The Effects of Chlorpheniramine and Antiallergic Drugs on *Ascaris suum* Antigen-Induced Active Cutaneous Anaphylaxis in Dogs

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Abstract—Effects of chlorpheniramine and two antiallergic drugs on the active cutaneous anaphylaxis (ACA) reaction induced by intradermal injection of *Ascaris suum* antigen in naturally sensitized dogs were investigated. Chlorpheniramine (10 mg/kg, intraduodenally (i.d.)) almost abolished the ACA reaction. NCO-650 (100 mg/kg, i.d.) had no inhibitory effect, while tranilast (300 mg/kg, i.d.) showed a weak inhibitory effect. These findings show that (i) the ACA reaction is almost totally mediated by histamine and (ii) ACA reaction is considerably resistant to antiallergic drugs such as tranilast and NCO-650.

Most bronchial asthmas in humans are based on the type I allergic reaction in which chemical mediators released from mast cells play a major role in the response. Some allergic reactions in the skin are also relevant to type I allergic reaction, and skin anaphylaxis, especially that of the rat, has been widely used to evaluate effects of antiallergic drugs (1).

For assessment of antiallergic activities and the pursuit of their mechanisms, dogs have been increasingly used in this area. It is known that most mongrel dogs are naturally sensitized by nematode parasites and possess reaginic antibodies against them (2). Inhalation of *Ascaris suum* antigen (Asc-Ag) to dogs causes a bronchial constriction (3); and furthermore, intradermal injection of Asc-Ag causes a skin reaction, that is, the active cutaneous anaphylaxis (ACA) reaction (2). In contrast with the Asc-Ag induced bronchoconstriction, there have been few studies to pharmacologically characterize the canine ACA reaction.

The present study was therefore attempted to investigate the effects of chlorpheniramine, an H₁-receptor antagonist, and two antiallergic agents, *trans*-4-guanidinomethyl-cyclohexanecarboxylic acid *p*-tert-butyl-phenyl ester hydrochloride (NCO-650), a newly synthesized antiallergic agent, and tranilast, on the ACA reaction to Asc-Ag in dogs.

Male mongrel dogs, weighing 7–15 kg, were anesthetized with pentobarbital-Na (30 mg/kg, i.v.) and given 5% Evans blue dye saline solution i.v. at a volume of 0.5 ml/kg 30 min prior to skin testing. Asc-Ag was prepared and diluted with veronal buffered saline (VBS) according to the previously described method (3). Asc-Ag dilutions (2⁻¹¹, 2⁻¹⁰, 2⁻⁹, ..., 2⁰ mg protein/ml) and histamine (1 and 3 μg/ml) dissolved in saline were injected intradermally on the shaved abdomen of the dog at a volume of 0.1 ml. VBS and saline were injected as controls for the antigen and histamine, respectively. Thirty min after intradermal injections of their solutions, the animals were sacrificed by an injection of saturated potassium chloride solution. The areas of the individual wheal reactions (detectable by blueing coloration) were calculated as an ellipse by measuring the two orthogonal diameters; and in part of
the experiments, the extravasated dye was extracted by acetone-5% Na₂SO₄ aqueous solution (7:3) after cutting the skin tissue into small size and determined colorimetrically at 620 nm. Chlorpheniramine, NCO-650 and tranilast were administered intraduodenally (i.d.) 60 min before the intradermal injections of Asc-Ag and histamine.

The drugs used were chlorpheniramine hydrochloride (Tokyo Kasei), NCO-650 (Nippon Chemiphar), tranilast (Kissei) and histamine dihydrochloride (Wako Pure Chemicals). Chlorpheniramine, NCO-650 and tranilast were dissolved or suspended in 5% gum arabic aqueous solution, and they were administered i.d. through a tube intubated from the stomach wall in a volume of 0.5 ml/kg. The doses of chlorpheniramine, NCO-650 and tranilast were expressed as the base, salt and acid, respectively. All values were represented as the mean with S.E. Statistical significance of difference was determined by Student's t-test or Mann-Whitney's U-test.

The intradermal injections of Asc-Ag and histamine caused blueing at the injection sites, while the solvents (VBS and saline) had negligible effects (Table 1, Fig. 1). Effects of chlorpheniramine and NCO-650 on the amount of Evans blue dye extravasated by the ACA reaction are shown in Fig. 1. In the control group, a dose-dependent dye permeation was observed by Asc-Ag injection at a dose range of 2⁻³–2⁻¹ mg protein/ml, and the maximal permeation (approximately 12–13 μg of dye/site) was observed in the range of 2⁻³–2⁻⁰ mg protein/ml. Chlorpheniramine at a dose of 10 mg/kg, i.d., almost abolished the ACA reaction as well as the histamine-induced wheals. On the other hand, NCO-650 at doses of 30 and 100 mg/kg, i.d., had no protective effect on the extravasations of Evans blue dye induced by both the ACA reaction and histamine.

The blueing areas of the ACA reaction are shown in Table 1. A significant correlation (r=0.6544; P<0.001) was observed between the dye amount extravasated and the area of wheal induced by the ACA reaction. As shown in the Table, a weak inhibitory effect of tranilast (100 and 300 mg/kg, i.d.) on the ACA reaction was found, and the effect of chlorpheniramine was also confirmed in this case. NCO-650 had no effect on the blueing area of skin induced by the ACA reaction at any concentration except for 2⁻³ mg protein/ml of Asc-Ag.

Asc-Ag produces allergic reactions in the airways and skin of the dog through the release of chemical mediators in reaginic immediate type hypersensitivity (2, 3). The Asc-Ag induced canine asthma has been proved to resemble human bronchial asthma pathologically and symptomatologically. Anti-allergic drugs widely used for the treatment of patients with bronchial asthma such as disodium cromoglycate and tranilast are effective (4), whereas antihistamines are only slightly effective (3, 5) on this asthma model.

### Table 1. Effects of chlorpheniramine, NCO-650 and tranilast on blueing area of skin induced by active cutaneous anaphylaxis (ACA) reaction against Ascaris suum antigen (Asc-Ag) in dogs

| Drug      | Dose (mg/kg) | N  | Area of blue spot (mm²)/Asc-Ag concentration (mg protein/ml) |
|-----------|--------------|----|-------------------------------------------------------------|
|           |              |    | 2⁻⁸            | 2⁻⁶            | 2⁻⁴            | 2⁻²            | 2⁰             |
| Control   | 21           | 2  | 16.1±8.6       | 33.1±8.9       | 97.8±15.1      | 133.8±16.3     | 126.3±17.7     |
| Chlorpheniramine | 1          | 5  | 19.1±14.2      | 25.6±18.5      | 56.5±25.4      | 66.9±29.2      | 53.8±24.1      |
|           | 10           | 5  | 0.0±0.0        | 0.0±0.0        | 0.0±0.0        | 14.1±14.1      | 6.6±6.6***     |
| NCO-650   | 30           | 5  | 43.3±17.8      | 54.1±20.1      | 89.7±7.5       | 108.8±11.2     | 107.3±50.1     |
|           | 100          | 7  | 32.8±15.7      | 80.6±21.1*     | 104.4±4.5      | 139.2±12.8     | 135.2±13.0     |
| Tranilast | 100          | 6  | 0.0±0.0        | 29.3±13.7      | 61.6±19.9      | 90.4±16.9      | 118.3±13.7     |
|           | 300          | 6  | 2.9±2.7        | 28.5±13.1      | 70.6±21.4      | 64.5±20.2*     | 90.5±30.2      |

The results with five antigen extract dilutions (2⁻⁸, 2⁻⁶, ..., 2⁰ mg protein/ml) are shown here. Chlorpheniramine, NCO-650 and tranilast were administered intraduodenally 60 min prior to the intradermal injections of Asc-Ag. Each value represents the mean±S.E. *P<0.05 vs. the control group using Student's t-test and **P<0.05 and ***P<0.01 vs. the control group using Mann-Whitney's U-test.
Fig. 1. Effects of chlorpheniramine and NCO-650 on the Evans blue extravasation amount induced by intradermal injections of Ascaris suum antigen in dogs. The amount of Evans blue at each injection site was determined. Chlorpheniramine and NCO-650 were administered intraduodenally (i.d.) 60 min prior to the injections of Ascaris suum antigen and histamine. Each value represents the mean with S.E. of 11 (control group) and 5-7 experiments (other groups). *P<0.05 vs. the control group. VBS: veronal-buffered saline.

as is the case in human asthma.

On the other hand, chlorpheniramine, a histamine H₁-blocker, almost abolished the ACA reactions induced by Asc-Ag as well as those by histamine in the present study. This finding suggests that the Asc-Ag induced ACA reaction is almost exclusively mediated by histamine, contrary to the finding that the Asc-Ag induced bronchial asthma is mediated mostly by chemical mediators other than histamine.

In the case of the passive cutaneous anaphylaxis (PCA) reaction in rats, histamine and 5-hydroxytryptamine (5-HT) are reportedly involved (1, 6); and in the bronchial anaphylaxis reaction in actively sensitized rats, 5-HT is the major mediator (7, 8), and histamine acting upon H₁-receptors is contributory (7). These findings suggest that in both the PCA reaction and bronchial anaphylaxis reaction of the rat, histamine and 5-HT seem to commonly play a major role in mediating the responses. The present finding that histamine is the major mediator in the canine ACA reaction, therefore, might be different from the situation in the rat.

In the present experiment, two antiallergic drugs were used: One was NCO-650, a newly synthesized orally active antiallergic drug, and the other was tranilast, which has been proven to be useful in prophylactic treatment of patients with allergic bronchial asthma (9). These two drugs are reported to efficiently inhibit the bronchoconstriction induced by Asc-Ag inhalation in dogs (4). In the present study, however, NCO-650 showed only a slight effect on the ACA reaction and tranilast slightly inhibited it at the same doses which inhibit the bronchoconstriction (4), suggesting that the effects of these two drugs are not the same in the allergic reactions induced in two different sites: bronchial and cutaneous. The increase in blueing area at 2⁻⁶ mg protein/ml of Asc-Ag by NCO-650 is now difficult to explain.

It is now generally accepted that mast cells are heterogeneous morphologically and functionally, and they are classified into connective tissue mast cells and mucosal mast cells (10).
Patterson et al. (11) showed that disodium cromoglycate, a representative antiallergic agent, partially inhibits the bronchial anaphylaxis, but has no effect on the active cutaneous anaphylaxis to Asc-Ag in hypersensitive rhesus monkeys. They proposed the existence of more than one population of mast cells as a possible explanation for their experimental results. In the skin of the dog, Becker et al. (12) demonstrated the presence of “typical” mast cells and “atypical” mast cells. In the canine bronchial lavage cells, Patterson et al. (13) demonstrated the presence of two types of cells with granules having the staining characteristics of mast cells or basophils. It is not yet clear why the antiallergic effects of NCO-650 and tranilast in the skin were much weaker than that in the airways. The observed heterogeneous effects of NCO-650 and tranilast may be due to heterogeneity of mast cells in the two regions.

The above findings suggest that (i) the ACA reaction is almost exclusively mediated by histamine, (ii) the ACA reaction is considerably resistant to antiallergic drugs such as tranilast and NCO-650 and thus (iii) the mechanisms of allergic reactions and evaluation of the antiallergic effects of drugs should be considered separately between the skin and airway anaphylaxes.

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