In Vivo Pharmacologic Profile of ONO-1078: A Potent, Selective and Orally Active Peptide Leukotriene (LT) Antagonist

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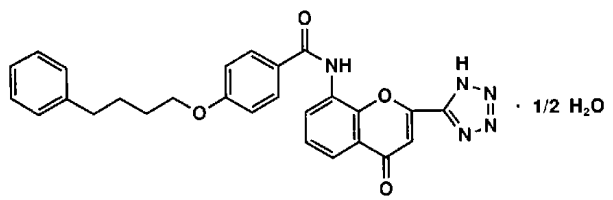
ABSTRACT—We investigated the in vivo antagonistic activity of ONO-1078 against peptide leukotrienes (LTs) in guinea pigs. ONO-1078, when administered p.o. (0.3–3 mg/kg), caused a dose-dependent reduction of LTC_4-, LTD_4- and LTE_4-induced bronchoconstriction, LTD_4-induced airway microvascular leakage and LTD_4-induced increase in cutaneous vascular permeability. When administered intravenously, ONO-1078 (3–30 μg/kg) inhibited these responses approximately 200–600 fold more potently than FPL55712. When guinea pigs were treated with indomethacin to examine the antagonism of ONO-1078 on the direct action against peptide LTs, intravenous (3–30 μg/kg) and oral (0.3–3 mg/kg) administration of ONO-1078 also inhibited LTC_4- and LTD_4-induced bronchoconstriction, and its activity was approximately 300–500 fold more potent than that of FPL55712. ONO-1078 (10 mg/kg, i.v.) had no inhibitory effect on bronchoconstrictions induced by histamine, acetylcholine, serotonin, arachidonic acid, LTB_4, prostaglandin (PG) $F_{2\alpha}$, PGD_2, 9α,11β-PGF_2, a stable thromboxane A_2 mimetic agent and platelet activating factor. Furthermore, oral administration of ONO-1078 (1–10 mg/kg) inhibited slow-reacting substance of anaphylaxis mediated bronchoconstriction induced by antigen in a dose-dependent manner. These results indicate that ONO-1078 is an extremely potent, selective and orally active peptide LT antagonist and that oral administration of ONO-1078 antagonizes not only exogenously administered peptide LTs but also endogenous peptide LTs.

Keywords: ONO-1078, Leukotrienes (peptide) (LTC_4, LTD_4 and LTE_4), Bronchoconstriction, Vascular permeability, FPL55712

Peptide leukotrienes (LTs) C_4, D_4 and E_4, the main components of the slow-reacting substance of anaphylaxis (SRS-A) (1–3), potently contract airway smooth muscle, induce mucus secretion and increase vascular permeability (4–7). Furthermore, they are found in the sputum, urine, bronchoalveolar lavage fluid, plasma and nasal secretions from asthmatic patients (8–12). Accordingly, they have been suggested to play pathophysiological roles in asthma. Orally effective antagonists which prevent the activity of peptide LTs might be expected to be useful for the therapy of bronchial asthma. FPL55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4*H*-1-benzopyran-2-carboxylate) as the first peptide LT antagonist was discovered by Augstein et al. (13). However, the therapeutic potential of this compound was limited by its poor oral bioavailability and its short biological half life (14, 15).

Our recent efforts have focused on the design of high-affinity and orally active peptide LT antagonists from the derivatives of (*p*-amylcinnamoyl) anthranilic acid (16). This resulted in the discovery of 4-oxo-8-[4-(4-phenylbutoxy)benzoylamino]-2-(tetrazol-5-yl)-4*H*-1-benzopyran hemihydrate (ONO-1078) (Fig. 1) as a pep-



ONO-1078

Fig. 1. Chemical structure of ONO-1078, 4-oxo-8-[4-(4-phenylbutoxy)benzoylamino]-2-(tetrazol-5-yl)-4*H*-1-benzopyran hemihydrate.

tide LT antagonist. In this report, we describe the in vivo pharmacologic profile of ONO-1078.

MATERIALS AND METHODS

Animals

Male Hartley guinea pigs (Nihon Rabbit Co.), weighing 250 to 400 g, were used throughout the experiments. Animals were housed in a temperature- and humidity-controlled room (12 hr light and 12 hr dark cycle) with free access to food and water.

Chemicals

The following drugs and chemicals were used: LTE_4 (Wako Pure Chemical Co.); histamine dihydrochloride, ovalbumin (OA, grade III), indomethacin and arachidonic acid sodium salt (Sigma Chemical Co.); serotonin creatinine sulfate (Merck Co.); acetylcholine chloride (Ovisot®; Daiichi Pharmaceutical Co.); mepyramine maleate (May & Baker Co.); killed organisms *Bordetella pertussis* (The Chemo-Sero-Therapeutic Research Institute); ketotifen fumarate (Zaditen®; Sankyo Co.); azelastine hydrochloride (Azeptin®; Eisai Co.); Evans blue (Tokyo Kasei Co.); polyoxyethyleneglycerin trioxystearic acid 40 (HCO-40) as a cationic surfactant (Nikko Chemical Co.); formamide (Katayama Chemical Co.). ONO-1078, FPL55712, LTB_4, LTC_4, LTD_4, prostaglandin (PG) $F_{2\alpha}$, PGD_2, $9\alpha,11\beta$-PGF_2, a stable thromboxane A_2 mimetic agent (STA_2) and platelet activating factor (PAF) were synthesized by Ono.

LTC_4, LTD_4 and LTE_4 were dissolved in 50% ethanol, and diluted with 1/15 M phosphate-buffered solution (pH 7.4). For the experiment of cutaneous vascular permeability, LTD_4 was diluted with 0.9% NaCl. Mepyramine maleate, OA and Evans blue were dissolved in 0.9% NaCl. Indomethacin was dissolved in 7% sodium bicarbonate solution. FPL55712, ketotifen fumarate and azelastine hydrochloride were dissolved in distilled water, and ONO-1078 for oral administration was suspended in 0.5% sodium carboxymethylcellulose solution. ONO-1078 for intravenous administration was initially dissolved in a solution of equimolar 1 N NaOH and 50% ethanol, prepared to a concentration of 50 mg/ml as a stock solution, and then diluted with 5% ethanol / 1% HCO-40 / 0.9% NaCl before use.

Measurement of bronchoconstriction

The method was essentially a modification of the Konzett-Rössler (17) technique. Guinea pigs were anesthetized with sodium pentobarbital (75 mg/kg, i.p.). A small cannula was inserted into trachea. The right jugular vein was cannulated for the administration of drugs and antigen. The tracheal cannula was connected to a constant volume respirator (Model SN-480-7®, Shinano Apparatus), and the animals were artificially ventilated with a constant volume of 5 ml at a frequency of 70 strokes/min. Changes in insufflation pressure at a constant airflow were measured by a pressure transducer (MFP-1T®, MFP-1100®; Nihon Kohden) connected to the side-arm of the tracheal cannula. Spasmogen- or antigen-induced bronchoconstriction was measured for 10 or 15 min and represented as a percentage of the maximal increase in insufflation pressure achieved by clamping-off the trachea.

Spasmogen-induced bronchoconstriction

Bronchoconstrictions were induced by intravenous administration of the spasmogens: LTC_4 (2 μg/kg), LTD_4 (2 μg/kg), LTE_4 (5 μg/kg), histamine (10 μg/kg), acetylcholine (30 μg/kg), serotonin (10 μg/kg), arachidonic acid (1000 μg/kg), LTB_4 (20 μg/kg), $PGF_{2\alpha}$ (300 μg/kg), PGD_2 (100 μg/kg), $9\alpha,11\beta$-PGF_2 (300 μg/kg), STA_2 (3 μg/kg) and PAF (0.3 μg/kg). Indomethacin (2 mg/kg), to eliminate the contribution of thromboxane A_2 (TXA_2) to peptide LTs-induced bronchoconstriction (18, 19), was administered intravenously 2 min before LTC_4 or LTD_4 challenge. ONO-1078, azelastine and ketotifen were administered orally 1 hr before spasmogen challenge. FPL55712 and ONO-1078 were administered intravenously 1 min before spasmogen challenge.

Antigen-induced SRS-A-mediated bronchoconstriction

Guinea pigs were actively sensitized by intraperitoneal administration (0.5 ml) of 1 mg OA containing 5×10^9 killed organisms of *Bordetella pertussis* on day 0. On days 14 to 18, the sensitized animals were pretreated with intravenous administration of indomethacin (2 mg/kg) at 3 min and mepyramine (1 mg/kg) at 2 min before OA challenge to eliminate the contribution by endogenous PGs, TXA_2 and histamine, and challenged with intravenous administration of OA (0.5 mg/kg). ONO-1078 was administered orally 1 hr before OA challenge.

LTD_4-induced airway microvascular leakage

Guinea pigs were anesthetized with sodium pentobarbital at the dose of 75 mg/kg, intraperitoneally. A tracheal cannula was inserted and attached to a constant volume respirator (Model SN-480-7®, Shinano Apparatus). Animals were respirated with a constant volume of 5 ml at a rate of 70 strokes/min. A catheter was inserted into the jugular vein for the administration of LTD_4 and Evans blue. One min after the administration of Evans blue (20 mg/kg), the animal was challenged with LTD_4 (2 μg/kg) and 10 min later, exsan-

guinated. The chest cavity was opened, and Evans blue dye was washed out by perfusing with 0.9% NaCl from the pulmonary artery into the left atrium. The airways and lung were then removed and cleared of extraneous connective tissues. The extrapulmonary airway was separated into trachea and main bronchi, and the intrapulmonary airways stripped of parenchyma. Wet weights of all tissues were taken. Evans blue dye was extracted by 2 ml of formamide for more than 24 hr. The amount of Evans blue dye was determined by measuring the optical density at 620 nm with a spectrophotometer (UV-240®, Shimadzu) and expressed as ng/mg tissue. The baseline was determined by the administration of 0.9% NaCl containing Evans blue dye. ONO-1078 was given orally 1 hr before LTD_4 challenge.

LTD_4-induced increase in cutaneous vascular permeability in conscious guinea pigs

The backs of guinea pigs were shaved with an electric clipper 3 hr before the experiment. Evans blue (20 mg/ml) in 0.5 ml of 0.9% NaCl was administered intravenously to the guinea pigs and immediately, LTD_4 (50 ng/site) and 0.9% NaCl (to indicate the amount of vascular leakage that occurs as an injection artifact) were injected intradermally to the backs of the animals. The animals were challenged with LTD_4 and 30 min later, exsanguinated. The dorsal skin was removed, and the site of Evans blue dye leaked was placed in 1 ml of 1 N KOH and incubated at 37°C for 16 to 20 hr. After addition of 6.5 ml of acetone and 2.5 ml of 0.6 N H_3PO_4, the mixture was vortexed and then filtered through filter paper (ADVANTEC No. 2, Toyo Roshi). The optical density of the filtrate at 620 nm was measured with a spectrophotometer (UV-240®, Shimadzu). The concentration of Evans blue dye leaked was calculated from a standard curve constructed with four known concentrations of Evans blue dye. ONO-1078 and FPL55712 were administered intravenously 1 min before LTD_4 injection, and ONO-1078 for oral administration was given 1 hr before LTD_4 injection.

Statistical analyses

Results were expressed as the mean ± standard error (S.E.). Either Student's *t*-test or two-way analysis of variance followed by Dunnett's *t*-test was used to determine statistical significance ($P < 0.05$) between means.

RESULTS

Effect of ONO-1078 on peptide LT-induced bronchoconstriction in guinea pigs

Intravenous administration of peptide LTs produced a biphasic bronchoconstriction with peaks that appeared at 30–60 sec and 4–5 min. Intravenous and oral administration of ONO-1078 inhibited LTC_4-, LTD_4- and LTE_4-induced bronchoconstriction in a dose-dependent manner (Figs. 2 and 3). FPL55712, when administered

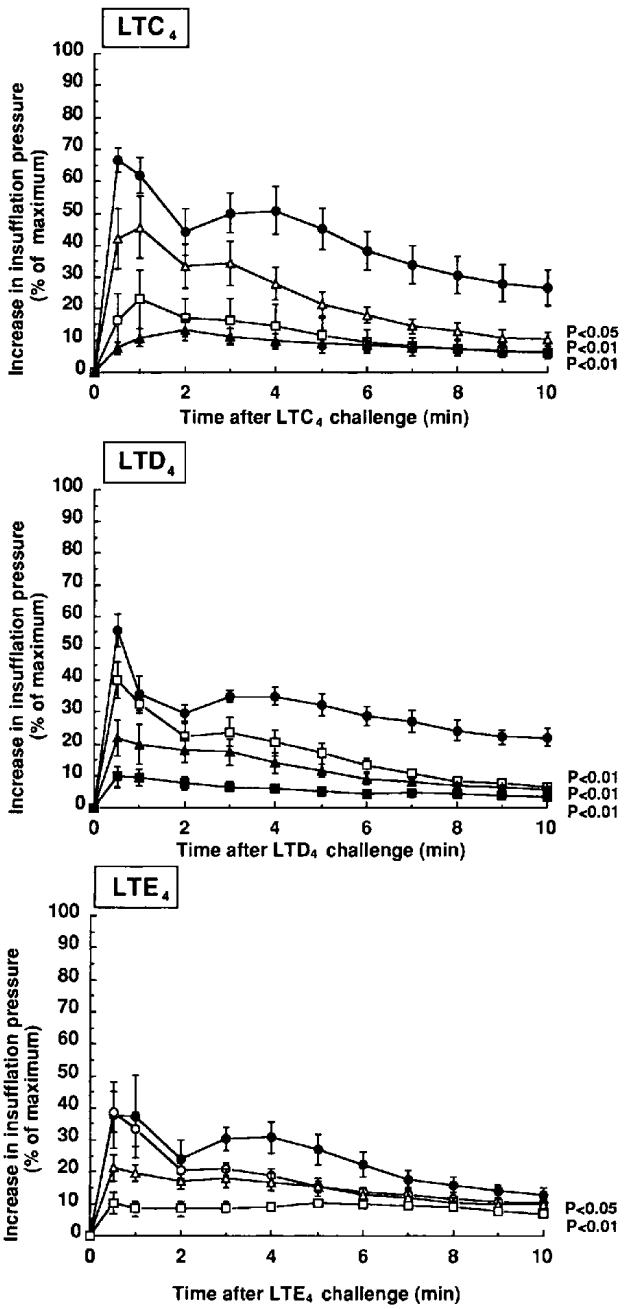


Fig. 2. Effect of ONO-1078 on LTC_4-, LTD_4- and LTE_4-induced bronchoconstriction in guinea pigs. Vehicle (●) or ONO-1078 (○: 1 μg/kg, △: 3 μg/kg, □: 10 μg/kg, ▲: 30 μg/kg, ■: 100 μg/kg) was administered intravenously 1 min before peptide LT challenge. Each value represents the mean ± S.E. of 5 animals. $P < 0.05$, $P < 0.01$: significant difference compared to the vehicle using two-way analysis of variance followed by Dunnett's *t*-test.

intravenously, inhibited these responses. Their ED_{50} values are shown in Table 1. Significant antagonistic activity of ONO-1078 (10 mg/kg, p.o.) against LTD_4 lasted for 3 hr (Fig. 4). Neither azelastine nor ketotifen inhibited LTD_4-induced bronchoconstriction at 10 mg/kg, p.o. (Fig. 5 and Table 1).

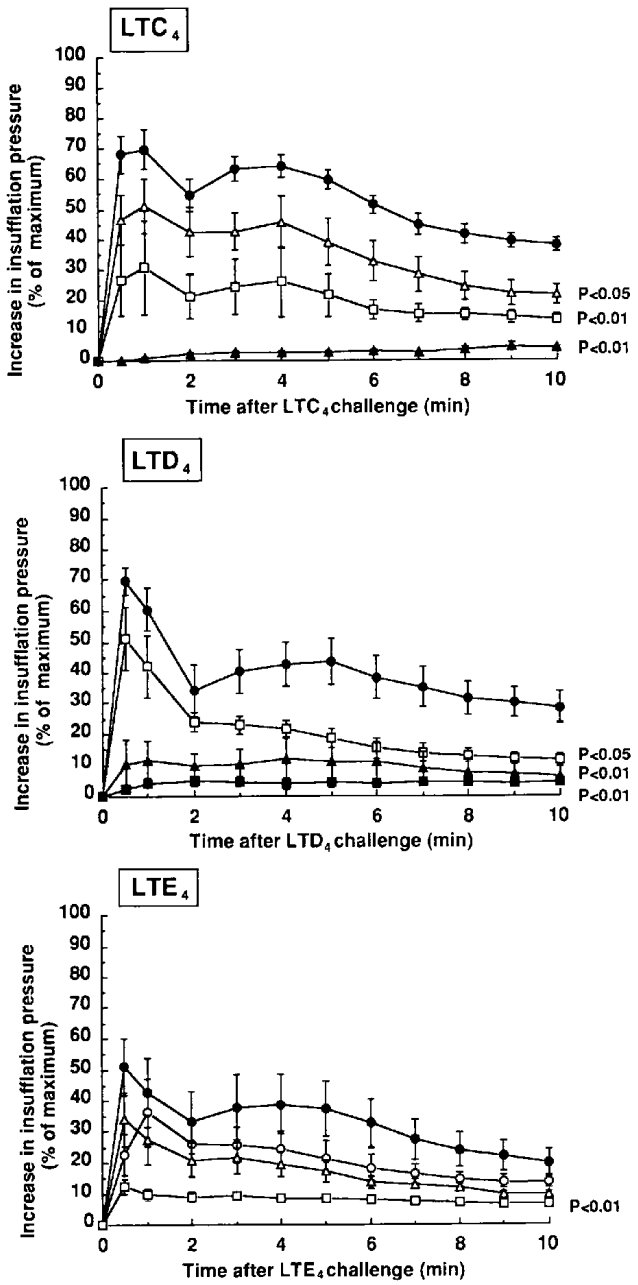


Fig. 3. Effect of ONO-1078 on LTC_4-, LTD_4- and LTE_4-induced bronchoconstriction in guinea pigs. Vehicle (●) or ONO-1078 (○: 0.1 mg/kg, △: 0.3 mg/kg, □: 1 mg/kg, ▲: 3 mg/kg, ■: 10 mg/kg) was administered orally 1 hr before peptide LT challenge. Each value represents the mean ± S.E. of 5 animals. $P < 0.05$, $P < 0.01$: significant difference compared to the vehicle using two-way analysis of variance followed by Dunnett's *t*-test.

When guinea pigs were pretreated with indomethacin, intravenous administration of LTC_4 or LTD_4 produced a slowly developing but maximal bronchoconstriction; After the initial phase of this bronchoconstriction disappeared, a new peak appeared at 2 to 3 min. Intravenous and oral administration of ONO-1078 inhibited both LTC_4- and LTD_4-induced bronchoconstriction in a dose-dependent manner (Figs. 6 and 7). FPL55712, when administered intravenously, inhibited these responses. Their ED_{50} values are shown in Table 2. However, ONO-1078 (10 mg/kg, i.v.) had no effect on bronchoconstrictions induced by spasmogens other than peptide LTs (Table 3).

Table 1. Effects of ONO-1078, FPL55712, azelastine and ketotifen on LTC_4-, LTD_4- and LTE_4-induced bronchoconstriction in guinea pigs

Drugs	Route	ED_{50} (μg/kg)		
		LTC_4	LTD_4	LTE_4
ONO-1078	i.v.	3.8	15.8	4.8
FPL55712	i.v.	1440	2650	1040
ONO-1078	p.o.	590	970	270
Azelastine	p.o.	ND	NE at 10 mg/kg	ND
Ketotifen	p.o.	ND	NE at 10 mg/kg	ND

NE = No effect, ND = Not done. The ED_{50} value was calculated by linear regression analysis of a plot of log drug dose versus the percent inhibition of the area under the curve of the bronchoconstriction response of the treated animals as compared with the vehicle-treated controls.

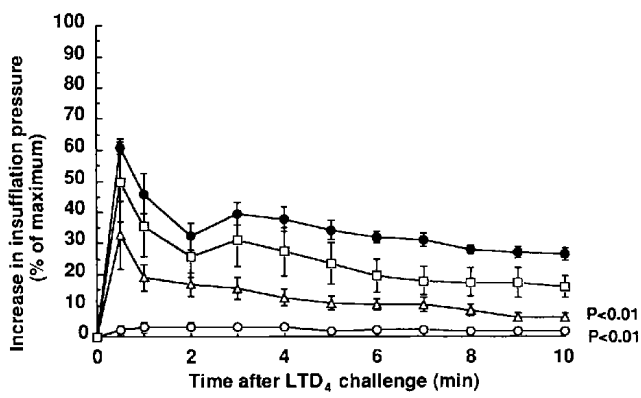


Fig. 4. Duration of inhibitory effect of orally administered ONO-1078 on LTD_4-induced bronchoconstriction in guinea pigs. ONO-1078 was administered orally at a dose of 10 mg/kg 1 hr (○), 3 hr (△) or 5 hr (□) before LTD_4 challenge. Each value represents the mean ± S.E. of 5 animals. $P < 0.01$: significant difference compared to the vehicle (●) using two-way analysis of variance followed by Dunnett's *t*-test.

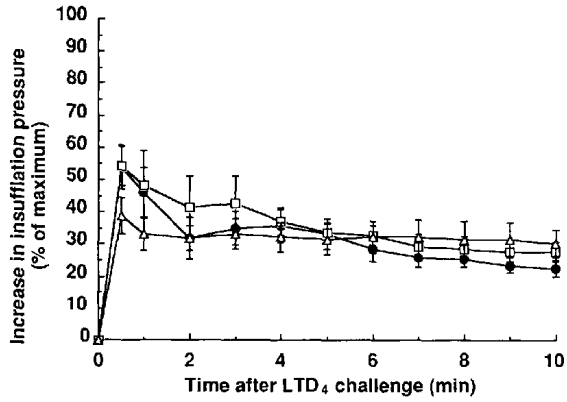


Fig. 5. Effects of ketotifen and azelastine on LTD_4-induced bronchoconstriction in guinea pigs. Vehicle (●), ketotifen (△: 10 mg/kg) or azelastine (□: 10 mg/kg) was administered orally 1 hr before LTD_4 challenge. Each value represents the mean ± S.E. of 5 animals.

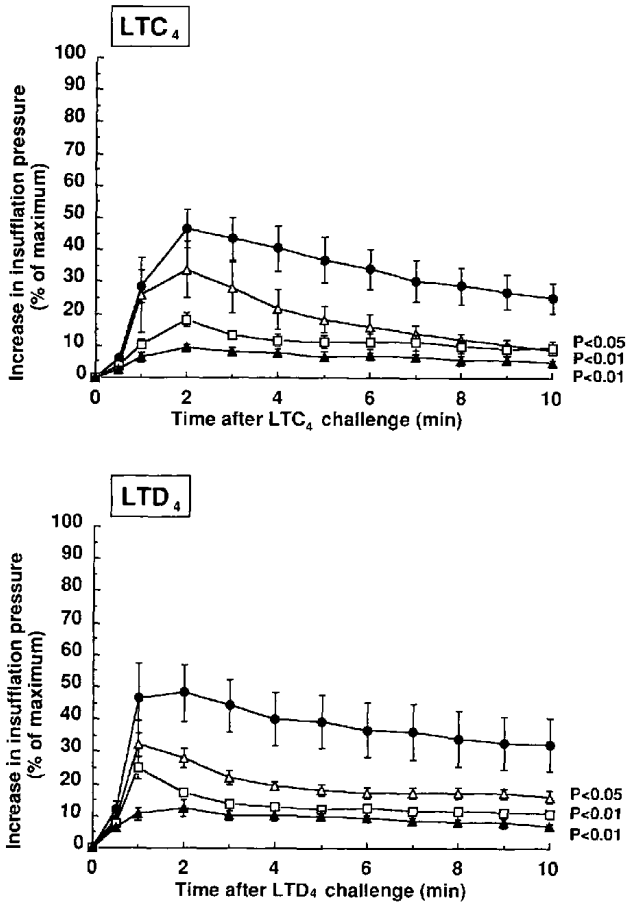


Fig. 6. Effect of ONO-1078 on LTC_4- and LTD_4-induced bronchoconstriction in indomethacin (2 mg/kg, i.v.)-treated guinea pigs. Vehicle (●) or ONO-1078 (△: 3 μg/kg, □: 10 μg/kg, ▲: 30 μg/kg) was administered intravenously 1 min before peptide LT challenge, and indomethacin was administered intravenously 2 min before peptide LT challenge. Each value represents the mean ± S.E. of 5 animals. $P < 0.05$, $P < 0.01$: significant difference compared to the vehicle using two-way analysis of variance followed by Dunnett's *t*-test.

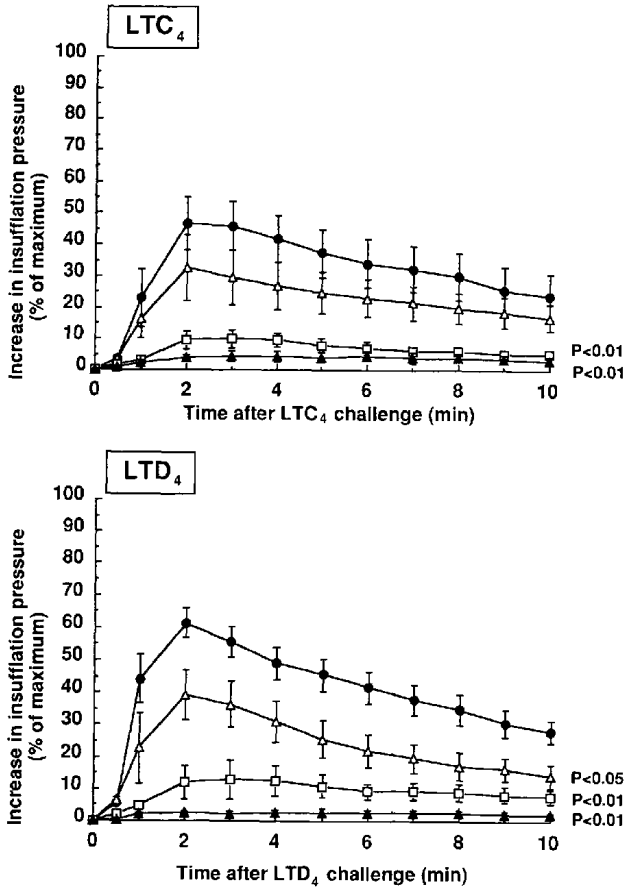


Fig. 7. Effect of ONO-1078 on LTC_4- and LTD_4-induced bronchoconstriction in indomethacin (2 mg/kg, i.v.)-treated guinea pigs. Vehicle (●) or ONO-1078 (△: 0.3 mg/kg, □: 1 mg/kg, ▲: 3 mg/kg) was administered orally 1 hr before peptide LT challenge, and indomethacin was administered intravenously 2 min before peptide LT challenge. Each value represents the mean ± S.E. of 5 animals. $P < 0.05$, $P < 0.01$: significant difference compared to the vehicle using two-way analysis of variance followed by Dunnett's *t*-test.

Table 2. Effects of ONO-1078 and FPL55712 on LTC_4- and LTD_4-induced bronchoconstriction in indomethacin-treated guinea pigs

Drugs	Route	ED_{50} (μg/kg)	
		LTC_4	LTD_4
ONO-1078	i.v.	4.0	3.4
FPL55712	i.v.	1340	1730
ONO-1078	p.o.	490	380

The ED_{50} value was calculated by linear regression analysis of a plot of log drug dose versus the percent inhibition of the area under the curve of the bronchoconstriction response of the treated animals as compared with the vehicle-treated controls.

Table 3. Effect of ONO-1078 on various spasmogen-induced bronchoconstrictions in guinea pigs

Spasmogens	Doses of spasmogens (μg/kg, i.v.)	Dose of ONO-1078 (mg/kg, i.v.)	N	Increase in insufflation pressure (the maximal response, %) Vehicle	ONO-1078
Histamine	10	10	3	63.6 ± 5.0	66.4 ± 1.7
Acetylcholine	30	10	3	50.6 ± 5.6	49.8 ± 5.4
Serotonin	10	10	3	59.0 ± 6.3	60.0 ± 7.8
Arachidonic acid	1000	10	5	71.4 ± 5.0	66.3 ± 6.7
LTB_4	20	10	3	55.7 ± 17.6	53.7 ± 6.1
$PGF_{2\alpha}$	300	10	3	50.4 ± 2.1	58.7 ± 7.8
PGD_2	100	10	3	53.4 ± 6.0	53.3 ± 17.3
$9\alpha,11\beta$-PGF_2	300	10	3	63.6 ± 3.9	58.1 ± 18.7
STA_2	3	10	5	47.9 ± 3.1	43.8 ± 3.8
PAF	0.3	10	5	72.6 ± 8.4	69.9 ± 3.7

ONO-1078 was administered intravenously 1 min before injection of each spasmogen. N = Number of animals. Each value represents the mean ± S.E. Significant difference compared to the vehicle was determined using Student's unpaired *t*-test for arachidonic acid, LTB_4, $PGF_{2\alpha}$, PGD_2, $9\alpha,11\beta$-PGF_2 and PAF or the paired *t*-test for histamine, acetylcholine, serotonin and STA_2.

Effect of ONO-1078 on SRS-A-mediated bronchoconstriction induced by antigen in actively sensitized guinea pigs

When actively sensitized guinea pigs were pretreated with indomethacin and mepyramine, intravenous administration of OA produced a typical anaphylactic bronchoconstriction which peaked at 7 min. Oral administration of ONO-1078 inhibited this response in a dose-dependent manner (Fig. 8). The ED_{50} value was 1.63 mg/kg.

Inhibition of LTD_4-induced airway microvascular leakage in guinea pigs by ONO-1078

LTD_4 produced significant airway microvascular leakage into the trachea, main bronchi and intrapulmonary airways. ONO-1078 significantly inhibited this response in the trachea at 3 mg/kg, p.o. and in the main bronchi and intrapulmonary airways, at 1–3 mg/kg, p.o. (Fig. 9); the respective ED_{50} values of ONO-1078 were 0.74, 0.40 and 0.69 mg/kg.

Inhibition of LTD_4-induced increase in cutaneous vascular permeability in guinea pigs by ONO-1078

LTD_4 produced a marked increase in cutaneous vascular permeability. Oral and intravenous administration of ONO-1078 inhibited this response in a dose-dependent manner. FPL55712, when administered intravenously, inhibited this response in a dose-dependent manner (Fig. 10). The ED_{50} values of ONO-1078 for oral and intravenous administration were 0.63 mg/kg and 10 μg/kg, respectively, and the ED_{50} value of FPL55712 for intravenous administration was 5700 μg/kg.

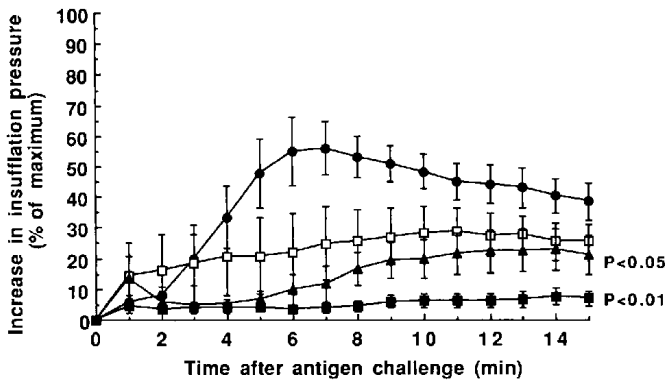


Fig. 8. Effect of ONO-1078 on antigen-induced SRS-A-mediated bronchoconstriction in guinea pigs. Vehicle (●) or ONO-1078 (□: 1 mg/kg, ▲: 3 mg/kg, ■: 10 mg/kg) was administered orally 1 hr before antigen challenge. Each value represents the mean ± S.E. of 5–7 animals. $P < 0.05$, $P < 0.01$: significant difference compared to the vehicle using two-way analysis of variance followed by Dunnett's *t*-test. The ED_{50} value was calculated by linear regression analysis of a plot of log drug dose versus the percent inhibition of area under the curve of the bronchoconstriction response of the treated animals as compared with the vehicle-treated controls.

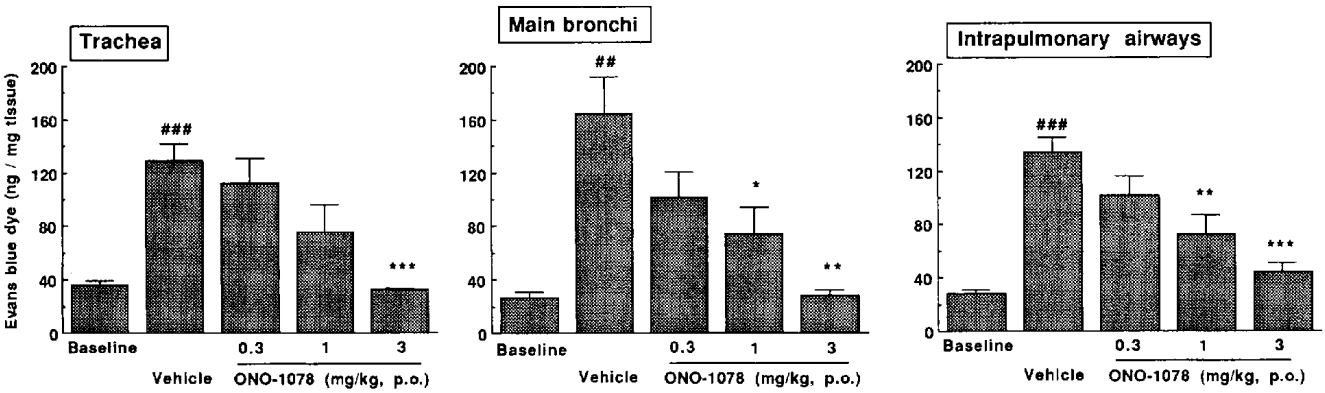


Fig. 9. Effect of ONO-1078 on airway microvascular leakage into trachea, main bronchi and intrapulmonary airways induced by LTD_4 in guinea pigs. Each value represents the mean ± S.E. of 5 animals. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$: significant difference compared to the vehicle using Student's unpaired *t*-test. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$: significant difference compared to the baseline value using Student's unpaired *t*-test. The ED_{50} value was calculated by linear regression analysis of a plot of log drug dose versus the percent inhibition of airway microvascular leakage of the treated animals as compared with the vehicle-treated controls.

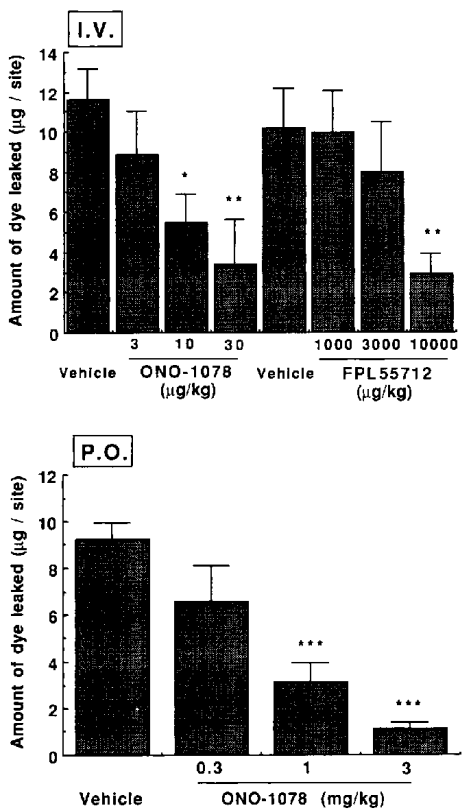


Fig. 10. Effects of ONO-1078 (i.v. and p.o.) and FPL55712 (i.v.) on the increased cutaneous vascular permeability induced by LTD_4 in guinea pigs. Each value represents the mean ± S.E. of 4–10 animals. $^{*}P < 0.05$, $^{**}P < 0.02$, $^{***}P < 0.001$: significant difference compared to the vehicle using Student's unpaired *t*-test. The ED_{50} value was calculated by linear regression analysis of a plot of log drug dose versus the percent inhibition of increased cutaneous vascular permeability of the treated animals as compared with the vehicle-treated controls.

DISCUSSION

The present study showed that ONO-1078 was orally effective against peptide LT, and it did not antagonize the activity of spasmogens other than peptide LT. The antagonistic activity of ONO-1078 against peptide LTs were approximately 200–400 fold more potent than that of FPL55712. These results demonstrate that ONO-1078 is a potent, selective and orally active peptide LT antagonist. Neither azelastine nor ketotifen as anti-allergic agents inhibited LTD_4-induced bronchoconstriction, although in particular, azelastine has been reported to possess peptide LT antagonistic activity in vitro (20) and in vivo (21).

It has been reported that TXA_2 contributes to the bronchoconstriction induced by intravenous administration of peptide LTs (18, 22–26). In the present study, bronchoconstriction induced by LTC_4 (2 μg/kg) or LTD_4 (2 μg/kg) was blocked only at the initial phase by indomethacin as a cyclooxygenase inhibitor. It is speculated that the initial phase of this bronchoconstriction is contributed to TXA_2. Among the reports described above, it has been shown that bronchoconstriction induced by a low concentration of peptide LT (0.5 μg/kg) is mostly inhibited by indomethacin (24, 25). On the other hand, it has been reported that bronchoconstriction at higher concentrations of peptide LT ($>$ 0.8 μg/kg) is only partially blocked by indomethacin (18, 23, 26). Therefore, it is suggested that bronchoconstrictions at a higher concentration of peptide LT (2 μg/kg) were induced by both the direct action of peptide LT and the release of TXA_2. We examined the antagonism of ONO-1078 on the direct action against peptide LTs in guinea pigs pretreated with indometh-

acin. ONO-1078 also inhibited LTC_4- and LTD_4-induced bronchoconstriction in the pretreatment with indomethacin approximately 300- to 500-fold more potently than FPL55712. Therefore, these results suggest that ONO-1078 has an antagonistic activity against the direct action of peptide LTs. The ED_{50} value of ONO-1078 against LTD_4-induced bronchoconstriction was higher than that against LTC_4-induced response in non-treated animals, in spite of almost the same respective ED_{50} values in indomethacin-treated animals. Since ONO-1078 had no effect on the direct bronchoconstriction induced by TXA_2, this difference of ED_{50} value may be due to the magnitude of the contribution of the indirect action by TXA_2 to LTD_4-induced bronchoconstriction in non-treated animals. This is currently under investigation in our laboratories.

We examined the effect of ONO-1078 on antigen-induced bronchoconstriction in actively sensitized guinea pig in the presence of indomethacin and mepyramine to eliminate any contribution by endogenous PGs, TXA_2 and histamine. Since it has been reported that both BW A4C, a 5-lipoxygenase inhibitor, and FPL55712 inhibited anaphylactic bronchoconstriction in guinea pigs pretreated with indomethacin and mepyramine (27), SRS-A is one of the main mediators of anaphylactic bronchoconstriction in this widely used model of "asthma" (28–30). From the fact that ONO-1078 (3–10 mg/kg, p.o.) significantly inhibited SRS-A-mediated bronchoconstriction, it is suggested that ONO-1078 antagonizes the endogenous peptide LT response in addition to exogenous peptide LT.

Peptide LT possesses the ability to increase vascular permeability in airways and skin (31, 32). Airway microvascular leakage is a primary feature of inflammation and leads to the formation of mucosal edema, followed by bronchial narrowing. ONO-1078 significantly inhibited LTD_4-induced microvascular leakage into all airway levels. These results suggest that the inhibition of airway microvascular leakage by ONO-1078 may contribute to the relief of antigen-induced bronchoconstriction. Since LTD_4-induced increase in cutaneous vascular permeability has been reported to be independent of the release of histamine, serotonin or products of the cyclooxygenase pathway of arachidonic acid metabolism (31), it appears that it is mediated by the direct action of LTD_4. ONO-1078 also inhibited the LTD_4-induced increase in cutaneous vascular permeability, and the antagonistic activity of ONO-1078 against this response was approximately 570-fold more potent than that of FPL55712. This result suggests that ONO-1078 potently antagonizes the direct action against LTD_4.

In conclusion, ONO-1078 is a highly potent, selective and orally active peptide LT antagonist in vivo. Furthermore, ONO-1078 antagonizes not only exogenous peptide LT but also endogenous peptide LT. Therefore, it is suggested that ONO-1078 may be useful for the therapy of allergic and inflammatory diseases such as bronchial asthma to which peptide LT is greatly related.

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