The Effect of trans-4-Guanidinomethylcyclohexanecarboxylic Acid p-tert-Butylphenyl Ester Hydrochloride (NCO-650) on Ascaris suum Antigen-Induced Bronchoconstriction in Dogs

Miwa MISAWA, Kiyoteru TAKENOUCHI, Masaru SATO* and Saizo YANAURA
Department of Applied Pharmacology, School of Pharmacy, Hoshi University, Ebara, Shinagawa-ku, Tokyo 142, Japan *Research Institute of Nippon Chemiphar Co., Ltd., Misato, Saitama 341, Japan
Accepted October 6, 1986

Abstract—Antiallergic asthma effect of trans-4-guanidinomethylcyclohexancarboxylic acid p-tert-butylphenyl ester hydrochloride (NCO-650), a new anti-allergic drug, was investigated in comparison with those of tranilast and disodium cromoglycate (DSCG) in anesthetized dogs. The asthmatic bronchoconstriction was induced by inhalation of Ascaris suum antigen (Asc-Ag) to naturally Ascaris sensitive dogs. The airway resistance was determined using the modified Konzett-Rössler method. Both intravenous (1 and 5 mg/kg) and intraduodenal (10, 30 and 100 mg/kg) administrations of NCO-650 prior to the antigen challenge markedly inhibited the asthmatic bronchoconstriction induced by Asc-Ag inhalation. The antiasthmatic effect of NCO-650 was much stronger than that of DSCG (10 mg/kg, i.v.) and was about three-fold stronger than that of tranilast. On the other hand, when NCO-650 was administered after the antigen challenge, the agent had no inhibitory effect on the Asc-Ag induced bronchoconstriction. As for the effects on increased airway secretion at the time of asthmatic attack, NCO-650 inhibited the excessive secretions without any remarkable change in the viscosity of the secretions. NCO-650 had no effect on the bronchoconstriction induced by inhalation of acetylcholine, suggesting that NCO-650 appears to have no anticholinergic effect and thus no effect on the vagal reflex that occurred during the asthmatic responses. The above findings show that NCO-650 may be useful for the treatment of bronchial asthma as an orally active drug.

It is generally supposed that most bronchial asthmas are concerned with an immediate hypersensitivity reaction, known also as an anaphylactic or Type I allergic reaction. Mast cell stabilizers such as disodium cromoglycate (DSCG) and tranilast (N-5') have been proven to be useful in prophylactic treatment of patients with allergic bronchial asthma (1, 2). Trans-4-guanidinomethylcyclohexanecarboxylic acid p-tert-butylphenyl ester hydrochloride (NCO-650) (Fig. 1) is a newly synthesized anti-allergic agent with splendid oral absorption (3). A few in vitro and in vivo antiallergic effects of NCO-650 have hitherto been reported, and it inhibits the release of histamine from rat peritoneal mast cells and histamine-containing liposomes (3, 4). It is known that most mongrel dogs are naturally sensitized by nematode parasites and possess reaginic antibodies (5). Inhalation of Ascaris suum antigen (Asc-Ag) to dogs causes anaphylactic

![Fig. 1. Chemical structure of trans-4-guanidiniumethylcyclohexanecarboxylic acid p-tert-butylphenyl ester hydrochloride (NCO-650).](image-url)
bronchoconstriction, and thus this response has been used as an experimental asthma model (6, 7). In the present study, the effects of NCO-650 on Asc-Ag induced bronchoconstriction and bronchosecretion were examined in comparison with those of DSCG and tranilast, of which effects on Asc-Ag induced asthma have also been poorly investigated.

Materials and Methods

Experimental animals: Male mongrel dogs weighing 7.5–22.0 kg were anesthetized with pentobarbital sodium (30 mg/kg, i.v.) and immobilized with decamethonium bromide (initial dose of 0.4 mg/kg, i.v., and supplemental doses of 0.2 mg/kg, i.v., every hour). The animals were placed in the supine position and ventilated artificially through a specialized endotracheal cannula (called “stopper” (7)) at a frequency of 30 breaths/min.

Antigen preparation and skin reactivity test: Ascaris suum antigen (Asc-Ag) was prepared according to the previously described method (7). Dogs naturally sensitive to Asc-Ag were selected by means of a skin reactivity test using antigen extract solutions diluted with veronal buffered saline (VBS) (3×10⁻³ to 10⁻⁷ g protein/ml). Anesthetized dogs were given 1% Evans Blue dye in a volume of 0.2 ml/kg, i.v., 15 min before skin testing. The skin test was done by giving an intracutaneous injection of 0.1 ml of each dilution of antigen solution. Thirty min after the injections of the antigen solutions, blueing diameters at the injected sites were measured. Dogs that showed a significant blueing to the solutions in a concentration range of 10⁻⁷ to 10⁻⁵ g protein/ml were determined to be suitable for the experiment. In the experiments where the effect of posttreatment with NCO-650 was investigated, dogs that showed a moderate bronchoconstriction to inhalation of Asc-Ag were used, regardless of skin test reactivity.

Determination of airway constriction: In anesthetized and immobilized dogs, the bronchomotor tone was measured by a modification of the Konzett-Rössler method (8) (Fig. 2). The lung was inflated at a fixed volume of air under a constant pressure (10 cmH₂O), and ventilation overflow was continuously recorded with a combination of a pneumotachograph (MFP-1T, Nihon Kohden) and an integrator (RFJ-5, Nihon Kohden) as an index of change in airway resistance. Systemic arterial blood pressure was monitored with a pressure transducer (MPU-0.5, Nihon Kohden) from an arterial cannula inserted into the left femoral artery, and heart rate was measured with a tachometer (RT-5, Nihon Kohden) using systolic blood pressure as the trigger. The above parameters were all recorded using a polygraph (Nihon Kohden, RM-85).

Induction of allergic asthma attacks (7): Asc-Ag (1.5 mg protein/ml; total 3.0 mg protein) was aerosolized and inhaled for 10 min with the use of an ultrasonic nebulizer (TUR-3000, Nihon Kohden) introduced into the respiratory circuit.

Measurement of volume and viscosity of airway secretions: Bronchial secretion was measured by the stopper method (9, 10). Sixty min after the end of 10-min Asc-Ag inhalation, airway secretions which had accumulated at the stopper were collected, and the secretory volume and its viscosity
were measured.

**Acetylcholine induced airway constriction:** Acetylcholine was inhaled for 10 min with the ultrasonic nebulizer. In each animal, one concentration of the agent that caused a moderate bronchoconstriction was determined in a concentration range of 0.005–0.08%. Effects of NCO-650 and atropine were determined by comparison of the acetylcholine induced bronchoconstrictions before and after administration of these drugs.

**Drugs:** The drugs used were NCO-650 (Nippon Chemiphar), disodium cromoglycate (Fujisawa), tranilast (synthesized by Nippon Chemiphar), acetylcholine chloride (Ovisot, Daiichi Seiyaku) and atropine sulphate (Tokyo Kasei). All these drugs were dissolved in saline when used for i.v. administration, and suspended in 5% arabic gum solution when used for intraduodenal (i.d.) administration at the time of usage. In the experiments of i.d. administration, the animals were fasted overnight before use. The doses of NCO-650 and DSCG were expressed as the respective salt. The dose, route and timing of drug administration in the experiments are shown in the "Results".

**Statistical analysis:** All values were expressed as the mean with S.E. Statistical significance of difference was determined by Student's t-test.

**Results**

Effects of NCO-650, disodium cromoglycate and tranilast on Asc-Ag induced bronchoconstriction: Asc-Ag was inhaled for 10 min at a total dose of 3 mg protein. Asc-Ag caused a remarkable increase in ventilation overflow, sometimes accompanied by slight falls in blood pressure and heart rate (Fig. 3). The bronchoconstrictor response attained to the peak near the end of Asc-Ag inhalation and lasted for more than 60 min. Inhalation of VBS, instead of Asc-Ag, had no effect on ventilation overflow as well as on blood pressure and heart rate.

Intravenous administrations of NCO-650 at doses of 1 and 5 mg/kg 10 min before Asc-Ag inhalation and administration of DSCG at a dose of 10 mg/kg 30 sec before the inhalation significantly inhibited the increase in ventilation overflow (Fig. 4).

Effects of drugs were estimated from Fig. 4 by the integrated value of increase in ventilation overflow as expressed by the area under the increasing airway resistance curve over the preinhalation value in the 30 min period after the end of Asc-Ag inhalation. The integrated value in the control group was 1286±215 (ml-min) (Fig. 5). NCO-650 at doses of 1 and 5 mg/kg i.v. showed a dose-dependent inhibitory effect and the inhibition ratios were 78% and 95%, respectively. In the DSCG (10 mg/kg, i.v.) treated group, the integrated value was 25±6 ml-min (Fig. 5).

**Fig. 3.** Typical recording of the bronchomotor response to inhalation of *Ascaris suum* antigen (Asc-Ag). B.P.: systemic blood pressure. H.R.: heart rate and V.O.: ventilation overflow as an index of airway resistance. Asc-Ag (total of 3.0 mg protein) was inhaled for 10 min.
Fig. 4. Effects of i.v. pretreatments with NCO-650 and disodium cromoglycate (DSCG) on the increase in ventilation overflow induced by inhalation of Ascaris suum antigen (Asc-Ag). Each value represents the mean with S.E. *P<0.05, **P<0.01 and ***P<0.001 vs. the control group.

Fig. 5. Effects of i.v. pretreatments with NCO-650 and disodium cromoglycate (DSCG) on the integrated increase in ventilation overflow induced by inhalation of Ascaris suum antigen (Asc-Ag). The integrated increase was expressed by the area under the increasing airway resistance curve over the pre-inhalation value in the 30 min-period after the end of Asc-Ag inhalation. Each column represents the mean with S.E. Numbers of experiments are as in Fig. 4. *P<0.05 and **P<0.01 vs. the control group.

The inhibition ratio was 58%.

On the other hand, when NCO-650 at the same doses was postadministered immediately after the end of Asc-Ag inhalation, the agent caused no significant effect on the increase in ventilation overflow (Fig. 6).

Effects of intraduodenal (i.d.) administration of these drugs are shown in Fig. 7. The integrated value of the increase in ventilation overflow in the control group was 1113±136 (ml-min). In the NCO-650 treated groups in which 10, 30 and 100 mg/kg i.d. were administered 60 min before the inhalation of Asc-Ag, the integrated values were 1180±265, 387±144 and 453±105 (ml-min), respectively. The inhibitions were significant in doses of 30 and 100 mg/kg, and the inhibition ratios were 65% and 59%, respectively. When tranilast at doses of 30 and 100 mg/kg i.d. was administered 60 min before the inhalation of Asc-Ag, the integrated values of 998±357 and 484±109 (ml-min) were obtained, respectively. The inhibition was significant at a dose of 100 mg/kg, i.d., and the inhibition ratio was 57%. NCO-650 and tranilast by themselves did not affect the bronchomotor tone.

Effects of NCO-650 and tranilast on Asc-Ag induced bronchosecretory response: The results obtained are summarized in Fig. 8. The basal secretory volume for 70 min was 0.040±0.020 ml (N=6). In the control
Fig. 6. Effects of i.v. posttreatment with NCO-650 on the increase in ventilation overflow induced by inhalation of *Ascaris suum* antigen (Asc-Ag). NCO-650 was administered immediately after the end of Asc-Ag inhalation. Each value represents the mean with S.E.

Fig. 7. Effects of intraduodenal pretreatments with NCO-650 and tranilast on the integrated increase in ventilation overflow induced by inhalation of *Ascaris suum* antigen. Each column represents the mean with S.E. The numbers in the columns are those of experiments. **P<0.01 vs. the control group.

Fig. 8. Effects of intraduodenal pretreatments with NCO-650 and tranilast on the increase in airway secretion induced by inhalation of *Ascaris suum* antigen. White columns represent the airway secretion volume. Hatched columns represent the viscosity of airway secretions. Each column represents the mean with S.E. The numbers in the columns are those of experiments. In the NCO-650 (30 mg/kg, i.d.) and tranilast (100 mg/kg, i.d.) treated groups, the secretion volume of each animal was so small that determination of viscoelasticity could not be done. *P<0.05 and **P<0.01 vs. the control group.
group, the inhalation of Asc-Ag provoked a hypersecretion in the airways; the total secretion volume for 70 min was 0.22±0.07 ml. Intraduodenal administration of NCO-650 at doses of 30 and 100 mg/kg caused a strong inhibition of the Asc-Ag induced hypersecretion; the secretion volumes were 0.04±0.01 and 0.04±0.02 ml, respectively. Tranilast at doses of 30 and 100 mg/kg caused a dose-dependent inhibition of hypersecretion, although not significantly. No significant change in viscoelastisity was observed with NCO-650 (10 and 100 mg/kg, i.d.) and tranilast (30 mg/kg). In the NCO-650 (30 mg/kg, i.d.) and tranilast (100 mg/kg, i.d.) treated groups, the secretion volume of each animal was so small that determination of viscoelastisity could not be done.

**Effects of NCO-650 and atropine on acetylcholine induced bronchoconstriction:** Inhalation of acetylcholine for 10 min induced a transient increase in ventilation overflow, accompanied by no change in systemic blood pressure and heart rate. When acetylcholine was inhaled again 60 min after administration of NCO-650 at a dose of 100 mg/kg, i.d., the increase in ventilation overflow evoked (19.3±3.8 ml) was almost the same degree as that seen before treatment (20.8±3.4 ml) (N=4). On the other hand, atropine at a dose of 1 mg/kg, i.p., inhibited the increase in ventilation overflow induced by acetylcholine by more than 90% (from 27.3±8.7 ml to 1.7±0.9 ml) (N=3).

**Discussion**

The bronchoconstriction induced by inhalation of Asc-Ag to dogs is one of the models of bronchial asthma. IgE type reaginic antibodies are involved in this reaction, probably through a cross-sensitivity between Asc-Ag and *Toxocara canis* antigen by which most dogs are naturally sensitized. Hitherto, clinically used antiasthmatic drugs such as dyphylline (11) and anticholinergic agents (7, 12, 13) have been proven to be effective in this experimental asthma model. Antihistamines are only slightly effective on this model (14), as is the case in human asthma.

DSCG and tranilast are widely used for the treatment of patients with bronchial asthma. Antiasthmatic effects of DSCG and tranilast in laboratory animals have been investigated in various experiments (15–20). There were, however, very few experimental asthma models with which the effectiveness of these drugs could be ascertained. Especially, it is generally known that DSCG has no effect on allergy induced bronchoconstrictions in guinea pigs (16), although this asthma model is often used as a screening model for antiallergic agents. As for the Asc-Ag induced asthma in dogs, the two standard antiallergic drugs significantly inhibited the bronchial response as shown in the present study. Thus, it is supposed that with this asthma model clinical antiallergic effects of this type of drugs can be predicted. As for DSCG, this agent is extensively administered inhalationally in clinical use. In laboratory studies concerning allergy, an i.v. dose range of DSCG used to inhibit the rat PCA reaction is 0.1 to 10 mg/kg (17). A dose-related antiallergic activity was in that case obtained. In the present study, a higher dose (10 mg/kg) was used with the intention of obtaining a clearer result.

NCO-650, a newly synthesized antiallergic drug, inhibits active systemic anaphylaxis in mice, at doses of 50 to 500 mg/kg, p.o.; and it inhibits experimental asthma in passively or actively sensitized guinea pigs at doses of 125 to 500 mg/kg, p.o. (3). In their experiments, death was used as the index of response.

In the present study, NCO-650 remarkably reduced the bronchoconstriction induced by Asc-Ag inhalation at doses of 30 and 100 mg/kg, i.d., as well as at doses of 1 and 5 mg/kg, i.v. when used prophylactically. On the other hand, when administered after Asc-Ag inhalation, NCO-650 showed no inhibitory effect on Asc-Ag induced asthma at the doses which had a prophylactic effect. This result suggests that NCO-650 produces the antiasthmatic effect neither through an antagonistic effect on chemical mediators involved in Asc-Ag induced asthma nor through a bronchodilating effect.

It is unexplainable why the antiasthmatic effectiveness of prophylactically administered NCO-650 at 30 and 100 mg/kg, i.d., was almost the same at either dose. Possibly, the effect of NCO-650 might attain to the maximal
level at a dose of 30 mg/kg when administered i.d.

It is reported that NCO-650 inhibits the release of chemical mediators from rat peritoneal mast cells (3, 4) and stabilizes the membrane of liposomes (4, 5, 21). The antiasthmatic effect of NCO-650 is therefore assumed to result from the inhibition of release of chemical mediators from mast cells like the cases of DSCG and tranilast.

The vagal reflex is reportedly involved in the Asc-Ag induced bronchoconstriction (6), and the bronchoconstriction is inhibited by anticholinergic drugs such as atropine (7) and its derivatives (12, 13). NCO-650 did not inhibit acetylcholine induced bronchoconstriction even at a high dose (100 mg/kg, i.d.), suggesting that NCO-650 has no significant anticholinergic effect, and that the antiasthma effect of NCO-650 is not due to an inhibitory effect on the vagal reflex, unlike the effects of atropine and ipratropium.

Patients with allergic asthma frequently suffer from an excessive mucus secretion in the airways, which contributes significantly to the obstruction of airways. Asc-Ag induced asthma in dogs also results in hypersecretion of airway mucus (7). The increase in the respiratory mucus output evoked by Asc-Ag was effectively prevented by an i.d. administration of NCO-650, with no change in the viscoelasticity of mucus. Since NCO-650 does not affect the normal tracheal secretion in dogs (22), it is suggested that the inhibitory effect of NCO-650 on the hypersecretion induced by Asc-Ag inhalation is not due to a direct effect on secretory tissues, but due to an inhibition of chemical mediator release from airway mast cells.

NCO-650 appears to be useful as an orally active antiasthma drug like tranilast, the activity of NCO-650 probably exceeding that of tranilast.

References
2 N-5’ Study Group in Children: A double blind controlled trial of N-(3′,4’-dimethoxy-cinnamoyl) anthranilic acid on children with bronchial asthma. Allergy 34, 213–219 (1979)
12 Yamatake, Y., Sasagawa, S., Yanaura, S. and Okamiya, Y.: Antiallergic asthma effect of ipratropium bromide (Sch 1000) in dogs. Folia Pharmacol. Japon. 73, 786–791 (1977) (Abs. in English)
13 Yanaura, S., Mizuno, H., Goto, K., Kamei, J., Hosokawa, T., Ohtani, K. and Misawa, M.: Effects of 8-(2-fluoroethyl)-1H,5H-tropaniumbromide benzilate (Ba598Br) on


