Peptide Leukotriene Antagonistic Activity of AS-35, a New Antiallergic Drug

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ABSTRACT — The effects of 9-[(4-acetyl-3-hydroxy-2-n-propylphenoxy)methyl]-3-(1H-tetrazol-5-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (AS-35), a newly synthesized compound, on leukotrienes (LTs) antagonistic activities were investigated in vitro and in vivo. In isolated guinea pig preparations, AS-35 antagonized LTC4-, LTD4 and LTE4-induced contractions of the ileum with IC50 values of 8 nM, 4 nM and 3 nM, respectively. In the trachea, the agent also antagonized LTD4- and LTE4-induced contractions with IC50 values of 10 nM and 20 nM, respectively. However, LTC4-induced tracheal contraction in the presence of L-serine borate was not antagonized by AS-35. Histamine-, acetylcholine-, serotonin and bradykinin-induced contractions of the ileum, carbachol-, prostaglandin D2-, prostaglandin F2α-induced contractions of the trachea and LTE4-induced chemotaxis of rat polymorphonuclear leukocytes were not inhibited by AS-35. As to the in vivo models, AS-35 (i.v.) dose-dependently antagonized bronchoconstriction induced by i.v.-injection of LTC4 and LTD4 in anesthetized guinea pigs, but did not inhibit histamine-induced bronchoconstriction. Oral administration of AS-35 also antagonized LTD4 as well as antigen-induced LT-mediated bronchoconstriction. In addition, LTD4-induced increase in the cutaneous vascular permeability of guinea pig was inhibited by the drug (p.o.). These results indicate that AS-35 is an orally effective, potent and selective peptide LT antagonist.

Extensive studies have demonstrated that peptide leukotrienes (LTs) such as LTC4 and LTD4 play a major role in the pathogenesis of allergic disorders (1–13).

Peptide LTs are released immunologically or non-immunologically from the lung tissue of a variety of species including humans, and they display various biological activities. For example, peptide LTs were shown to possess much more potent bronchoconstriction activity than histamine or methacholine, to induce secretion of bronchial mucus and to increase vascular permeability. Furthermore, the severity of the allergic symptoms in asthmatic patients was shown to correlate with LTs levels in plasma, sputum and bronchial lavages (14–17). These findings suggest that LTs antagonists may be effective in controlling bronchial asthma. Although FPL-55712 was first reported to be a specific LT antagonist, this compound was not used clinically because of its brief biological half-life (18, 19).

In the present study, the effects of a newly synthesized LT antagonist, 9-[(4-acetyl-3-hydroxy-2-n-propylphenoxy)methyl]-3-(1H-tetrazol-5-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (AS-35, see structural formula shown in Fig. 1) on isolated smooth muscle and bronchoconstriction were mainly investigated in guinea pigs. The results obtained here indicate that AS-35
CH₃C
\[ \text{O} \]
\[ \text{HO} \]
\[ \text{C}_3\text{H}_7 \]
\[ \text{OCH}_2 \]
\[ \text{N} \]
\[ \text{O} \]
\[ \text{N} \]
\[ \text{H} \]

Fig. 1. Chemical structure of AS-35.

is an orally effective, potent and selective peptide LT antagonist.

MATERIALS AND METHODS

Animals
Male Hartley guinea pigs weighing 270 to 600 g and male Sprague-Dawley rats weighing 250 to 330 g were used. Guinea pigs were purchased from Tokyo Laboratory Animals and rats, from Charles River.

Chemicals
AS-35 (Tokyo Tanabe) is a pale yellowish white crystalline powder, insoluble in water. FPL-55712 was synthesized by Tokyo Tanabe Company. Other chemicals used were as follows: acetylcholine chloride, serotonin creatinine, bradykinin, carbamylcholine chloride (carbachol), boric acid, LTC₄, LTD₄, LTE₄, and histamine dihydrochloride (Wako); propranolol hydrochloride, pyrilamine maleate, indomethacin, prostaglandin D₂ (PGD₂), and prostaglandin F₂α (PGF₂α) (Sigma); l-serine and l-cysteine (Nihon Rikagaku); and LTB₄ (Paesel GmbH & Co.). AS-35 was dissolved in dimethyl sulfoxide or potassium hydroxide and diluted in saline or Tyrode’s solution. PGs and LTs were dissolved in ethanol and methanol, respectively, and they were diluted in saline or Tyrode’s solution before use.

Contractile studies on isolated preparations
Guinea pigs were sacrificed by bloodletting, and the ileum and trachea were removed. The ileum was divided into small segments of approximately 2 to 3 cm, and then the segments were transferred to organ baths and maintained for 1 hr at 30°C under a load of 0.5 g. Tracheal strip chains were prepared according to the method of Takagi and Takayanagi (20) and equibrated for 1 hr at 37°C under a load of 1.5 g. Isolated preparations were suspended in 5–10 ml organ baths containing Tyrode’s or modified Krebs’ solution (4.6 mM KCl, 10 mM KH₂PO₄, 1.1 mM MgSO₄, 118 mM NaCl, 24.9 mM NaHCO₃, 11.1 mM glucose, 1.8 mM CaCl₂), which was gassed with a mixture of 95% O₂ and 5% CO₂. Indomethacin (3 μM) was added to the buffer to inhibit the release of bronchoactive prostanoids when trachea was used. In some experiments, l-serine borate (45 mM), an inhibitor of γ-glutamyltranspeptidase, or l-cysteine (3 mM), an aminopeptidase inhibitor, was added to prevent the metabolism of LTC₄ to LTD₄ and LTD₄ to LTE₄, respectively. Contractile responses of the ileum and trachea were measured with a model TD-112S isotonic transducer (Nihon Kohden). For LT antagonist studies, the LTs responses of the tissues treated with drugs were compared with mean values of the control responses which were obtained before and after the treatment of drugs. LTs were applied 3 to 5 times in each preparation at 30- or 40-min intervals for the ileum and at 60- to 90-min intervals for trachea after washing.

Determination of IC₅₀ values and dissociation constants
LT antagonistic activity of AS-35 was evaluated in terms of the concentration of AS-35 at which the contraction of the ileum and trachea induced by LTs was inhibited by 50%. Ileum or trachea was pretreated with AS-35 for 30 sec or 5 min before the addition of agonist. In the experiment with trachea, the dissociation constant (Kₐ) was calculated by the method of Furchgott (21) using the equation Kₐ = [antagonist]/dose ratio − 1. Dose ratio refers to the concentration of agonist required to elicit 50% of the maximum response in the presence of antagonist.
Bronchoconstriction induced by LTs and histamine

Guinea pigs were anesthetized with i.p.-injection of urethane (1.5 g/kg), trachea was cannulated for the measurement of bronchoconstriction, and spontaneous breathing was fully arrested with gallamine triethiodide (1 mg/kg, i.v.). Animals were then ventilated with a small animal respirator (Ugo Basile) at the rate of 70 breaths/min (5.0 to 8.5 ml of stroke volume). Airflow was measured with a bronchospasm transducer (Ugo Basile) connected to a side arm of the tracheal cannula and expressed as a percentage of the maximum bronchoconstriction obtained by clamping off the trachea. LTC4 (2 μg/kg), LTD4 (0.5 μg/kg) or histamine (5 μg/kg) was injected into the jugular vein.

Antigen-induced LT-mediated bronchoconstriction

Guinea pigs were passively sensitized with i.v.-injection of 0.15 ml/kg of homologous anti-dinitrophenyl (DNP) IgE serum (1:1024 of 8 day homologous passive cutaneous anaphylaxis titer) which had been prepared as reported (22). After 48 hr, they were treated with i.v.-injection of 3 mg/kg of indomethacin, 5 mg/kg of pyrilamine and 1 mg/kg of propranolol, which were given 10 min, 5 min and 5 min before the antigen challenge, respectively. Bronchoconstriction was then induced by i.v.-injection of 1 mg/kg of DNP-conjugated bovine serum albumin, and airflow was measured as described above.

Cutaneous vascular permeability induced by LTD4

Guinea pigs were injected with 10 ng/site of LTD4 into the shaved back immediately after i.v.-injection of 1 ml/body of 1% Evans blue solution. The animals were killed 30 min after the injection, and the skin samples were removed for the colorimetric measurement of the blueing spot. The amount of the dye leaked was determined according to the method of Katayama et al. (23).

Chemotaxis of rat polymorphonuclear leukocytes (PMNs) induced by LTB4

Rats were injected i.p. with 2% casein, and peritoneal fluids were entirely collected 14–16 hr after the injection and centrifuged at 720 × g for 4 min. The cell pellet was washed with Hank's balanced solution and adjusted to a concentration of 4 × 10⁶ cells/ml in Eagle's MEM medium containing 10% fetal calf serum. Chemotactic activity was determined by using Boyden chemotactic chamber (Sanki Kogei). Briefly, 1 ml of LTB4 (10⁻⁸ M) was placed in the lower compartment of the chamber, and the nuleopore membrane (2.0 μm pore size, 25 mm diameter) was then attached. Finally, 1 ml of casein-induced PMNs, which was preincubated with AS-35 or FPL-55712 at 37°C for 15 min, was applied to the upper compartment. The chamber was incubated at 37°C in an atmosphere of 5% CO₂ and 95% air. After 2 hr, the cells on the bottom glass coverslip passed through the membrane were counted in 5 random 300× microscopic fields.

Statistical analysis

Results are expressed as the mean ± S.E. Statistical significance was determined by Student's t-test.

RESULTS

Effect on LTs-induced contractions of isolated guinea pig smooth muscle

The antagonistic activities of AS-35 against peptide LTs were first evaluated in guinea pig ileum and trachea. As summarized in Table 1, AS-35 strongly antagonized the contractions of ileum induced by 2 nM LTC4, 0.8 nM LTD4 and 9 nM LTE4; IC₅₀ values against LTC4, LTD4 and LTE4 were 8 nM, 4 nM and 3 nM, respectively. FPL-55712 also blocked LTC₄-, LTD₄- and LTE₄-induced contractions of the ileum, although its potencies against peptide LTs were weaker than those of AS-35. Similarly, AS-35 antagonized the contractile responses of trachea induced by 1 nM LTD4 and 10 nM LTE4. IC₅₀ values against LTD₄ and LTE₄ were 10 nM and 20 nM for AS-35.
nM and 80 nM for FPL-55712, respectively. In contrast, the contraction of trachea induced by 1 nM LTC₄ in the presence of 45 mM L-serine borate was not inhibited by AS-35. However, AS-35 inhibited the LTC₄-induced contraction with an IC₅₀ value of 40 nM in the absence of L-serine borate. Next, the specificity of action of AS-35 was assessed by using various agonists. AS-35 had no effects on the contractions induced by histamine (10 ng/ml), acetylcholine (10 ng/ml), serotonin (10 μg/ml), bradykinin (0.1 μg/ml), carbachol (0.1 μM), PGE₂ (0.3 μM) and PGF₂α (0.3 μM) (Table 1).

Table 1. Effect of AS-35 and FPL-55712 on contractions of isolated guinea pig ileum and trachea elicited by various pharmacologic agonists

| Agonist        | Concentration | Preparation | IC₅₀ (μM) |
|----------------|---------------|-------------|-----------|
|                |               |             | AS-35     | FPL-55712 |
| LTC₄           | 2 nM          |             | 0.008     | 0.2       |
| LTD₄           | 0.8 nM        |             | 0.004     | 0.06      |
| LTE₄           | 9 nM          |             | 0.003     | 0.04      |
| Histamine      | 10 ng/ml      | Ileum       | > 10      | > 10      |
| Acetylcholine  | 10 ng/ml      |             | > 10      | > 10      |
| Serotonin      | 10 μg/ml      |             | > 10      | > 10      |
| Bradykinin     | 0.1 μg/ml     |             | > 10      | NT        |
| LTC₄ (+ serine borate) | 1 nM |             | 0.04      | NT        |
| LTD₄           | 1 nM          |             | > 10      | 5         |
| LTE₄           | 10 nM         | Trachea     | 0.02      | 0.08      |
| Carbachol      | 0.1 μM        |             | > 10      | NT        |
| Prostaglandin D₂ | 0.3 μM   |             | > 10      | NT        |
| Prostaglandin F₂α | 0.3 μM |             | > 10      | NT        |

NT: not tested. Contractile responses of isolated guinea pig ileum and trachea suspended in an organ bath were measured with an isotonic transducer. Each value represents the mean of 3 or 4 experiments. Details are described in Materials and Methods.

Effect on cumulative concentration-response curve to LTD₄ in trachea

Contractile responses are expressed as a percentage of the maximum response induced by 30 μM carbachol. Figure 2 illustrates the effect of AS-35 and FPL-55712 on the cumulative concentration-response curve obtained by successive increases in the bath concentration of LTD₄. Cumulative concentration-response curves for LTD₄ in trachea were effectively shifted to the right by AS-35 (0.1 and 0.3 μM) with Kᵦ values of 23 ± 4 nM and 36 ± 9 nM, respectively, suggesting that AS-35 antagonizes LTD₄ in a competitive manner. Similar results were obtained by FPL-55712 (1 and 3 μM) with Kᵦ values of 325 ± 161 nM and 325 ± 91 nM, respectively.

Effect on bronchoconstriction induced by LTs and histamine in anesthetized guinea pigs

As indicated in Fig. 3, the bronchoconstriction induced by i.v.-injection of LTD₄ was inhibited in a dose-dependent fashion by AS-35 (0.0375 to 0.3 mg/kg, i.v.) and FPL-55712 (0.15 to 1.2 mg/kg, i.v.) when the drugs were administered 2 min before the LTD₄ challenge. ID₅₀ values were 97 μg/kg for AS-35 and 488 μg/kg for FPL-55712, respectively (Fig. 3A). LTC₄-induced bronchoconstriction was also inhibited dose-dependently by AS-35 (0.01 to 0.3 mg/kg, i.v.) and FPL-55712 (0.1 to 3.0 mg/kg, i.v.), which were given 2 min
before challenge. ID₅₀ values of AS-35 and FPL-55712 were 38 μg/kg and 790 μg/kg, respectively (Fig. 3). Note that the inhibitory activity of AS-35 on the LTD₄ or LTC₄-induced bronchoconstrictions was much higher than that of FPL-55712. In addition, AS-35 (3 to 30 mg/kg), administered orally 2 hr before challenge with LTD₄, produced dose-dependent inhibition of the bronchoconstriction, but FPL-55712 (30 mg/kg, p.o.) did not (Fig. 3C). On the other hand, histamine-induced bronchoconstriction was inhibited by FPL-55712 (10 mg/kg, i.v.), but not by AS-35 (1 and 10 mg/kg, i.v.) (Table 2).

**Effect on antigen-induced LT-mediated bronchoconstriction in anesthetized guinea pigs**

As shown in Fig. 4, antigen challenge produced typical anaphylactic bronchoconstriction, and air overflow volume nearly reached a plateau at 6 min after challenge. Oral administration of AS-35 (3 to 30 mg/kg) 2 hr prior to antigen challenge resulted in the dose-dependent inhibition of endogenous LT-mediated bronchoconstriction, and the significant inhibition was observed with the drug at a dose of 10 mg/kg and over. The ID₅₀ value of AS-35 at 12 min after challenge was 12.0 mg/kg.
Table 2. Effect of intravenous administration of AS-35, FPL-55712 and pyrilamine on histamine-induced bronchoconstriction in anesthetized guinea pigs

| Drug        | Dose (mg/kg, i.v.) | No. of Animals | Air overflow volume (%) |
|-------------|--------------------|----------------|-------------------------|
| Control     |                    | 6              | 89.4 ± 2.47             |
| AS-35       | 1                  | 5              | 93.2 ± 2.29             |
|             | 10                 | 5              | 89.5 ± 1.15             |
| FPL-55712   | 10                 | 5              | 44.5 ± 7.86**           |
| Pyrilamine  | 0.1                | 5              | 12.1 ± 6.73**           |

Drugs were administered 2 min before histamine (5 μg/kg, i.v.) injection. Each column represents the mean ± S.E. **: Statistically significant difference from the control at P < 0.05.

Fig. 5. Effect of AS-35 on LTD4-induced cutaneous reaction in guinea pigs. AS-35 was administered p.o. 2 hr before LTD4 (10 ng/site, i.d.) injection. Each column represents the mean ± S.E. of 5 or 6 animals. **: Statistically significant difference from the control at P < 0.05 and P < 0.01, respectively.

Effect on LTD4-induced cutaneous vascular permeability in guinea pigs

The results are shown in Fig. 5. Intradermal injection of LTD4 produced marked increases in vascular permeability. Oral administration of AS-35 (3 to 30 mg/kg) 2 hr before LTD4 injection produced dose-dependent inhibition of the vascular permeability induced by LTD4.

Effect on chemotaxis of rat PMNs induced by LTB4

LTB4 at a concentration of 10^{-8} M was selected from the preliminary experiment. The number of migrating cells in the control counted after the incubation for 2 hr was 290 ± 32. Neither AS-35 nor FPL-55712 at concentrations of 10^{-8} to 10^{-5} M inhibited chemotaxis of rat PMNs induced by LTB4 (Table 3).
The present results clearly demonstrate that AS-35, a newly synthesized compound, is an orally effective, potent and selective peptide LT antagonist. Indeed, AS-35 antagonized the contractions induced by LTC₄, LTD₄ and LTE₄ in isolated guinea pig ileum or trachea more effectively than FPL-55712. Concentration-response curves for LTD₄ in the trachea were shifted in the presence of AS-35 to the right in a concentration-related manner without depressing the maximal response, thus suggesting that AS-35 is competitive with LTD₄. In contrast, AS-35 failed to antagonize the LTC₄-induced contraction in isolated trachea in the presence of L-serine borate, known to prevent the conversion of LTC₄ to LTD₄ (24). As to LTC₄-induced contraction in the absence of L-serine borate, it was inhibited by AS-35, and a consistent result was also obtained with FPL-55712 as already reported (24). Failure of antagonism of AS-35 against LTC₄-induced contraction in the presence of L-serine borate in trachea does not result from its interaction with L-serine borate, because the inhibition of LTD₄-induced contraction by AS-35 was not affected by L-serine borate in the preliminary experiment (data not shown). These data suggest that when the conversion of LTC₄ to LTD₄ and subsequently to LTE₄ is blocked by L-serine borate in the trachea, AS-35 is ineffective in antagonizing the action of LTC₄. The result obtained in the trachea seems to be contradictory to that in the ileum, because LTC₄-induced contraction of the ileum was antagonized by AS-35. LTC₄ has been shown to display an inherent spasmodic activity and to be predominantly independent of its bioconversion to LTD₄ in the ileum (25). Thus L-serine borate was not added to the medium when the ileum was used. The reason for the difference between the ileum and trachea in the LTC₄-antagonizing activity of AS-35 is still unknown. However, in human airway smooth muscle, contractions induced by LTC₄, LTD₄ and LTE₄ were found to occur via activation of a homogenous receptor population (26).

The specificity of action of AS-35 was shown by the results that contractile responses to histamine, acetylcholine, serotonin, bradykinin, carbachol, PGD₂ and PGF₂α were not antagonized in guinea pig ileum or trachea by AS-35. Chemotaxis of rat PMNs induced by LTB₄ was also not inhibited by AS-35. On the other hand, FPL-55712 at a high dose of 10 mg/kg (i.v.) but not AS-35 at the same dose inhibited histamine-induced bronchoconstriction. High doses of FPL-55712 have been reported to non-specifically inhibit histamine-, serotonin- and acetylcholine-induced contractions of guinea pig ileum (18). Therefore, the specificity of AS-35 in antagonizing peptide LTs was considered to be higher than that of FPL-55712.

LTD₄-induced bronchoconstriction in anesthetized guinea pigs was dose-dependently inhibited by i.v.-injection of AS-35, and its potency was approximately 5 times more than that of FPL-55712. In addition, this bronchoconstriction was inhibited by oral administration of AS-35. Of interest was the observation that i.v.-administration of AS-35 inhibited

| Drug  | Concentration (M) | Number of migrating cells |
|-------|-------------------|--------------------------|
| Control | —                 | 290 ± 32                 |
| AS-35   | 10⁻⁸              | 281 ± 28                 |
|        | 10⁻⁷              | 290 ± 38                 |
|        | 10⁻⁶              | 275 ± 28                 |
|        | 10⁻⁵              | 299 ± 35                 |
| FPL-55712 | 10⁻⁸          | 286 ± 31                 |
|        | 10⁻⁷              | 283 ± 38                 |
|        | 10⁻⁶              | 281 ± 22                 |
|        | 10⁻⁵              | 272 ± 29                 |

The Boyden chamber was incubated at 37°C for 2 hr, and the number of cells on the bottom glass coverslip the ones that had passed through the membrane, were counted. Drugs were preincubated with rat PMNs at 37°C for 15 min. Each value represents the mean ± S.E. of 5 experiments.

The present results clearly demonstrate that AS-35, a newly synthesized compound, is an orally effective, potent and selective peptide LT antagonist. Indeed, AS-35 antagonized the contractions induced by LTC₄, LTD₄ and LTE₄ in isolated guinea pig ileum or trachea more effectively than FPL-55712. Concentration-response curves for LTD₄ in the trachea were shifted in the presence of AS-35 to the right in a concentration-related manner without depressing the maximal response, thus suggesting that AS-35 is competitive with LTD₄. In contrast, AS-35 failed to antagonize the LTC₄-induced contraction in isolated trachea in the presence of L-serine borate, known to prevent the conversion of LTC₄ to LTD₄ (24). As to LTC₄-induced contraction in the absence of L-serine borate, it was inhibited by AS-35, and a consistent result was also obtained with FPL-55712 as already reported (24). Failure of antagonism of AS-35 against LTC₄-induced contraction in the presence of L-serine borate in trachea does not result from its interaction with L-serine borate, because the inhibition of LTD₄-induced contraction by AS-35 was not affected by L-serine borate in the preliminary experiment (data not shown). These data suggest that when the conversion of LTC₄ to LTD₄ and subsequently to LTE₄ is blocked by L-serine borate in the trachea, AS-35 is ineffective in antagonizing the action of LTC₄. The result obtained in the trachea seems to be contradictory to that in the ileum, because LTC₄-induced contraction of the ileum was antagonized by AS-35. LTC₄ has been shown to display an inherent spasmodic activity and to be predominantly independent of its bioconversion to LTD₄ in the ileum (25). Thus L-serine borate was not added to the medium when the ileum was used. The reason for the difference between the ileum and trachea in the LTC₄-antagonizing activity of AS-35 is still unknown. However, in human airway smooth muscle, contractions induced by LTC₄, LTD₄ and LTE₄ were found to occur via activation of a homogenous receptor population (26).

The specificity of action of AS-35 was shown by the results that contractile responses to histamine, acetylcholine, serotonin, bradykinin, carbachol, PGD₂ and PGF₂α were not antagonized in guinea pig ileum or trachea by AS-35. Chemotaxis of rat PMNs induced by LTB₄ was also not inhibited by AS-35. On the other hand, FPL-55712 at a high dose of 10 mg/kg (i.v.) but not AS-35 at the same dose inhibited histamine-induced bronchoconstriction. High doses of FPL-55712 have been reported to non-specifically inhibit histamine-, serotonin- and acetylcholine-induced contractions of guinea pig ileum (18). Therefore, the specificity of AS-35 in antagonizing peptide LTs was considered to be higher than that of FPL-55712.

LTD₄-induced bronchoconstriction in anesthetized guinea pigs was dose-dependently inhibited by i.v.-injection of AS-35, and its potency was approximately 5 times more than that of FPL-55712. In addition, this bronchoconstriction was inhibited by oral administration of AS-35. Of interest was the observation that i.v.-administration of AS-35 inhibited
LTC₄-induced bronchoconstriction. This is inconsistent with the in vitro observation that AS-35 failed to inhibit the LTC₄-induced contraction in trachea in the presence of L-serine borate. Similar results were obtained with FPL-55712 (27). It is considered that LTC₄ is rapidly converted to LTD₄ by γ-gulutamyl transpeptidase (28). In fact, this is supported by the in vitro finding that [³H]-LTC₄ is rapidly metabolized to [³H]-LTD₄ and [³H]-LTE₄ by guinea pig lung parenchyma (28, 29). Therefore, it is suggested that AS-35 or FPL-55712 might block the response to the converted LTD₄ in LTC₄-induced bronchoconstriction. In addition, antigen-induced bronchoconstriction in guinea pigs pretreated with indomethacin, pyrilamine and propranolol was blocked by oral administration of AS-35. Anderson et al. (30) have demonstrated that this response is mediated by endogenous peptide LTs. Accordingly, inhibition of antigen-induced bronchoconstriction by AS-35 results from the inhibition of endogenous LTs released by antigen stimulation. Oral administration of AS-35 also inhibited the increase of guinea pig vascular permeability caused by intradermal injection of LTD₄. Since LTD₄ is not considered to be dependent on the release of endogenous histamine, serotonin and products of the cyclooxygenase pathway (31, 32), it is clear that AS-35-mediated inhibition is based on the ability to block peptide LTs-induced responses both in the skin and airway.

In summary, AS-35 is a novel, potent and selective peptide LT antagonist that shows higher activity than FPL-55712. In addition, this compound is orally effective in preventing the responses induced by exogenous and endogenous peptide LTs. Therefore, AS-35 may be therapeutically useful in bronchial asthma, in which peptide LTs are highly implicated in the pathogenesis of allergic disorders.

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