In Vitro Antagonism of ONO-1078, a Newly Developed Anti-Asthma Agent, against Peptide Leukotrienes in Isolated Guinea Pig Tissues

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ABSTRACT — We evaluated the antagonist activity of ONO-1078 against peptide leukotrienes (LTs) by a radioligand binding assay and functional experiments in guinea pigs. In the radioligand binding assay, ONO-1078 inhibited [^{3}H]LTD_4 and [^{3}H]LTE_4 bindings to lung membranes (K_i = 0.99 and 0.63 nM, respectively) and was 2,000- to 3,000-fold more potent than FPL55712. Antagonism of ONO-1078 against [^{3}H]LTC_4 binding (K_i = 5640 nM) was approximately twofold more potent than that of FPL55712. The antagonism of ONO-1078 against [^{3}H]LTD_4 binding was competitive. In functional experiments, ONO-1078 showed competitive antagonism against the LTC_4- and LTD_4-induced contractions of guinea pig trachea and lung parenchymal strips with a pA_2 range of 7.70 to 10.71 and was approximately 400- to 3,300-fold more potent than FPL55712. Interestingly, in the presence of an inhibitor of the bioconversion of LTC_4 to LTD_4, ONO-1078 also antagonized the LTC_4-induced contraction of guinea pig trachea (pA_2 = 7.78). ONO-1078 significantly reversed the LTD_4-induced prolonged contraction without effect on the KCl- and $BaCl_2$-induced contractions of guinea pig trachea. Furthermore, ONO-1078 antagonized the antigen-induced SRS-A mediated contraction of guinea pig trachea. On the other hand, ONO-1078 showed no antagonism against histamine, acetylcholine, 5-hydroxytryptamine, prostaglandin D_2 and U-46619. In addition, ONO-1078 showed little or no effect on the activities of cyclooxygenase, 5-lipoxygenase and thromboxane synthetase. These in vitro studies indicate that ONO-1078 is a highly potent, selective and competitive antagonist of peptide leukotrienes that acts with higher affinity at LTD_4 and LTE_4 receptors than LTC_4 receptors.

Keywords: ONO-1078, Leukotriene antagonist (peptide), Anti-asthma agent, Leukotrienes (peptide) (LTC_4, LTD_4 and LTE_4), FPL55712

Peptide leukotrienes (LTC_4, LTD_4 and LTE_4) are 5-lipoxygenase products of arachidonic acid metabolism (1) and considered to be major constituents of slow reacting substance of anaphylaxis (SRS-A) (2, 3). They induce potent bronchoconstriction (4, 5), increase vascular permeability followed by the formation of mucosal edema (6, 7) and enhance mucus secretion (8). On the basis of their potent biological and pathophysiological effects, it has been hypothesized that peptide leukotrienes may play an important role in bronchial asthma (9). Consequently, peptide leukotriene antagonists or 5-lipoxygenase inhibitors have been suggested to be of therapeutic value in bronchial asthma. FPL55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4*H*-1-benzopyran-2-carboxylate) is the first peptide leukotriene (LT) antagonist with moderate potency and selectivity (10). More recently, highly potent peptide leukotriene antagonists, which appear to act as selective LTD_4/E_4 antagonists with poor or no antagonism against LTC_4 receptors, have been reported (11–17).

ONO-1078 (4-oxo-8-[4-(4-phenylbutoxy)benzoylamino]-2-(tetrazol-5-yl)-4*H*-1-benzopyran hemihydrate) is a new class of peptide LT antagonist among the series of 8-(benzoylamino)-2-tetrazol-5-yl-4-oxo-4*H*-1-benzopyrans (18). The present investigation describes in vitro antagonism of ONO-1078 against peptide LTs using a radioligand binding assay and functional experiments, and the effects of ONO-1078 were compared with those of FPL55712. The results indicate that ONO-1078 is a highly potent, selective and competitive antagonist of peptide LTs.

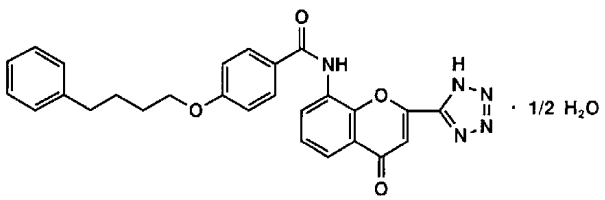
MATERIALS AND METHODS

Animals

Male Hartley strain guinea pigs (300–600 g, Keari Co. and Nakajima Experimental Animal Laboratories) were used throughout the experiments.

Drugs and chemicals

ONO-1078 (Fig. 1), FPL55712, OKY-046·HCl, LTC_4, LTD_4, LTB_4, prostaglandin D_2 (PGD_2) were synthesized by Ono Pharmaceutical Co., Ltd. Other chemicals and drugs used were as follows: [^{3}H]LTC_4 (specific activity, 39.3 Ci/mmol), [^{3}H]LTD_4 (specific activity, 39.3 and 40.0 Ci/mmol), [^{3}H]LTE_4 (specific activity, 39.3 Ci/mmol), [^{3}H]LTB_4 (specific activity, 39.3 Ci/mmol), [^{14}C]arachidonic acid (specific activity 55.0 mCi/mmol) and RIA kit for thromboxane B_2 (NEN Research Products and Amersham); indomethacin, carbachol, L-serine, 5-hydroxytryptamine creatinine sulfate (5-HT), phenylmethylsulfonyl fluoride, aprotinin, benzamidine, soybean trypsin inhibitor, glycine, ovalbumin and bovine serum albumin (Sigma Chemical Co.); acetylcholine chloride (Daiichi Pharmaceutical Co.); L-cysteine (Hayashi Pure Chemical Co.); nordihydroguaiaretic acid (NDGA) (Nacalai Tesque Co.); boric acid (Katayama Chemical Co.); sheep seminal vesicular microsomes (Hilran Biochemicals Co.); assay kit for thromboxane synthetase (Eldan Technologies Co.); U-46619 (Cayman Chemical Co.). LTs were diluted with either the buffer used in the radioligand binding assay or with 0.1 M phosphate buffer (pH 7.4) used in functional experiments. ONO-1078 was dissolved in ethanol by adding an equimolar amount of NaOH, and then diluted with either distilled water or 10% ethanol/distilled water. FPL55712 was dissolved in ethanol and then diluted with distilled water. Indomethacin, NDGA and OKY-046·HCl were dissolved and diluted with ethanol. Other chemicals were dissolved and diluted with physiological saline.



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.

Preparation of guinea pig lung membranes

The membrane fractions containing LT receptors were prepared as described by Mong et al. (19) and Aharony (20). Briefly, male Hartley strain guinea pigs (300–600 g) were decapitated and then their lungs were perfused with 20 ml of 0.1 M phosphate-buffered saline (PBS, pH 7.4) and excised. Large blood vessels and hemorrhagic and necrotized tissues were removed. The remaining tissue was minced and washed twice with 25 ml of ice-cold PBS and suspended in 10 mM Tris-HCl/0.25 M sucrose buffer (pH 7.4) containing protease inhibitors: 87 μg/ml phenylmethylsulfonyl fluoride, 10 mU/ml aprotinin, 157 μg/ml benzamidine, and 100 μg/ml soybean trypsin inhibitor. The suspended tissues were homogenized 4 times on ice by a Physcotron (Niti-on) at 21,000 rpm for 15 sec. The homogenate was centrifuged at 1,500 $\times$ *g* for 15 min at 4°C, and the supernatant was collected and centrifuged at 40,000 $\times$ *g* for 30 min at 4°C. The resulting pellet was resuspended in 10 mM Tris-HCl buffer (pH 7.4), homogenized for 3 strokes by a glass-Teflon motorized homogenizer and centrifuged again at 40,000 $\times$ *g* for 30 min at 4°C. The final pellet was resuspended in 10 mM Tris-HCl buffer (pH 7.4) to a final protein concentration of 10 mg/ml and stored at −80°C until use. The protein concentration of this crude membrane preparation was determined by the method of Lowry et al. (21) with bovine serum albumin as a standard.

Radioligand binding assay of [^{3}H]LTC_4, [^{3}H]LTD_4, [^{3}H]LTE_4 and [^{3}H]LTB_4

The [^{3}H]LT binding studies were carried out in the following buffer: [^{3}H]LTC_4 binding, 10 mM Tris-HCl buffer (pH 7.5) containing 5 mM cysteine, 5 mM glycine, 80 mM L-serine borate, 10 mM $CaCl_2$ and 10 mM $MgCl_2$; [^{3}H]LTD_4 binding, 10 mM piperazine-*N*,*N*'-bis(2-ethanesulfonic acid) buffer (pH 6.5) containing 10 mM $CaCl_2$, 10 mM $MgCl_2$, 5 mM cysteine and 5 mM glycine; [^{3}H]LTE_4 binding, 10 mM piperazine-*N*,*N*'-bis(2-ethanesulfonic acid) buffer (pH 6.5) containing 10 mM $CaCl_2$, 10 mM $MgCl_2$ and 10 mM cysteine; [^{3}H]LTB_4 binding, 50 mM Tris-HCl buffer (pH 7.5). The saturation binding experiments were performed by incubating an aliquot of lung membranes ([^{3}H]LTC_4 binding, 200 μg/ml; [^{3}H]LTD_4 binding, 400 μg/ml; [^{3}H]LTE_4 binding, 400 μg/ml) with various concentrations of [^{3}H]peptide LT ([^{3}H]LTC_4, 1 to 50 nM; [^{3}H]LTD_4, 0.1 to 5 nM; [^{3}H]LTE_4, 0.1 to 5 nM) at 25°C for 30 min. In the competition experiments, an aliquot of the lung membranes ([^{3}H]LTC_4 binding, 100 μg/ml; [^{3}H]LTD_4 binding, 400 μg/ml; [^{3}H]LTE_4 binding, 400 μg/ml; [^{3}H]LTB_4 binding, 300 μg/ml) was incubated at 25°C for 30 min with [^{3}H]LT ([^{3}H]LTC_4, 0.5 nM;

[^{3}H]LTD_4, 0.5 nM; [^{3}H]LTE_4, 1 nM; [^{3}H]LTB_4, 1 nM) and various concentrations of competing drugs.

The binding reaction was terminated by rapid filtration of samples through Whatman GF/C glass microfiber filter strips with a Brandel Cell Harvester followed by four rapid washes with 2.5-ml aliquots of ice-cold 10 mM Tris/100 mM NaCl buffer (pH 7.4). Filters were removed and allowed to dry before assaying filter bound radioactivity by liquid scintillation spectrophotometry. The specific binding was defined as the difference between the amount of [^{3}H]LT bound in the absence and presence of LT (5 μM LTC_4, 1 μM LTD_4, 1 μM LTE_4, 1 μM LTB_4).

Functional experiments using guinea pig trachea and lung parenchymal strips

Male Hartley strain guinea pigs (250–490 g) were sacrificed by a sharp blow to the head and appropriate tissue sections were removed immediately. Each trachea was cut into zigzag strips. Parenchymal strips, 2-mm wide, were prepared from the left and right lower lobe edges of lungs. The preparations were suspended in 10 ml tissue baths containing modified Krebs' solution of the following composition: 119 mM NaCl, 4.6 mM KCl, 1.8 mM $CaCl_2$, 0.5 mM $MgSO_4$, 24.9 mM $NaHCO_3$, 1.0 mM KH_2PO_4 and 11.1 mM glucose. The tissue baths were maintained at 37 ± 1°C and continuously aerated with 95% O_2–5% CO_2. The resting tension of guinea pig trachea was 2.0 g and that of lung parenchymal strips was 0.5 g. Each preparation was equilibrated for 60 min by washing with fresh physiologic solution every 15 min and pretreated with 10^{-6} M indomethacin to remove the influence of cyclooxygenase products on the responses to various agonists.

Contractile responses were recorded as a change of isometric tension by a force displacement transducer (Nihon Kohden, SB-1T). Concentration-response curves were constructed by cumulative increases in bath concentration of agonists according to the method of Van Rossum (22). Only one concentration-response curve was generated with each tissue. Contractile responses were expressed as a percentage of the response to 10^{-5} M carbachol for the trachea and 10^{-4} M carbachol for the lung parenchymal strips. ONO-1078 and FPL55712 were added 30 min before the addition of each agonist. In certain experiments with LTC_4, 45 mM L-serine borate complex, a γ-glutamyl transpeptidase inhibitor, was incubated with the guinea pig trachea for 30 min before the addition of LTC_4 to inhibit the bioconversion of LTC_4 to LTD_4. For the reverse experiment, the tracheal strips were contracted with LTD_4 (3×10^{-8} M), KCl (2.5×10^{-2} M), or $BaCl_2$ (10^{-3} M). When the response reached a steady state, ONO-1078 or FPL55712 was added to the bath.

Antigen-induced contraction of guinea pig trachea

Male Hartley strain guinea pigs (300–470 g) were passively sensitized with 1 ml/kg, i.v. of anti-ovalbumin serum (4 hr heterologous PCA titer = 1:8192) produced by the immunization of rabbits with ovalbumin. The trachea from the sensitized animal was removed 24 hr after the passive sensitization and cut into zigzag strips for recording isometric tension as indicated above. Tissues were pretreated with 5×10^{-6} M indomethacin and various concentrations of ONO-1078 for 30 min and contracted with the challenge of 10 μg/ml ovalbumin for 60 min. Contractile responses were expressed as a percentage of the response to 10^{-5} M carbachol.

Assay of cyclooxygenase, 5-lipoxygenase and thromboxane synthetase activities

Cyclooxygenase activity was measured using sheep seminal vesicular microsomes by the method of Miyamoto et al. (23). 5-Lipoxygenase activity was measured by the method of Ochi et al. (24). Cyclooxygenase and 5-lipoxygenase activities were expressed in terms of the amount of arachidonic acid oxygenated. Thromboxane synthetase activity was measured using a commercially available assay kit. The enzyme activity was expressed in terms of the amount of thromboxane B_2 biosynthesized. Thromboxane B_2 was measured by a commercially available RIA kit.

Statistics

Data from the radioligand binding assay were subjected to computer analysis using programs of Equilibrium Binding Data Analysis (EBDA by McPherson, Elsevier-BIOSOFT, 1983). In functional experiments, the antagonist potency was calculated and expressed as either pA_2 or pK_B. The pA_2 values were obtained by Schild plot analysis (25), whereas pK_B values were calculated by the method of Furchgott (26). In the enzyme assay, the IC_{50} values were determined by a linear least square regression analysis.

Results were expressed as the mean with standard error of the mean (S.E.M.). Statistical analysis was performed by Student's paired or unpaired *t*-test, with a probability value of $P < 0.05$ regarded as significant.

RESULTS

Characteristics of [^{3}H]peptide LT binding to guinea pig lung membranes

The specific bindings of [^{3}H]LTC_4, [^{3}H]LTD_4 and [^{3}H]LTE_4 to guinea pig lung membranes were satur-

able, and Scatchard analysis indicated a single population of binding sites with K_d (equilibrium dissociation constant) values of 25.7 ± 1.3, 0.24 ± 0.02 and 0.62 ± 0.08 nM and B_{max} (maximum number of binding sites) values of 15,800 ± 700, 254 ± 17 and 178 ± 12 fmol/mg protein (n = 5), respectively. When the data were analyzed by Hill plots, the Hill coefficients were not significantly different from unity ([^{3}H]LTC$_4$, 1.00 ± 0.001; [^{3}H]LTD$_4$, 0.91 ± 0.04; [^{3}H]LTE$_4$, 0.97 ± 0.09; n = 5), indicating that no cooperativity was detected.

Inhibition of [^{3}H]LTC$_4$, [^{3}H]LTD$_4$, [^{3}H]LTE$_4$ and [^{3}H]LTB$_4$ specific bindings to lung membranes by ONO-1078 and FPL55712

ONO-1078 concentration-dependently inhibited [^{3}H]LTC$_4$, [^{3}H]LTD$_4$ and [^{3}H]LTE$_4$ bindings to guinea pig lung membranes at concentration ranges of $10^{-6}-10^{-4}$ M, $10^{-10}-10^{-7}$ M and $10^{-10}-10^{-7}$ M, respectively, whereas ONO-1078 had little effect on the [^{3}H]LTB$_4$ binding at $10^{-6}-10^{-4}$ M (Fig. 2). When compared by K_i values, ONO-1078 selectively inhibited [^{3}H]LTD$_4$ and [^{3}H]LTE$_4$ bindings (Table 1). The rank orders of the inhibition of [^{3}H]LTD$_4$ and [^{3}H]LTE$_4$ bindings were LTD$_4$ > LTE$_4$ > ONO-1078 > LTC$_4$ > FPL55712 and LTD$_4$ > ONO-1078 > LTE$_4$ > LTC$_4$ > FPL55712, respectively. ONO-1078 was approximately 2,000-fold and 3,000-fold more potent than FPL55712 against [^{3}H]LTD$_4$ and [^{3}H]LTE$_4$ bindings, respectively. On the other hand, the inhibition of [^{3}H]LTC$_4$ binding by ONO-1078 was approximately 5,700-fold and 9,000-fold less potent than those of [^{3}H]LTD$_4$ and [^{3}H]LTE$_4$ bindings, respectively. The rank order of the inhibition of [^{3}H]LTC$_4$ binding was LTC$_4$ > ONO-1078 > LTD$_4$ > FPL55712 > LTE$_4$. ONO-1078 was about 2-fold more potent than FPL55712.

To analyze the nature of the interaction of ONO-1078 ($3 \times 10^{-10}-3 \times 10^{-9}$ M) with the LTD$_4$ receptors, K_d values and B_{max} values in the presence of ONO-1078 were determined by cold saturation experiments. As shown in Table 2, Scatchard analysis indicated that ONO-1078 significantly increased the K_d values at $10^{-9}-3 \times 10^{-9}$ M without any influence on the B_{max} values.

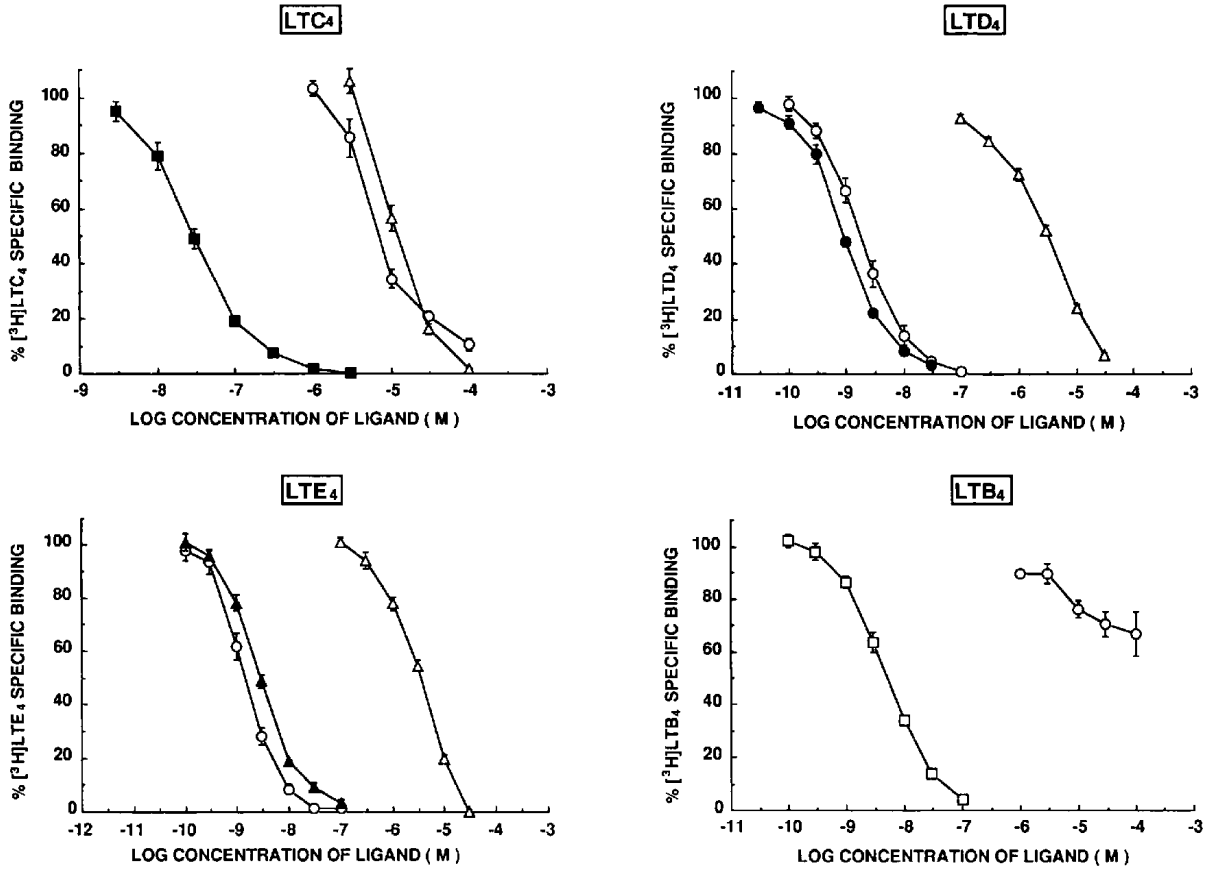


Fig. 2. Competition of ONO-1078 and FPL55712 for [^{3}H]LTC$_4$, [^{3}H]LTD$_4$, [^{3}H]LTE$_4$ and [^{3}H]LTB$_4$ specific bindings to guinea pig lung membranes. Results are means ± S.E.M. of 5 experiments. ○: ONO-1078; △: FPL55712; ■: LTC$_4$; ●: LTD$_4$; ▲: LTE$_4$; □: LTB$_4$.

Table 1. K_i values for competition of [^{3}H]LTC_4, [^{3}H]LTD_4 and [^{3}H]LTE_4 specific bindings and IC_{50} values for competition of [^{3}H]LTB_4 specific binding to guinea pig lung membranes

Compounds	K_i (nM)			IC_{50} (nM)
	[^{3}H]LTC_4	[^{3}H]LTD_4	[^{3}H]LTE_4	[^{3}H]LTB_4
ONO-1078	5640 ± 680	0.99 ± 0.19	0.63 ± 0.11	> 100,000
FPL55712	10980 ± 820	2040 ± 180	1910 ± 280	—
LTC_4	27.7 ± 2.9	38.0 ± 5.5	32.4 ± 2.6	—
LTD_4	6220 ± 290	0.48 ± 0.04	0.28 ± 0.05	—
LTE_4	34100 ± 1000	0.62 ± 0.01	1.12 ± 0.11	—
LTB_4	—	—	—	5.09 ± 0.44

Values are means ± S.E.M. of five experiments. —: Not done.

Table 2. Effect of ONO-1078 on K_d and B_{max} values for [^{3}H]LTD_4 specific binding to guinea pig lung membranes

ONO-1078 (nM)	K_d (nM)	B_{max} (fmol/mg of protein)
0	0.24 ± 0.02	254 ± 17
0.3	0.33 ± 0.03	251 ± 12
1	0.56 ± 0.05**	233 ± 12
3	1.59 ± 0.47*	228 ± 28

Values are means ± S.E.M. of five experiments. *, **: Significantly different from the control at $P < 0.05$ and $P < 0.001$.

Antagonism of ONO-1078 and FPL55712 against peptide LT-induced contractions of guinea pig trachea

ONO-1078 produced parallel rightward shifts of the LTC_4 and LTD_4 concentration-response curves in a concentration-dependent manner at 10^{-11}–10^{-8} M (Fig. 3, A and C). As shown in Table 3, the pK_B values were independent of concentrations of ONO-1078, and Schild plot analysis indicated that the slopes were not significantly different from unity. When compared in terms of pA_2 values, ONO-1078 was shown to

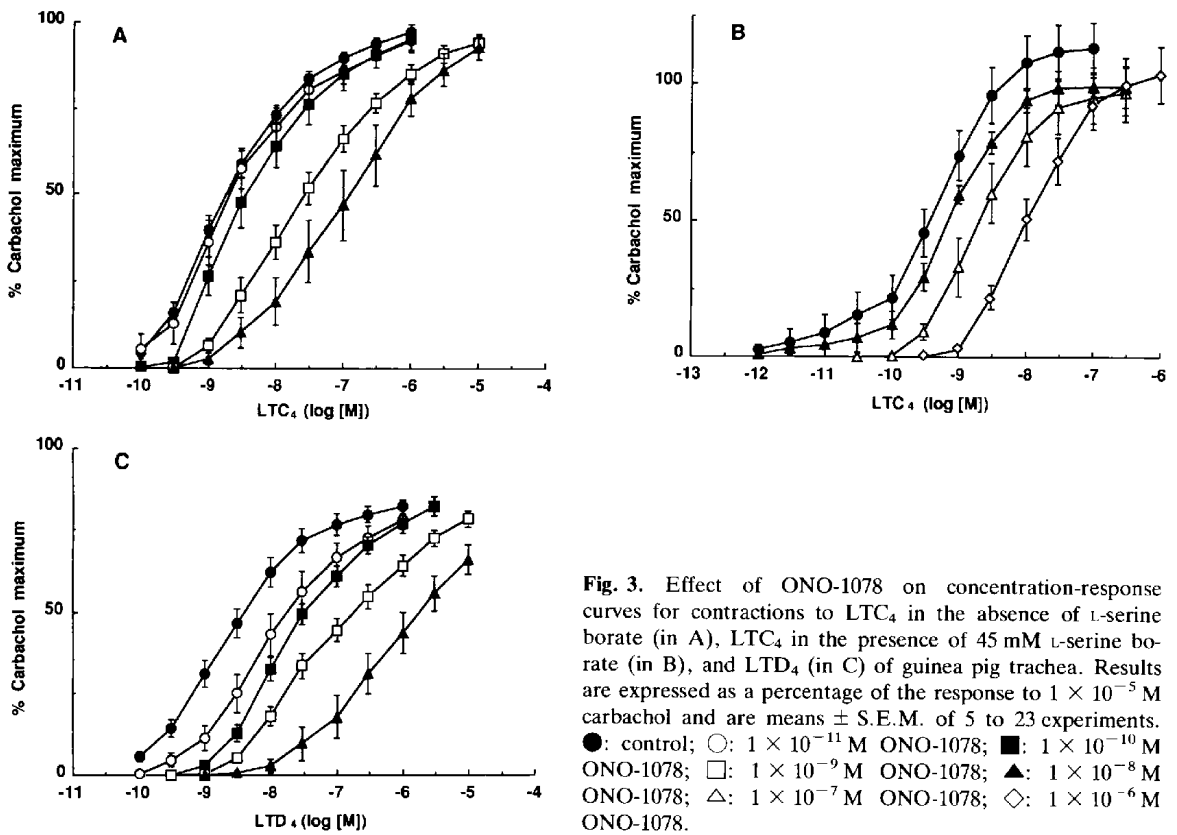


Fig. 3. Effect of ONO-1078 on concentration-response curves for contractions to LTC_4 in the absence of L-serine borate (in A), LTC_4 in the presence of 45 mM L-serine borate (in B), and LTD_4 (in C) of guinea pig trachea. Results are expressed as a percentage of the response to 1×10^{-5} M carbachol and are means ± S.E.M. of 5 to 23 experiments. ●: control; ○: 1×10^{-11} M ONO-1078; ■: 1×10^{-10} M ONO-1078; □: 1×10^{-9} M ONO-1078; ▲: 1×10^{-8} M ONO-1078; △: 1×10^{-7} M ONO-1078; ◇: 1×10^{-6} M ONO-1078.

Table 3. Interactions of ONO-1078 and FPL55712 with LTC_4 and LTD_4 in guinea pig trachea

	Compound Concn (M)	n	$pK_B \pm$ S.E.M.	Schild plot analysis pA_2 (95% C.L.)	Schild plot analysis Slope (95% C.L.)
	ONO-1078				
LTC_4	1×10^{-10}	8	10.30 ± 0.25	10.47	0.86
	1×10^{-9}	8	10.48 ± 0.19	(9.73–11.21)	(0.48–1.23)
	1×10^{-8}	8	10.01 ± 0.31		
LTC_4 + L-serine borate	1×10^{-8}	5	7.80 ± 0.40	7.78	0.82
	1×10^{-7}	5	7.67 ± 0.33	(7.11– 8.46)	(0.33–1.30)
	1×10^{-6}	5	7.43 ± 0.23		
LTD_4	1×10^{-10}	8	10.54 ± 0.28	10.71	0.78
	1×10^{-9}	8	10.38 ± 0.23	(9.88–11.54)	(0.44–1.23)
	1×10^{-8}	8	10.10 ± 0.20		
	FPL55712				
LTC_4	1×10^{-7}	8	7.54 ± 0.17	7.64	0.78
	1×10^{-6}	8	7.18 ± 0.14	(7.12– 8.17)	(0.55–1.00)
	1×10^{-5}	8	7.09 ± 0.15		
LTD_4	1×10^{-7}	6	7.51 ± 0.20	7.19	1.14
	1×10^{-6}	8	6.89 ± 0.15	(6.71– 7.66)	(0.78–1.45)
	1×10^{-5}	8	7.58 ± 0.28		

n: The number of experiments. pA_2 values and slopes are derived from linear least square regression analysis with 95% confidence limits.

Table 4. Interactions of ONO-1078 and FPL55712 with LTC_4 and LTD_4 in guinea pig lung parenchyma

	Compound Concn (M)	n	pK_B + S.E.M.	Schild plot analysis pA_2 (95% C.L.)	Schild plot analysis Slope (95% C.L.)
	ONO-1078				
LTC_4	1×10^{-8}	7	7.66 ± 0.17	7.70	0.90
	3×10^{-8}	9	7.78 ± 0.17	(7.45–7.94)	(0.39–1.42)
	1×10^{-7}	7	7.57 ± 0.10		
LTD_4	1×10^{-8}	12	8.41 ± 0.13	8.58	0.64
	3×10^{-8}	8	8.11 ± 0.11	(8.03–9.13)	(0.32–0.97)
	1×10^{-7}	8	8.06 ± 0.09		
	FPL55712				
LTC_4	3×10^{-5}	6	4.79 ± 0.16	—	—
	1×10^{-4}	6	5.28 ± 0.20		
LTD_4	3×10^{-5}	5	5.04 ± 0.13	—	—
	1×10^{-4}	5	5.23 ± 0.15		

n: The number of experiments. —: Not done. pA_2 values and slopes are derived from linear least square regression analysis with 95% confidence limits.

be approximately 700-fold and 3,300-fold more potent than FPL55712 against the LTC_4- and LTD_4-induced contractions of guinea pig trachea, respectively. In the presence of L-serine borate, which inhibits the bioconversion of LTC_4 to LTD_4, higher concentrations of ONO-1078 (10^{-8}–10^{-6} M) were required to antagonize the LTC_4-induced contractions (Fig. 3, B). ONO-1078 shifted the LTC_4 concentration-response curve to the right in a parallel manner. Schild plot analysis indicated that the slope of ONO-1078 was not significantly different from unity. When compared in terms of the pA_2 values, the antagonist potency of ONO-1078 against LTC_4 receptors was approximately 850-fold less potent than that against LTD_4 receptors (Table 3).

Antagonism of ONO-1078 and FPL55712 against peptide LT-induced contractions of guinea pig lung parenchymal strips

ONO-1078 inhibited the LTC_4- and LTD_4-induced contractions of guinea pig lung parenchymal strips at $10^{-8}-10^{-7}$ M. As shown in Table 4, Schild plot analysis indicated that the slopes of ONO-1078 were not significantly different from unity. When compared in terms of pK_B values, ONO-1078 was approximately 400-fold and 1,100-fold more potent than FPL55712 against LTC_4 and LTD_4, respectively.

Specificity of the action of ONO-1078

ONO-1078 was examined for its ability to reduce contractions induced by a variety of contractile agents. ONO-1078 at 10^{-5} M was without effect on responses induced by acetylcholine, histamine, 5-HT, PGD_2 and U-46619 (Table 5). ONO-1078 reversed the prolonged contraction induced by 3×10^{-8} M LTD_4 at $10^{-8}-10^{-6}$ M in a concentration-dependent manner, but showed no effect on the prolonged contractions induced by 2.5×10^{-2} M KCl and 10^{-3} M $BaCl_2$ (Fig. 4). The significant reversal was observed at higher concentrations than 10^{-8} M of ONO-1078 and the inhibitions of the LTD_4-induced prolonged contraction at 60 min by ONO-1078 were 14.8% at 10^{-8} M, 61.5% at 10^{-7} M and 85.2% at 10^{-6} M. FPL55712 at 10^{-6} M also significantly reversed the LTD_4-induced contraction with the inhibition of 54.4% at 60 min.

Effect of ONO-1078 on antigen-induced contraction of passively sensitized guinea pig trachea

The challenge with ovalbumin induced a biphasic response: a rapid early phase which reached a maximum at 5–7 min and a second sustained phase which was maintained at 20–60 min after the challenge (Fig. 5). ONO-1078 did not affect the first phase of the contraction at $10^{-7}-10^{-5}$ M, but significantly reduced the second sustained phase of the contraction to the initial

Table 5. Effect of ONO-1078 on contractile responses of guinea pig trachea induced by various agonists

Agonists	EC_{50} (−log [M])	
	Control	ONO-1078 1×10^{-5} M
Acetylcholine	5.63 ± 0.25	5.85 ± 0.45
Histamine	4.90 ± 0.09	5.07 ± 0.31
5-Hydroxytryptamine	7.00 ± 0.15	6.48 ± 0.13
Prostaglandin D_2	6.70 ± 0.31	5.98 ± 0.44
U-46619	8.49 ± 0.11	8.20 ± 0.06

Values are means ± S.E.M. of five experiments.

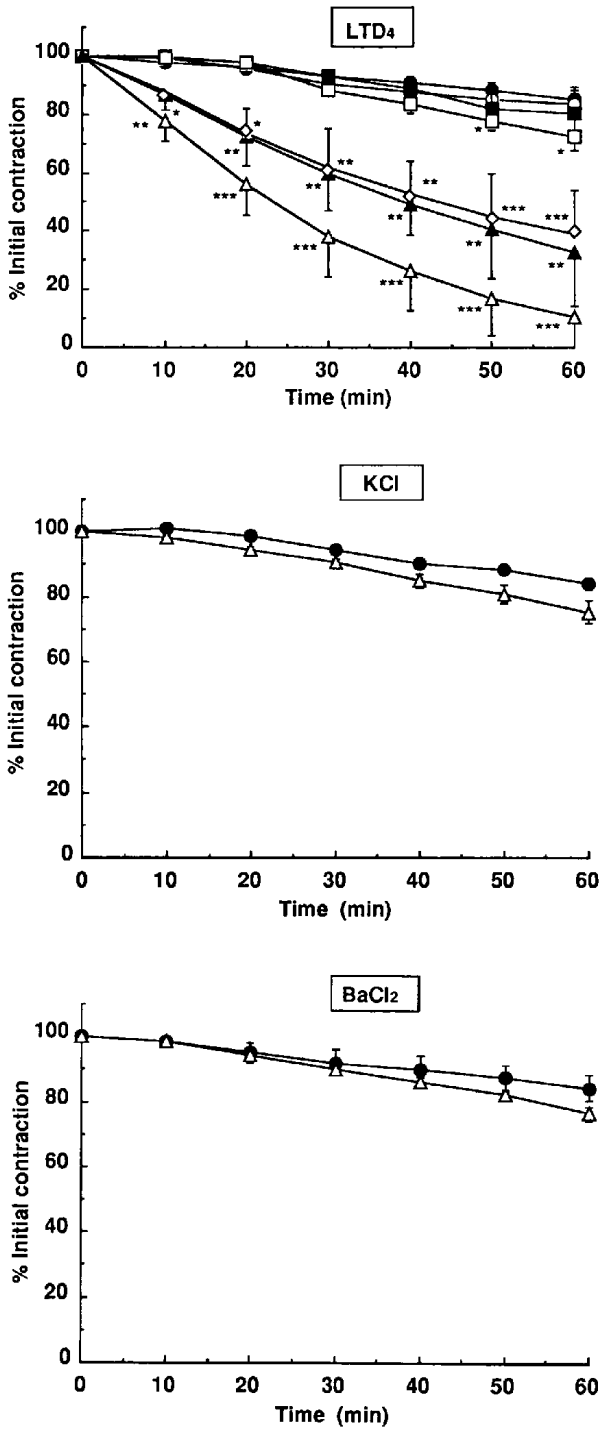


Fig. 4. The reversal effect of ONO-1078 on LTD_4 (3×10^{-8} M)-, KCl (2.5×10^{-2} M)-, and $BaCl_2$ (1×10^{-3} M)-induced contractions of guinea pig trachea. Results are expressed as a percentage of the initial contraction of each agonist. Results are means ± S.E.M. of 5 to 13 experiments. *, **, ***: Statistically significant difference from the control at $P < 0.05$, $P < 0.01$ and $P < 0.001$ by the unpaired *t*-test, respectively. ●: control; ○: 1×10^{-10} M ONO-1078; ■: 1×10^{-9} M ONO-1078; □: 1×10^{-8} M ONO-1078; ▲: 1×10^{-7} M ONO-1078; △: 1×10^{-6} M ONO-1078; ◇: 1×10^{-6} M FPL55712.

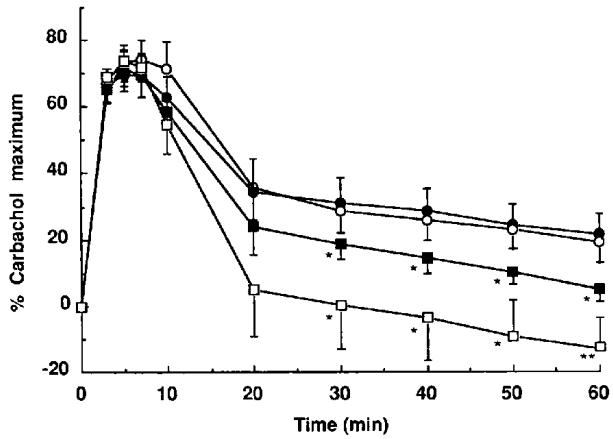


Fig. 5. Effect of ONO-1078 on the ovalbumin-induced contraction of passively sensitized guinea pig trachea. Results are expressed as a percentage of the response to 1×10^{-5} M carbachol and are means ± S.E.M. of 5 experiments. *, **: Statistically significant difference from the control at $P < 0.05$ and $P < 0.01$ by the paired *t*-test, respectively. ●: control; ○: 1×10^{-7} M ONO-1078; ■: 1×10^{-6} M ONO-1078; □: 1×10^{-5} M ONO-1078.

level before ovalbumin challenge at $10^{-6} - 10^{-5}$ M.

Inhibitory activities for cyclooxygenase, 5-lipoxygenase and thromboxane synthetase

ONO-1078 showed little or no effect on cyclooxygenase, 5-lipoxygenase and thromboxane synthetase activities ($IC_{50} = > 300$, > 300 and 44 ± 18 μM, $n = 3-5$, respectively). Indomethacin, NDGA and OKY-046·HCl as reference compounds showed potent inhibition with respective IC_{50} values of 0.13 ± 0.12, 0.25 ± 0.03 and 0.017 ± 0.003 μM ($n = 3-5$).

DISCUSSION

The antagonist activity of ONO-1078 against peptide LTs was examined in the radioligand binding assay and functional experiments using guinea pig lung and trachea. The radioligand binding assay on guinea pig lung membranes indicated that ONO-1078 selectively interacted with the LTD_4 and LTE_4 receptors and that the antagonist activity of ONO-1078 against LTD_4 and LTE_4 was approximately 2,000-fold and 3,000-fold more potent, respectively, than that of FPL55712. In functional experiments, ONO-1078 potently antagonized the LTD_4-induced contractions of guinea pig trachea and lung parenchymal strips. The antagonist activity of ONO-1078 against LTD_4 in guinea pig trachea and lung parenchymal strips was approximately 3,300-fold and 1,100-fold more potent than that of FPL55712. Data from the radioligand binding assay were in good agreement with those obtained from functional experiments. In particular, ONO-1078 showed potent antagonism against LTD_4 with a pA_2 value of 10.71 in guinea pig trachea, and ONO-1078 appears to be the most potent LTD_4 antagonist so far described (15). On the other hand, ONO-1078 showed antagonism against LTC_4 with a K_i value of 5640 ± 680 nM in guinea pig lung membranes and a pA_2 value of 7.78 in guinea pig trachea treated with L-serine borate. These results indicated that the interaction of ONO-1078 with LTC_4 receptors was approximately 850-fold in functional experiments and approximately 5,700-fold in the radioligand binding assay lower than that with LTD_4 receptors. However, it is interesting that ONO-1078 has antagonist activity against LTC_4 and that the pA_2 value of ONO-1078 against LTC_4 is higher than any other peptide LT antagonists so far reported (11–17). The selectivity of the action of ONO-1078 was indicated by the lack of antagonist activity against $[^3H]LTB_4$ binding to guinea pig lung membranes and against the contractions of guinea pig trachea induced by a variety of agonists other than peptide LTs. As for the nature of the antagonism of ONO-1078 against peptide LTs, the radioligand binding assay indicated that ONO-1078 was a competitive LTD_4 antagonist due to the significant increase in K_d values without any influence on the B_{max} values in the presense of ONO-1078. In addition, as the functional experiments indicated that the slopes in the LTC_4- and LTD_4-induced contractions obtained by Schild plot analysis were not significantly different from unity, ONO-1078 was a competitive antagonist of peptide LTs. Thus, it was demonstrated that ONO-1078 was a highly potent, selective and competitive antagonist of peptide LTs with higher affinity to LTD_4 and LTE_4 receptors than to LTC_4 receptors.

Radioligand binding assay demonstrated the existence of a single class of high affinity and saturable binding sites for peptide LT receptors in guinea pig lung membranes (27–29). Based on the findings that the K_d values for $[^3H]LTD_4$ and $[^3H]LTE_4$ specific bindings to guinea pig lung membranes were almost identical and that the rank order potency of agonist binding to $[^3H]LTE_4$ binding sites was $LTD_4 > LTE_4 \gg LTC_4$, identical to that for $[^3H]LTD_4$ binding, it has been suggested that LTE_4 binds to a subset population of high affinity LTD_4 receptors (30, 31). Our results are similar to those findings, and this suggestion would account for the ability of ONO-1078 to inhibit $[^3H]LTD_4$ and $[^3H]LTE_4$ bindings with almost equal potency. In addition, ONO-1078 showed almost the same affinity to LTD_4/E_4 receptors as the natural agonists, LTD_4 and LTE_4. As for the LTC_4 receptors, it is reported that LTC_4 receptors are biochemically different from LTD_4

receptors in guinea pig lung (29). The saturation experiments indicated that [^{3}H]LTC_4 binding sites were 40–70-fold lower in affinity and 40–90-fold higher in density than [^{3}H]LTD_4 and [^{3}H]LTE_4 binding sites. In addition, the competition experiments indicated that the rank order of potency of agonists for the inhibition of [^{3}H]LTC_4 binding was different from those for the inhibition of [^{3}H]LTD_4 and [^{3}H]LTE_4 bindings. The present results suggest that LTC_4 receptors are different from LTD_4 and LTE_4 receptors.

The existence of multiple LTD_4 receptors has been reported in guinea pig ileum and lung parenchymal strips using FPL55712 (32, 33). In functional experiments, the antagonist activity of ONO-1078 against LTD_4 in guinea pig trachea ($pA_2 = 10.71$) was approximately 2.1 log degrees higher than that in guinea pig lung parenchymal strips ($pA_2 = 8.58$). As there existed ONO-1078-sensitive or -less sensitive LTD_4 receptors, it is suggested that LTD_4 receptors in guinea pig lung parenchymal strips may differ from those present in guinea pig trachea.

LTC_4 and LTD_4 have been shown to produce thromboxane A_2 and PGs from guinea pig lung (13, 34) and to induce the contraction of guinea pig parenchymal strips mediated by thromboxane A_2 (35). ONO-1078 antagonized the LTC_4- and LTD_4-induced contractions of guinea pig lung in the presence of indomethacin and moreover, ONO-1078 had little or no effect on cyclooxygenase and thromboxane synthetase activities. Therefore, it appears that the inhibitory activity of ONO-1078 against the LTC_4- and LTD_4-induced contractions of guinea pig lung parencymal strips is not mediated by the blockade of thromboxane A_2 formation, but is the direct interaction with LTC_4 and LTD_4 receptors.

ONO-1078 reversed the LTD_4-induced sustained contraction of guinea pig trachea in a concentration-dependent manner. Since ONO-1078 showed no reversal effect on the KCl- and $BaCl_2$-induced contractions of guinea pig trachea, ONO-1078 appears to selectively interact with LTD_4 receptors in guinea pig trachea. This reversal effect of ONO-1078 was approximately 10-fold more potent than that of FPL55712. This result is inconsistent with the finding that ONO-1078 was about 3,300-fold more potent than FPL55712 in inhibiting the LTD_4-induced contraction of guinea pig trachea. This difference in the antagonist activity of FPL55712 against the LTD_4-induced sustained contraction may be due to the potent inhibition of phosphodiesterase (36) in addition to the antagonism against LTD_4.

The exposure of the sensitized guinea pig trachea to specific antigen produced a rapidly developing and prolonged contraction. It is suggested that histamine and SRS-A are involved in this response, with histamine mediating the early phase of the contraction and SRS-A primarily mediating the late protracted phase of the contraction (37). ONO-1078 selectively reduced the prolonged phase of antigen-induced contraction of guinea pig trachea without effect on the histamine-mediated early phase. Since ONO-1078 had no effect on 5-lipoxygenase activity and it is reported that a classical SRS-A antagonist, FPL55712, selectively reduced the prolonged phase of the antigen-induced contraction of guinea pig trachea (37), it appears that the selective reduction of the late phase contraction by ONO-1078 is due to the antagonism of peptide LTs. Recently, it is reported that selective antagonists of LTD_4 and LTE_4 only partially inhibited the antigen-induced contraction of guinea pig trachea, suggesting that this contraction may be partly mediated by the activation of LTC_4 receptors in guinea pig trachea (38, 39). On the contrary, ONO-1078 completely reduced the SRS-A mediated contraction of guinea pig trachea. This result could be explained by the antagonism of ONO-1078 against LTC_4 in addition to the potent antagonism against LTD_4. These results suggest that ONO-1078 potently antagonizes not only exogenous peptide LTs but also endogenously generated peptide LTs and it may be efficacious to possess antagonist activity against LTC_4 as well as LTD_4 and LTE_4.

In conclusion, ONO-1078 is a novel, highly potent, selective and competitive antagonist of peptide LTs. Furthermore, ONO-1078 also antagonizes endogenously generated peptide LTs. From these in vitro properties, ONO-1078 may have therapeutic value in the treatment of bronchial asthma and peptide LTs-related respiratory disorders.

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