

Inhibitory Effect of N-(3', 4'-dimethoxycinnamoyl) Anthranilic Acid (N-5') on SRS-A Mediated Bronchoconstriction in the Guinea Pig *in Vivo*

M. FUJIMURA, T. KOSHINO, T. ISHIZAKI*, S. MINAMI, T. SAGA and S. MIYABO*

Third Department of Internal Medicine, Kanazawa University School of Medicine, and

*Third Department of Internal Medicine, Fukui Medical School, Japan

Slow-reacting substance of anaphylaxis (SRS-A) is an important factor mediating bronchoconstriction in asthma. We developed a guinea pig model for SRS-A-mediated bronchoconstriction induced by antigen inhalation. Using this model, we investigated the effect of N-(3', 4'-dimethoxycinnamoyl) anthranilic acid (N-5'), a new anti-allergic drug, on the bronchoconstriction. FPL 55712 inhibited most of the bronchoconstriction induced by antigen inhalation. N-5' inhibited the antigen-induced bronchoconstriction in a dose-dependent fashion. Intraperitoneal administration of 200 mg/kg N-5' was effective for 40 min after antigen inhalation, while the effect of 60 mg/kg lasted only 7 min. On the other hand, 200 mg/kg N-5' showed no inhibitory effect on the bronchoconstriction caused by direct inhalation of leukotriene C₄, a component of SRS-A. These findings indicate that one of the anti-allergic actions of N-5' is due to inhibition of synthesis and/or release of SRS-A.

Key words: anti-allergic drug, N-5'; allergic bronchoconstriction; guinea pigs; LTC₄; SRS-A.

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N-(3', 4'-dimethoxycinnamoyl) anthranilic acid (N-5') introduced by Koda et al. (5) has been used therapeutically for bronchial asthma (8). On the other hand, slow-reacting substance of anaphylaxis (SRS-A) has been considered to be an important bronchoconstrictor in this allergic disease (2, 4). Furthermore, most research groups have concluded that leukotriene C₄ and D₄ are major constituents of SRS-A (7, 9).

In this study, the anti-allergic actions of N-5' on aerosol antigen-induced bronchoconstriction and bronchoconstriction produced by aerosol leukotriene C₄ (LTC₄) were examined in anesthetized, mechanically ventilated guinea pigs.

MATERIAL AND METHODS

Male albino Hartley strain guinea pigs weighing 300 to 460 g were used in this study.

Allergic bronchoconstriction

Passive sensitization of guinea pigs. Guinea pig homocytotropic antiserum was made by the method of Santives et al. (10). 500 µg ovalbumin (OA) was emulsified in complete Freund's adjuvant and injected intradermally into each guinea pig at multiple sites. Boostering was carried out in the same manner 2 weeks later. Serum was collected from each animal 2 weeks after the booster, pooled and kept frozen until use. The antibody titer of this serum was 1:12800 and 1:6400 as estimated by passive cutaneous anaphylaxis (PCA) at 4 and 24 h, respectively. Normal guinea pigs were passively sensitized with 1 ml/kg of the antiserum administered intraperitoneally.

Preparation of animals. 12 to 24 h after passive sensitization, the guinea pigs were anesthetized

with an intraperitoneal injection of 75 mg/kg sodium pentobarbital. They were placed in the supine position, the trachea was cannulated with a polyethylene tube (o.d. 2.5 mm; i.d. 2.1 mm) and one jugular vein was cannulated for the administration of drugs.

Airway opening pressure (Pao), the lateral pressure of the tracheal tube, was measured to estimate the overall bronchoconstriction (Model TP-603T, Nihon Koden Kogyo Co., Ltd., Tokyo) (3). The animals were artificially ventilated by a small animal ventilator (Model 1680, Harvard Apparatus Co. Inc., South Natick, MA.) adjusted to a tidal volume of 10 ml/kg at a rate of 60 breaths/min. Mechanical dead space in this system was 0.5 ml.

When all procedures were completed, the animals were given 60 mg/kg diphenhydramine hydrochloride intraperitoneally to block the action of histamine completely, and received a vital capacity maneuver in order to unify the volume history of the lung (3).

Aerosol antigen challenge. 15 min later, when Pao was stable, the animals were challenged with nebulized ovalbumin dissolved in saline (1 mg/ml) without interrupting the constant ventilation. The ovalbumin aerosol was generated for 30 sec by an apparatus equipped with an ultrasonic nebulizer, developed for small animal experiments (6). Since the amount of aerosolized ovalbumin solution (1 mg/ml) was 15.2 μ l/min, and 46.4% of the aerosol was deposited in the lung (6), approximately 3.5 μ g ovalbumin was considered to be deposited in the lung during the 30 sec.

Effects of FPL 55712. Intravenous administration of FPL 55712 dissolved in distilled water (0.25 vol%) was started 10 min before the antigen challenge and lasted for 40 min; the total dose given was 20 mg/kg. Control animals were given distilled water in the same manner.

Effects of N-5'. The passively sensitized guinea pigs were pretreated with N-5' (60 or 200 mg/kg) intraperitoneally 20 min before anesthetization.

LTC₄ induced bronchoconstriction

LTC₄ and acetylcholine provocation. Normal guinea pigs were prepared as described above. LTC₄ dissolved in saline (0.1 μ g/ml) was given by aerosol. This concentration of LTC₄ was selected from the result of a previous study (3). 40 min after the inhalation, a vital capacity maneuver was done and acetylcholine i.v. (16 μ g/animal) administered.

Effects of N-5'. The normal guinea pigs were given 200 mg/kg N-5' intraperitoneally 20 min before anesthetization.

Statistical analysis

Statistical differences were determined by Mann-Whitney's U test, with a *P* value of 0.05 or less as significant.

Chemicals

The following chemicals were used: ovalbumin (Sigma, St. Louis, U.S.A.); diphenhydramine hydrochloride (Sigma, St. Louis, U.S.A.); complete Freund's adjuvant (Difco Laboratories, Detroit, MI, U.S.A.); sodium pentobarbital (Abbott Laboratories, North Chicago, IL); acetylcholine chloride (Daichi Pharmaceutical Co., Ltd., Tokyo, Japan); N-(3', 4'-dimethoxycinnamoyl) anthranilic acid (N-5') (Kissei Pharmaceutical Co., Ltd., Matsumoto, Japan); leukotriene C₄ (Ono Pharmaceutical Co., Ltd., Osaka, Japan); FPL 55712 (Fisons Pharmaceuticals, Ltd., U.K.).

RESULTS

Aerosol antigen challenge produced gradual and long-lasting increase in Pao. Maximal increase in this index was observed at 10 min after the challenge.

FPL 55712 did not affect Pao value before the challenge. The increase in Pao induced by antigen challenge was significantly inhibited by FPL 55712 (Fig. 1). The inhibitory rate was 84.6% as estimated 10 min after the challenge.

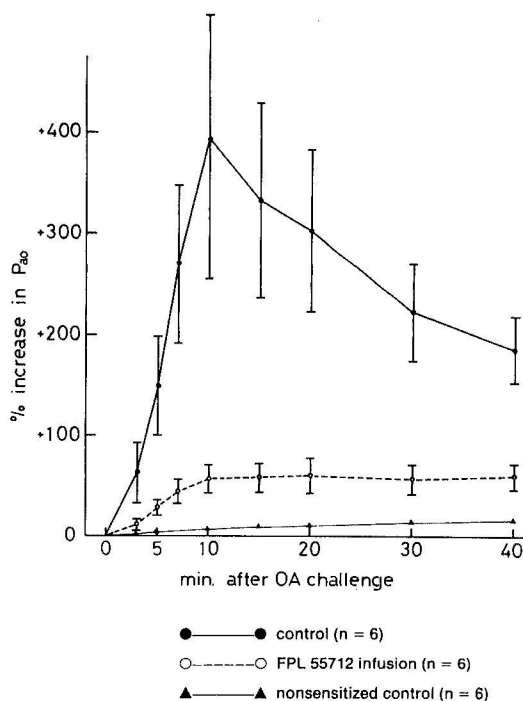


Fig. 1. Inhibition of allergic bronchoconstriction by FPL 55712 in mechanically ventilated guinea pigs pretreated with 60 mg/kg diphenhydramine hydrochloride. Each value represents mean $\pm$ SD. $n = 6$ in all groups.

Pretreatment with N-5' (60 and 200 mg/kg) did not affect Pao value before antigen challenge. Pretreatment with 60 mg/kg N-5' significantly inhibited increase in Pao at 3 to 7 min after the challenge (Fig. 2). The inhibitory rate was 49.3% as estimated at 10 min after the challenge. 200 mg/kg of N-5' significantly inhibited Pao increase throughout the observation period (Fig. 3). The inhibitory rate was 93.6% as estimated at 10 min.

Inhaled LTC₄ produced an increase in Pao which reached maximum at 5 to 6 min after the inhalation. Pretreatment with 200 mg/kg of N-5' did not affect the increase in Pao at all (Fig. 4).

Transient increase in Pao was observed after intravenous administration of acetylcholine, which was not significantly inhibited by 200 mg/kg N-5' (Fig. 4).

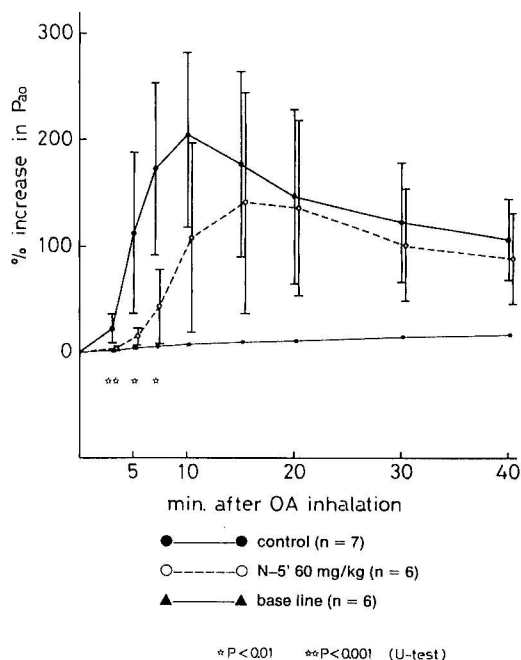


Fig. 2. Inhibitory effect of 60 mg/kg N-5' on allergic bronchoconstriction. Each value represents mean $\pm$ SD. Baseline $n = 6$.

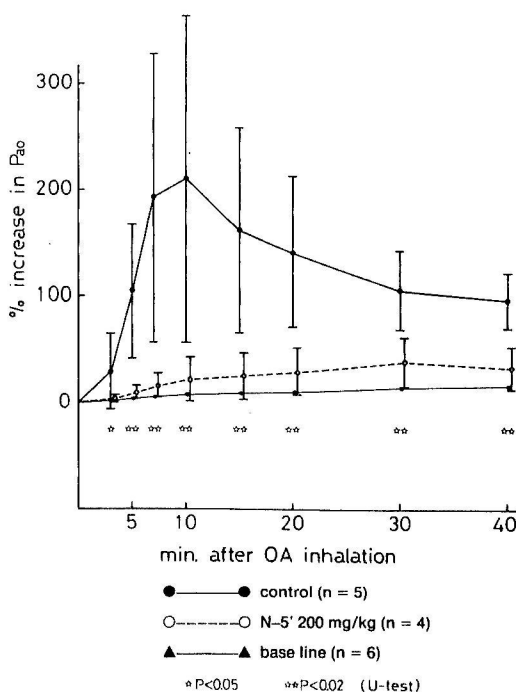


Fig. 3. Inhibitory effect of 200 mg/kg N-5' on allergic bronchoconstriction. Each value represents mean $\pm$ SD. Baseline $n = 6$.

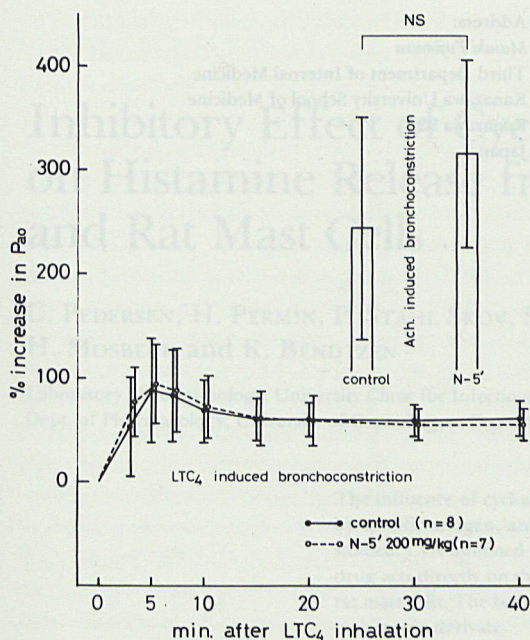


Fig. 4. Influence of N-5' on LTC₄ and acetylcholine-induced bronchoconstriction. Each value represents mean $\pm$ SD.

DISCUSSION

The guinea pigs used in this study were passively sensitized with homocytotropic antiserum and pretreated with a high dose of diphenhydramine hydrochloride. The hypersensitivity reaction occurred when animals were challenged with the aerosol antigen. The bronchoconstriction developed slowly and lasted for 40 min. Our previous results revealed that this procedure caused greater change in dynamic compliance than in mean pulmonary resistance (3). In this study, this type of allergic bronchoconstriction was inhibited for the most part by FPL 55712, a selective inhibitor of SRS-A (1). These findings indicate that the allergic bronchoconstriction in this study is mediated mainly by SRS-A.

N-5' decreases anaphylactic mediator release from guinea pig lung *in vitro* and inhibits homologous passive cutaneous anaphylaxis (PCA) both in rats and guinea pigs (5). Clinically, this agent has been proved useful for the prevention

of asthmatic attacks (8). However, little has been done to elucidate the mechanisms of the anti-allergic action of this drug. The results of this study revealed that pretreatment with N-5' apparently inhibited aerosol antigen-induced bronchoconstriction which was mediated mainly by SRS-A in a dose-dependent fashion. In contrast, bronchoconstriction caused by inhalation of LTC₄ was not inhibited by the pretreatment at all. Furthermore, N-5' did not change bronchial reactivity to acetylcholine. These facts clearly indicate that an important part of the anti-allergic actions of N-5' is due to inhibition of synthesis and/or release of SRS-A.

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REFERENCES

- Augstein, J., Farmar, J. B., Lee, T. B., Sheard, P. & Tattersall, M. L.: Selective inhibitor of slow reacting substance of anaphylaxis. *Nature* **245**, 215-217, 1974.
- Brocklehurst, W. E.: The release of histamine and formation of a slow reacting substance (SRS-A) during anaphylactic shock. *J. Physiol.* **151**, 416-435, 1960.
- Fujimura, M.: Inhibitory effects of steroids on slow-reacting substance of anaphylaxis (SRS-A) mediated bronchoconstriction in the guinea pig *in vivo*. *Jpn. J. Allergol.* **32**, 365-375, 1983.
- Kellaway, C.H. & Trethewie, E. R.: The liberation of a slow-reacting smooth muscle-stimulating substance in anaphylaxis. *Q. J. Exp. Physiol.* **30**, 121-145, 1940.
- Koda, A., Nagai, H., Watanabe, S., Yanagihara, Y. & Sakamoto, L.: Inhibition of hypersensitivity reactions by a new drug, N (3', 4'-dimethoxycinnamoyl) anthranilic acid (N-5'). *J. Allergy Clin. Immunol.* **57**, 396-407, 1976.
- Minami, S., Okafuji, K., Saga, T., et al.: A new quantitative inhalation apparatus for small animals. *Jpn. J. Chest Dis.* **21**, 252-258, 1983.
- Murphy, R. C., Hammarström, S. & Samuelsson, B.: Leukotriene C: A slow reacting substance from murine mastocytoma cells. *Proc. Natl. Acad. Sci.* **76**, 4275-4279, 1979.
- N-5' Study Group in Children: A double blind controlled trial of N-(3', 4'-dimethoxycinnamoyl) anthranilic acid on children with bronchial asthma. *Allergy* **34**, 213-219, 1979.

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