the human bronchial contraction induced by $10^{-10}$ to $10^{-7}$ g/ml LTC₄ appeared to be partially antagonized by $10^{-8}$ g/ml MCI-826 as shown in Fig. 5. FPL 55712 at $10^{-8}$ g/ml also seemed to slightly antagonize the contraction. pA₂ values for MCI-826 versus p-LTs in the human bronchial experiments were not calculated because of the lack of sufficient tissue that could reproducibly respond to the repeated cumulative application of p-LTs.

Table 1. Schild analysis for MCI-826 and FPL 55712 on LTD₄- and LTE₄-induced contractions of the isolated guinea pig trachea

| LTs    | MCI-826     | FPL 55712   |
|--------|-------------|-------------|
|        | pA₂ (mean)  | slope (mean ± S.E.) |
| LTD₄   | 8.32, 1.04 ± 0.24 (N = 30) | 6.49, 1.10 ± 0.19 (N = 27) |
| LTE₄   | 8.91, 1.06 ± 0.04 (N = 21) | 6.89, 1.12 ± 0.13 (N = 19) |

The antagonistic activity on LTD₄-induced contraction was assayed in the presence of 10 mM cysteine.
Fig. 3. Effect of MCI-826 on the LTD₄-induced contraction of the isolated human bronchus. The preparations of a, a' (1st and 2nd application of LTD₄ without MCI-826, control, respectively) and b, b' (1st and 2nd application of LTD₄ without and with MCI-826, respectively) were adjacent spiral strips from the same bronchus. Similar results were obtained from another two separate experiments. W: wash.

Fig. 4. Effect of MCI-826 on the LTD₄-induced contraction of the isolated human bronchus. The preparations of a (control) and b (MCI-826 treatment) were adjacent spiral strips from the same bronchus. H: histamine, W: wash. Similar results were obtained from another three separate experiments.
Effect on the contractions induced by the other agonists of the isolated guinea pig trachea and human bronchus

Table 2 shows the effect of MCI-826 and FPL 55712 at $10^{-6}$ and $10^{-5}$ g/ml on the various agonist-induced contractions of the isolated guinea pig trachea. MCI-826 at $10^{-6}$ g/ml slightly inhibited the contraction induced by $10^{-6}$ g/ml of either ACh, histamine or PGD$_2$, but not those by $10^{-8}$ g/ml U-46619 and $10^{-6}$ g/ml bradykinin. FPL 55712 at $10^{-6}$ g/ml showed similar results to those of MCI-826 on ACh, histamine and U-46619. However, it produced significantly stronger inhibition on the contraction by PGD$_2$ and tended to inhibit the contraction by bradykinin more than that by MCI-826. MCI-826 at $10^{-5}$ g/ml inhibited the contractions by PGD$_2$ and bradykinin by 15.5 and 12.9%, respectively. On the other hand, the same concentration of FPL 55712 significantly inhibited or tended to inhibit more the contraction by U-46619, PGD$_2$ or bradykinin.

The effect of MCI-826 on the contractions by $4 \times 10^{-4}$ g/ml BaCl$_2$ and $10^{-5}$ g/ml histamine of isolated human bronchi is illustrated in Fig. 6. Even very high concentrations such as $10^{-5}$ or $10^{-4}$ g/ml of MCI-826 did not affect these contractions, while $10^{-5}$ g/ml mepyramine and $10^{-8}$ g/ml isoproterenol obviously antagonized the respective contractions.
Table 2. Effect of MCI-826 and FPL 55712 on various agonist-induced contractions of the isolated guinea pig trachea

| Agonist (g/ml) | MCI-826 (g/ml) | FPL 55712 (g/ml) |
|---------------|---------------|------------------|
|               | 10⁻⁶          | 10⁻⁵            | 10⁻⁶          | 10⁻⁵          |
| ACh (10⁻⁶)    | 6.7 ± 1.15    | ND              | 4.2 ± 0.48    | ND             |
| ACh (10⁻⁵)    | 4.0 ± 1.18    | ND              | 4.5 ± 0.99    | ND             |
| U-46619 (10⁻⁸) | 1.5 ± 0.67   | 2.4 ± 1.75      | 0.9 ± 1.98    | 12.7 ± 2.72** |
| PGD₂ (10⁻⁶)   | 5.2 ± 1.74    | 15.5 ± 1.93     | 19.6 ± 0.77** | 29.8 ± 4.40*  |
| Bradykinin (10⁻⁴) | −3.5 ± 7.31 | 12.9 ± 7.21     | 10.4 ± 11.6*  | 42.8 ± 17.8   |

Drugs were administered 5 min before agonist addition. Each value represents the mean ± S.E. of 4 experiments. ND: not done. * and **: Statistically significant difference from 10⁻⁶ or 10⁻⁵ g/ml MCI-826 at P < 0.1 and 0.05, respectively.

Introduction

The treatment of MCI-826 at the concentration of 10⁻⁶ g/ml modestly increased the resting tonus of the isolated guinea pig trachea. On the other hand, the same concentration of FPL 55712 gradually lowered the tonus with time. At 20 min after the treatment, the relaxation reached 60% of the maximum induced by 10⁻⁶ g/ml isoproterenol (Fig. 7).

Discussion

The present experiments using isolated guinea pig tracheas and human bronchi showed that MCI-826 is a much more potent antagonist of LTD₄ and LTE₄ than FPL 55712. In addition, the slope of the Schild plot for MCI-826 against LTD₄ and LTE₄ for the isolated guinea pig trachea obtained in the present studies and this compound’s highly potent inhibition of the specific binding of LTD₄ (Kᵢ: MCI-826 = 1.8 × 10⁻⁹ M, FPL 55712 = 9.4 × 10⁻⁷ M) and LTE₄ (Kᵢ: MCI-826 = 6.0 × 10⁻¹⁰ M, FPL 55712 = 9.7 × 10⁻⁷ M) but not that of LTC₄ (Kᵢ: MCI-826 > 3 × 10⁻⁴ M, FPL 55712 = 1.8 × 10⁻³ M) obtained in our laboratory using guinea pig lung crude membranes strongly suggests that the compound is a competitive LTD₄ and LTE₄ antagonist. The low pA₂ values obtained for FPL 55712 versus LTD₄ and LTE₄, using isolated guinea pig trachea, are within the range of values reported previously (13).
On the contrary, MCI-826 has no substantial antagonistic property against the LTC₄-induced contraction in isolated guinea pig trachea. However, FPL 55712 antagonized the LTC₄-induced contraction concentration-dependently, like the results on the specific binding of the guinea pig lung crude membrane, as mentioned above. These results indicate that MCI-826 has a distinctive property that FPL 55712 does not have and that the compound may be able to distinguish the p-LT receptor subclasses. Specific binding sites of LTC₄ on the guinea pig crude lung membrane have been reported to be obviously different from those of LTD₄ or LTE₄ (14, 15). The results of the present experiments and the almost entirely non-inhibitory activities of MCI-826 on the binding assay of LTC₄ in the guinea pig crude lung membrane strongly suggested that the LTC₄ specific binding site is the LTC₄ receptor.

It has been proposed that LTE₄ receptors on the airway smooth muscle that mediate the contraction are identical or quite similar to the high affinity receptors of LTD₄ because LTE₄ binding to the guinea pig crude lung membrane is completely dissociated by low concentrations of LTD₄ (16, 17). No contradictory results to the hypothesis were obtained from the present experiments on the antagonistic effect of MCI-826 on the airway tissue contraction. Much lower concentrations of either compound were needed to antagonize the contraction induced by LTE₄ than that by LTD₄.

From the results of the binding assay using guinea pig crude lung membrane (18) and the inability of FPL 55712 to antagonize the LTD₄-induced guinea pig tracheal contraction (14), it has been postulated that the low affinity receptors for LTD₄ exist on airway smooth muscles. However, we did not obtain evidence that low affinity receptors (or specific binding sites) are coupled to smooth muscle contraction because the slope of the Schild plot for MCI-826 as well as that for FPL 55712 against LTD₄ using isolated guinea pig tracheas in the presence of cysteine was approximately one. Although MCI-826 hardly or slightly affected the contraction by LTC₄ of the guinea pig trachea or human bronchus, this compound strongly antagonized either LTD₄ or LTE₄-induced contraction. On the other hand, FPL 55712 did concentration-dependently inhibit the LTC₄-induced contraction and showed comparatively weak potencies of p-LT antagonism, indicating that FPL 55712 is not a specific antagonist to LTD₄ and LTE₄ or p-LTs. In addition to this, FPL 55712 significantly lowered the resting tonus of the isolated guinea pig trachea, while MCI-826 had little effect on it.

Taken together, these results indicate that in contrast to FPL 55712, MCI-826 is a highly potent and selective LTD₄ and LTE₄ antagonist, which is expected to be a very useful tool for research on p-LT field and effective for treating bronchial asthma.

Acknowledgments

We thank Dr. K. Takahashi of Mitsubishi Kasei Co., Ltd. for providing MCI-826 and peptide leukotrienes and Mr. P. Sheard of Fisons Co., Ltd., for FPL 55712.

REFERENCES

1. Dahlén, S.-E., Hedqvist, P., Hammarström, S. and Samuelson, B.: Leukotrienes are potent constrictors of human bronchi. Nature 288, 484–486 (1980)
2. Weiss, J.W., Drazen, J.M., Coles, N., McFadden, E.R., Jr., Wellner, P.F., Corey, E.J., Lewis, R.A. and Austen, K.F.: Bronchoconstrictor effects of leukotriene C in humans. Science 216, 196–198 (1982)
3. Hanna, C.J., Bach, M.K., Pare, P.D. and Schellenberg, R.R.: Slow-reacting substances (leukotrienes) contract human airway and pulmonary smooth muscle in vitro. Nature 290, 343–344 (1981)
4. Dahlén, S.-E., Hansson, G., Hedqvist, P., Björck, T., Granström, E. and Dahlén, B.: Allergen challenge of lung tissue from asthmatics elicits bronchial contraction that correlates with the release of leukotrienes C₄, D₄ and E₄. Proc. Natl. Acad. Sci. U.S.A. 80, 1712–1716 (1983)
5. Maron, Z., Shelhamer, J.H., Bach, M.K., Morton, D.R. and Kaliner, M.: Slow-reacting substances, leukotrienes C₄ and D₄, increase the release of mucus from human airways in vitro. Am. Rev. Respir. Dis. 126, 449–451 (1982)
6. Coles, S.J., Neill, K.H., Reid, L.M., Austen, K.F., Nii, Y., Corey, E.J. and Lewis, R.A.: Effects of leukotrienes C₄ and D₄ on glycoprotein and lysozyme secretion by human bronchial mucosa. Prostaglandins 25, 155–170 (1983)
7. Drazen, J.M., Austen, K.F., Lewis, R.A., Clark, D.A., Goto, G., Marfat, A. and Corey, E.J.: Comparative airway and vascular activities of leukotriene C₄ and D in vivo and in vitro. Proc. Natl. Acad. Sci. U.S.A. 77, 4354–4358 (1980)
8. Augustin, J., Farmer, J.B., Lee, T.B., Sheard, P. and Tattersall, M.L.: Selective inhibitor of slow reacting substance of anaphylaxis. Nature (New Biol.) 245, 215–217 (1973)
9. Sheard, P., Lee, T.B. and Tattersall, M.L.: Further studies on the SRS-A antagonist, FPL 55712. Monogr. Allergy 12, 245–249 (1977)
10. Tate, S.S. and Meister, A.: Serine-borate complex as a transition-state inhibitor of y-glutamyl transpeptidase. Proc. Natl. Acad. Sci. U.S.A. 75, 4806–4809 (1978)
11. Morris, H.R., Taylor, G.W., Jones, C.M., Piper, P.J., Samhoun, M.N. and Tippins, J.R.: Slow reacting substances (leukotrienes): Enzymes involved in their biosynthesis. Proc. Natl. Acad. Sci. U.S.A. 79, 4383–4384 (1982)
12. Sok, D.-E., Pai, J.-K., Atrash, V. and Sih, C.J.: Characterization of slow reacting substances (SRSs) of rat basophilic leukemia (RBL-I) cells: Effect of cyscine on SRS-profile. Proc. Natl. Acad. Sci. U.S.A. 77, 6481–6485 (1980)
13. Krell, R.D., Tsai, B.S., Berdouley, A., Barone, M. and Giles, R.E.: Heterogeneity of leukotriene receptors in guinea-pig trachea. Prostaglandins 25, 171–178 (1983)
14. Snyder, D.W. and Krell, R.D.: Pharmacological evidence for...
a distinct leukotriene C₄ receptor in guinea-pig trachea. J. Pharmacol. Exp. Ther. 231, 616–622 (1984)

15 Hogaboom, G.K., Mong, S., Wu, H.-L. and Crooke, S.T.: Peptide leukotrienes: Distinct receptors for leukotriene C₄ and D₄ in the guinea-pig lung. Biochem. Biophys. Res. Commun. 116, 1136–1143 (1983)

16 Cheng, J.B. and Townley, R.G.: Evidence for similar receptor site for binding of [³H]leukotriene E₄ and [³H]leukotriene D₄ to the guinea-pig crude lung membrane. Biochem. Biophys. Res. Commun. 122, 949–954 (1984)

17 Mong, S., Scott, M.O., Lewis, M.A., Wu, H.-L., Hogaboom, G.K., Clark, M.A. and Crooke, S.T.: Leukotriene E₄ binds specifically to LTD₄ receptors in guinea pig lung membranes. Eur. J. Pharmacol. 109, 183–192 (1985)

18 Cheng, J.B. and Townley, R.G.: Identification of leukotriene D₄ receptor binding sites in guinea pig lung homogenate using [³H]leukotriene D₄. Biochem. Biophys. Res. Commun. 118, 20–26 (1984)