Inhibitory Effect of the Newly Synthesized Pyridazinone Derivative NZ-107 on Bronchoconstriction Induced by Slow Reacting Substance of Anaphylaxis in the Guinea Pig

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Abstract—We have investigated the effect of a newly synthesized compound NZ-107, 4-bromo-5-(3-ethoxy-4-methoxybenzylamino)-3(2H)-pyridazinone, on bronchoconstriction induced by slow reacting substance of anaphylaxis (SRS-A) in the guinea pig. Orally administered NZ-107 (10 mg/kg, 2 hr) inhibited antigen-induced SRS-A-mediated bronchoconstriction in sensitized guinea pigs. NZ-107 (2 mg/kg, i.v., 1 min) prevented the antigen-induced response about as well as the SRS-A antagonist FPL-55712 and rapidly reversed it. This rapid reversal by NZ-107 but not FPL-55712 also appeared with the leukotriene (LT) D4-induced contraction of the isolated trachea. NZ-107 more selectively inhibited the LTD4 response than those of histamine, acetylcholine and KCl. Compared to FPL-55712, NZ-107 was one-fifteenth less potent in inhibiting the LTD4 response, but two-fold more potent in inhibiting the LTC4 response. NZ-107 inhibited the LTD4 response of the trachea 10-fold more potently than that of the ileum (IC50: trachea 5.61, ileum 4.56). The combination of NZ-107 (1 µM) with the β-agonist isoproterenol had no synergistic effect on the LTD4 response, but those of theophylline and papaverine had large effects. From these results, NZ-107 is a selective inhibitor of the SRS-A response and may be useful in the therapy of bronchial asthma and other diseases in which the LTs are thought to be involved.

It is well known that the allergic response is closely implicated in bronchial asthma. Slow reacting substance of anaphylaxis (SRS-A), consisting of the three arachidonic acid lipooxygenase products leukotriene (LT) C4, D4 and E4 (1–3), is considered to be an important mediator of allergic bronchoconstriction. Indeed, these substances are more potent bronchoconstrictors than histamine and cause prolonged contraction of human bronchial smooth muscle (4–6).

Recently, SRS-A antagonists have been produced for use in bronchial asthma therapy. FPL-55712 was the first SRS-A antagonist discovered (7), but because FPL-55712 has a short biologic half-life and lacks p.o. bioavailability, it is unsuitable for clinical use (8, 9). In contrast, LY171883, an analog of FPL-55712, is an orally active LTD4 antagonist (10) and is currently undergoing clinical evaluation (11). Because LY171883 is not only a moderate antagonist of the LTD4 response but also a potent inhibitor of phosphodiesterase, more potent and selective LTD4 antagonists have been developed (12, 13).

In this paper, we investigated the inhibitory effect of NZ-107, 4-bromo-5-(3-ethoxy-4-methoxybenzylamino)-3(2H)-pyridazinone (Fig. 1), newly synthesized in our laboratory, on SRS-A-induced bronchoconstriction in
the guinea pig in vivo and in vitro.

Materials and Methods

Materials: LTC₄-monomethylester and LTD₄-monomethylester (Paesel GmbH & Co., West Germany); rabbit anti-chicken egg albumin serum (Cooper Biochemical, PA); indomethacin, propranolol hydrochloride, pyrilamine maleate and aminophylline (Sigma Chemical Co., MO); theophylline, papaverine hydrochloride and histamine dihydrochloride (Wako Pure Chemicals, Japan); acetylcholine chloride (Dal-ichi Pharmaceuticals, Japan) NZ-107 and FPL-55712 were synthesized by the Central Research Laboratory, Nissan Chemical Ind. (Funabashi, Japan).

In vivo experiment: Male albino Hartley guinea pigs (350–450 g) were passively sensitized with i.v. injections of 125 μl of rabbit anti-chicken egg albumin 1 day preceding the experiment. Antigen-induced SRS-A-mediated bronchoconstriction was determined by the modified method of Anderson et al. (14). Sensitized animals were anesthetized with urethane (1.5 g/kg, i.p.). The right jugular vein was cannulated for the i.v. injection, and the trachea was cannulated to record total pulmonary resistance. Animals were artificially ventilated by a small respirator (Shinano, Model SN-480-7) set at a stroke volume of 4 to 5 ml and a rate of 50 breaths per min. The change in pulmonary resistance was measured with a differential pressure transducer (Nihon Kohden, Model TP-602T) connected to a T-tube on the tracheal cannula. The increase of air overflow volume, as a measure of bronchoconstriction, was expressed as a percentage of the maximal bronchoconstriction obtained by clamping off the trachea. Following surgical preparation, the animals were pretreated with indomethacin (2 mg/kg, 10 min), pyrilamine (2 mg/kg, 6 min) and propranolol (0.1 mg/kg, 5 min) before the chicken egg albumin challenge (0.2 mg/kg). NZ-107 was suspended in 5% gum arabic for p.o. administration and dissolved in 100% polyethylene glycol 400 for i.v. injection.

In vitro experiment: Male albino Hartley guinea pigs (250–350 g) were killed by a blow to the head. The trachea, lungs and ileum were removed. The trachea was sparsely cut and divided into three or four equal segments (one is the control), each was suspended under 1 g tension in an 8-ml organ bath containing a modified Tyrode solution maintained at 37°C and aerated with 95% O₂+5% CO₂. The composition of the modified Tyrode solution was: 137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.0 mM MgCl₂, 0.3 mM NaH₂PO₄, 20 mM NaHCO₃ and 11 mM dextrose. Responses were recorded isotonically (Nihon Kohden, Type TD-112S). Tissues were equilibrated for 50–60 min, and then the constant maximal responses to histamine (100 μM) were obtained. The following contractile responses were expressed as the percentage of the maximal response to histamine. The tissues were washed several times for 30 min until the resting level tone was restored and then incubated for 30 additional min with 5 μM indomethacin to inhibit the release of bronchoactive prostanoids (15, 16). For the reverse experiment, a sustained contraction was obtained by 30 nM LTD₄, and then the test compound was added singly. The reversal activity was determined by the pEC50 value, the negative logarithm of the concentration (molar) of test compound needed to relax 50% of the maximal response induced by 1 mM aminophylline. For the preventive experiment, tissues were incubated with the test compound in the presence of indomethacin for 30 min before an agonist was added singly or cumulatively. The preventive activity was determined by the pIC50 value, which is the negative logarithm of the concentration (molar) of the test compound needed to prevent 50% of the contractile response elicited by the following agonists: 30 nM LTC₄ in the presence of 45 mM mM -serine-borate complex (SB), 30 nM LTD₄, 30 μM histamine, 30 μM acetylcholine and 30 mM KCl. Lung parenchymal strips (3x3x30 mm) were dissected from the outer edges of the lung lobes and the experiment was performed.
in a manner similar to that of with the trachea. Ileal strips (20 mm) were suspended under 0.5 g tension at 30°C. The LTD\textsubscript{4}-induced contraction was obtained after 5 min incubation with the test compound in the absence of indomethacin. The response was expressed as a percentage of the 1 μM histamine response. NZ-107 was dissolved and diluted in 100% dimethylsulfoxide.

Statistics: Results are expressed as the mean±S.E.M. Statistical comparisons were made by Student’s t-test for paired and unpaired response, as appropriate. A P value of less than 0.05 was considered to be significant.

Results
Effects of NZ-107 on antigen-induced SRS-A-mediated bronchoconstriction in anesthetized guinea pigs: NZ-107 orally administered to passively sensitized guinea pigs 2 hr before antigen challenge prevented the antigen-induced SRS-A-mediated bronchoconstriction (Fig. 2). NZ-107 at doses of 10 mg/kg significantly inhibited the antigen-induced response and the magnitude of the inhibition was similar to that of 30 mg/kg NZ-107. The inhibitory effect of 30 mg NZ-107/kg on the antigen-induced response was observed 4 hr after the p.o. administration. Its effect disappeared at 6 hr (Fig. 3).

NZ-107 at doses from 2 to 5 mg/kg, i.v.,...
1 min before antigen challenge, significantly inhibited the SRS-A response. The magnitude of the inhibition was similar to that of the SRS-A antagonist FPL-55712 (Fig. 4). For the reversal experiment, NZ-107 was intravenously administered to the guinea pigs after the antigen-induced bronchoconstriction reached a steady level. NZ-107 at 2 mg/kg, i.v., rapidly reversed the antigen-induced response, but the same dose of FPL-55712 only gradually reversed it (Fig. 5).

These results show that NZ-107 not only has an orally active and a long-lasting inhibitory effect on the antigen-induced SRS-A-mediated bronchoconstriction in the guinea pig but the compound also rapidly reverses it when administered i.v.

Effects of NZ-107 on isolated guinea pig smooth muscle preparations: NZ-107 caused dose-dependent and rapid reversal of the contraction elicited by 30 nM LTD₄ in isolated trachea, but FPL-55712 gradually reversed it (Fig. 6).

The potency (pEC50) of NZ-107 to reverse the LTD₄-induced contraction was increased by about 1.3-fold at 60 min compared with that at 15 min, whereas the potency of FPL-55712 was greatly increased, by about 2.4-fold at 30 min and 4.8-fold at 60 min (Fig. 6, legend). This rapid reversal of NZ-107 on the LTD₄ response corresponded to that of i.v.

Fig. 4. Effect of i.v. administration of NZ-107 and FPL-55712 on the SRS-A-mediated bronchoconstriction in the guinea pig. NZ-107 and FPL-55712 were intravenously administered 1 min before antigen challenge. Each point represents the mean±S.E.M. of 6-8 animals. *P<0.05, compared to the control.

Fig. 5. Effect of i.v. administration of NZ-107 and FPL-55712 on reversing the SRS-A-mediated bronchoconstriction in the guinea pig. NZ-107 and FPL-55712 at a dose of 2 mg/kg was intravenously administered when the SRS-A response reached a plateau. Each point represents the mean±S.E.M. of 4-11 animals. *P<0.05, compared to the control.
Fig. 6. Effect of NZ-107 and FPL-55712 on reversing the LTD₄-induced contraction of isolated guinea pig trachea. NZ-107 and FPL-55712 was added when the 30 nM LTD₄-induced contraction reached a plateau. Each point represents the mean±S.E.M. of 4–5 experiments. pEC₅₀ values of NZ-107 at 15, 30 and 60 min were 5.68, 5.73 and 5.78, respectively; and those of FPL-55712 were 5.72, 6.11 and 6.40, respectively.

Table 1. Inhibitory effect of NZ-107 and FPL-55712 on the LTD₄-induced contraction of isolated guinea pig trachea, lung parenchyma and ileum

| Tissue                  | NZ-107  | FPL-55712 |
|-------------------------|---------|-----------|
| Trachea                 | 5.61ₐ (100)ₔ | 6.77 (100) |
| Lung parenchyma         | 4.43 (7) | 4.67 (0.8) |
| Ileum                   | 4.56 (9) | 6.60 (68)  |

ₐThe contractile response was induced by 30 nM LTD₄ for the trachea and lung parenchyma and 3 nM LTD₄ for the ileum. òpIC₅₀ is the negative logarithm of the concentration (molar) of NZ-107 and FPL-55712 required to prevent 50% of the contractile response elicited by LTD₄. ²Values are the means of four experiments. ₔRelative potency was determined as follows: IC₅₀ (trachea)/IC₅₀ (lung or ileum) x 100.

NZ-107 inhibits SRS-A-mediated contraction.

administration of NZ-107 on the antigen-induced response. The reversal effect of LTD₄-induced contractions by NZ-107 was not inhibited by the β-blocker propranolol (1 μM) (data not shown).

Table 1 shows the inhibitory effect of NZ-107 on the contraction elicited by LTD₄ in trachea, lung parenchyma and ileum (Table 1). The inhibitory activity (pIC₅₀) of NZ-107 against the LTD₄ response was one-fifteenth less potent than that of FPL-55712 in the trachea. The ability of NZ-107 to inhibit the LTD₄ response in the ileum was about one-tenth less potent than that in the trachea, whereas FPL-55712 had the same inhibitory effect on the LTD₄ response in both the ileum and the trachea. In lung parenchyma, the inhibition by NZ-107 of the LTD₄ response was equivalent to that of FPL-55712. NZ-107 had the same inhibitory effect on the LTD₄-induced contraction of the trachea in the absence of indomethacin (pIC₅₀=5.63).

The ability of NZ-107 to inhibit the LTC₄-induced contraction of the trachea was about one-fifth less potent than that of LTD₄, but this inhibitory activity of NZ-107 on the LTC₄
response was about 2-fold more potent than that of FPL-55712 (pIC50=4.66). NZ-107 showed moderate selectivity for inhibiting LTD4-induced contraction of the trachea compared with histamine-, acetylcholine- and KCl-induced responses (Table 2).

NZ-107 not only caused a rightward shift in the LTD4 concentration-response curve, but also suppressed the maximal response of LTD4 in the trachea, lung and ileum (Fig. 7). These results indicate that the inhibitory effect of NZ-107 against the LTD4-induced response may be non-competitive antagonism.

The combination of 1 \( \mu \text{M} \) NZ-107, which significantly inhibited the LTD4-induced contraction, with the \( \beta \)-agonist isoproterenol (3 nM) did not have a synergistic effect on the LTD4 response in the trachea, but the combination of the phosphodiesterase inhibitor theophylline (100 \( \mu \text{M} \)) with isoproterenol had a significant synergistic effect on it. This synergistic effect on the LTD4 response also appeared in the combination of the other phosphodiesterase inhibitor papaverine (1 \( \mu \text{M} \)) with isoproterenol (Fig. 8).

These results show that NZ-107 selectively inhibits the contraction elicited by LTD4 and its inhibitory effect is more potent in the trachea than in the ileum.

### Table 2. Inhibitory effect of NZ-107 on the contractile response elicited by various pharmacological agents in the isolated guinea pig trachea

| Agonist          | pIC50\(^a\) | Relative potency\(^b\) |
|------------------|-------------|------------------------|
| LTD4             | 5.61 \(c\) | 100                    |
| LTC4+45 mM SB    | 4.93        | 20                     |
| His              | 5.08        | 30                     |
| ACh              | 3.60        | 1                      |
| KCl              | 4.47        | 7                      |

\(^a\)pIC50 is the negative logarithm of the concentration (molar) of NZ-107 required to prevent 50% of the contractile response elicited by various agonists. \(^b\)Relative potency was determined as follows: IC50 (LTD4)/IC50 (tested agonist) \times 100. \(^c\)Values are the means of four to six experiments.

![Fig. 7. Effect of NZ-107 on LTD4-induced contraction of isolated trachea, lung parenchyma and ileum. The contractile response was expressed as the percentage of the response obtained by 100 \( \mu \text{M} \) histamine for the trachea and lung parenchyma or with 1 \( \mu \text{M} \) histamine for the ileum. Each point represents the mean±S.E.M. of 4–9 experiments.](image)
Fig. 8. Combined effect of 1 μM NZ-107, 100 μM theophylline and 1 μM papaverine with 3 nM isoproterenol (Iso) on LTD₄-induced contraction of isolated trachea. The contractile response is expressed as shown in Fig. 7. Each point represents the mean±S.E.M. of 5 experiments. *P<0.05, compared to the control; tP<0.05, compared to each drug alone.

Discussion

NZ-107 produced orally active and long-lasting inhibitory effects on antigen-induced SRS-A-mediated bronchoconstriction in the guinea pig. The inhibitory effect of NZ-107, i.v., on the antigen-induced response was almost the same as that of FPL-55712, but the ability of NZ-107 to inhibit the LTD₄-induced contraction of isolated trachea was about one-fifteenth less potent than that of FPL-55712. These results may be related to the inhibitory action of NZ-107 on the LTC₄ response. NZ-107 inhibits LTC₄-induced contraction of the isolated trachea two times more potently than FPL-55712. LTC₄ was reported to be released from sensitized guinea pig trachea stimulated by antigen (17) and metabolized to LTD₄ (18). NZ-107 seems to also act as an inhibitor of the LTD₄ response. It is also conceivable that 1) NZ-107 may inhibit SRS-A synthesis and/or its release from mediator cells, and 2) NZ-107 may inhibit the contraction induced by other chemical mediators, e.g., platelet activating factor (19). Further investigations are needed to elucidate the pharmacological profile of NZ-107. It is unlikely that the short half-life of FPL-55712 may be related to its weak inhibitory effect on the antigen-induced bronchoconstriction, because the potency of the preventive effect of i.v. administration of FPL-55712 on the antigen-induced response was equivalent to that of the reversal effect.

NZ-107 rapidly reversed not only the antigen-induced response in anesthetized guinea pigs but also reversed the LTD₄ response in isolated trachea, whereas the SRS-A antagonist FPL-55712 caused a slowly developed decrease in the antigen-induced/LTD₄ response. The rapid reversal by NZ-107 is not mediated by β-adrenoceptor stimulation, since the NZ-107-induced rapid reversal of the LTD₄ response was not inhibited by the presence of the β-blocker propranolol. NZ-107 not only shifted the LTD₄ concentration-response curve to the right, but also suppressed its maximal response in isolated trachea. We, therefore, suggest that the rapid reversal effect by NZ-107 of the antigen-induced/LTD₄ response is due to another action rather than LTD₄ antagonism. This rapid reversal effect of the LTD₄ response was also observed with cyclic AMP-dependent bronchodilators such as the β-adrenoceptor agonist isoproterenol and the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) (K. Shikada et al., unpublished data). It is conceivable that the rapid reversal effect of NZ-107 on the antigen-induced/LTD₄ response is not related to the
stimulation of \(\beta\)-adrenoceptors, but may be partly accompanied by increasing intracellular cyclic AMP level.

It has been demonstrated that the synergic action occurs by the combination of the phosphodiesterase inhibitor theophylline with the \(\beta_2\)-agonist salbutamol on LTD4-induced contractions of the guinea pig trachea (20). However, 1 \(\mu\)M NZ-107, which rapidly reversed and significantly prevented the LTD4 response, did not produce a synergistic effect by combination with isoproterenol, whereas theophylline and the other phosphodiesterase inhibitor papaverine did so markedly. From these results, it is unlikely that the rapid reversal effect of 1 \(\mu\)M NZ-107 on the LTD4 response is due to the increase of cyclic AMP level in the trachea.

NZ-107 more selectively inhibited the LTD4-induced contraction of the trachea than those of lung parenchyma and ileum, whereas the LTD4 receptor antagonist FPL-55712 more selectively inhibited LTD4-induced contractions of the trachea and ileum than that of the lung. FPL-55712 (21) and LY-171883 (10) have been reported to be more effective in antagonizing the LTD4 response in the trachea and ileum than in lung parenchyma. It is unclear why NZ-107 had little effect on the LTD4-induced contraction in the ileum. In the guinea pig trachea, the LTD4 response has been reported to be modulated by endogenous prostaglandins (16, 22–24) in the guinea pig trachea. Since the inhibition by NZ-107 of the LTD4 response in the trachea was not changed in the presence or absence of indomethacin, the effect of NZ-107 on the LTD4 response is not mediated by the modification of the endogenous release of dilator prostaglandins. The weak activity of NZ-107 in inhibiting the LTD4 response in the ileum may account for the fewer side effects encountered in NZ-107 therapy, because LTD4 may play a significant physiological role in the gastrointestinal track. Moreover, to the moderate inhibition by NZ-107 of the histamine-induced contraction must be added the beneficial profile on bronchial asthma in the clinical trial of NZ-107.

In conclusion NZ-107 is an orally active inhibitor for antigen-induced endogenous SRS-A-mediated bronchoconstriction in the guinea pig and the inhibitory effect of NZ-107 on the LTD4-induced contraction is more selective towards the tracheal muscle than the ileal muscle. These profiles of NZ-107 could be beneficial in treatment of SRS-A-predominant bronchial asthma.

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