Effects of Mast Cell Stabilizers on a New Bronchial Asthma Model Using Compound 48/80 in Dogs

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Abstract—Development of a nonimmunologically induced experimental asthma model using compound 48/80 was attempted. Male mongrel dogs anesthetized with pentobarbital-Na were immobilized with decamethonium bromide under artificial respiration. Airway resistance was measured with a modified Konzett-Rössler method and expressed as a change in ventilation overflow (VO). Inhalation of compound 48/80 caused no change in VO even in high concentrations up to a 1% solution. Infusion of compound 48/80 into the bronchial artery at a dose of 0.2 mg/min for 10 min by using the right bronchial perfusion method caused a marked increase in VO accompanied by decreases in perfusion pressure and systemic blood pressure. The compound 48/80-induced bronchoconstriction was inhibited 58% by surgical vagotomy and was almost abolished by chlorpheniramine (10 mg/kg, intraduodenally (i.d.)). Disodium cromoglycate (inhalation of 1% solution along with 5 mg/kg, i.v.), tranilast (300 mg/kg, i.d.) and NCO-650, a new antiallergic drug (100 mg/kg, i.d.) significantly inhibited the compound 48/80-induced bronchoconstriction. These results indicate that 1) compound 48/80 infusion into the bronchial artery produces an asthma-like bronchoconstriction, 2) the main chemical mediator involved in this response would be histamine acting through H1-receptors, and 3) effects of mast cell stabilizers can be evaluated with this model.

Bronchial asthma attack is known to be initiated not only by inhalation of some specific allergens but also by nonimmunological stimuli such as cold air, exercise, mental stress and infection. In vivo nonimmunological asthma models have not been, however, so much available as compared with allergic asthma models.

Compound 48/80 has been widely used to release chemical mediators such as histamine and leukotrienes from mast cells in in vitro experiments (1). However, the in vivo effect of compound 48/80 on the respiratory tract has been poorly documented. Leff et al. (2) reported that administration of compound 48/80 into the cranial thyroid arteries caused an increase in tracheal tension and a release of histamine. This is the only report in which reactivity of the upper trachea to this agent was investigated. On the other hand, reactivities of bronchi and bronchioles are much more significant in bronchial asthma.

In the present study, we attempted to induce a bronchoconstriction by inhalation or by direct infusion into the bronchial artery of compound 48/80 in dogs. Then with this model, the participations of the vagal reflex and histamine and the effects of mast cell stabilizers were investigated.

Materials and Methods

Experimental animals: Male mongrel dogs weighing 8.5–18.5 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 with a respirator (Natsume, KN-50) at a frequency of 30 breaths/min. Respiratory volume was adjusted at the beginning of the experiment for ventilation overflow to be 2.5 ml/kg in each dog.

Two types of experiments were done: one was inhalation of compound 48/80, and the other was infusion of the agent into the bronchial artery.

The bronchial artery perfusion (BAP) method (3) was used to infuse compound 48/80 into the bronchial artery. The chest was opened at the right fourth intercostal space. After heparinization (initial dose of 1000 units/kg, i.v., and supplemental doses of 500 units/kg, i.v., every hour), the right bronchial artery originating from one of the right intercostal arteries was cannulated and perfused with the dog's own blood delivered from the right femoral artery, using a constant flow pump (Tokyo Rika Kikai, MP-201). The flow was adjusted at the beginning of each experiment so that the perfusion pressure was approximately equal to the systemic arterial blood pressure, and it was kept constant throughout the experiment. The perfusion pressure was measured between the pump and the perfused artery via a pressure transducer (Nihon Kohden, MPU-0.5), and it was used as an index of the change in bronchial arterial resistance. The systemic arterial blood pressure and heart rate were monitored from the cannulated left femoral artery via a pressure transducer and tachometer (Nihon Kohden RT-5), respectively. These recordings were made on a polygraph (Nihon Kohden, RM-85). The perfused area in the lungs of the cannulated right bronchial artery was confirmed at the end of each experiment by injecting 1% Evans blue solution at a volume of 5 ml into the artery. It was found that almost all of the bronchioles in the right lung and the right main bronchus were stained by the dye in most cases, while the left lung and alveoli in either lung were not substantially stained.

Acute surgical vagotomy was performed by sectioning the bilateral cervical vagi and superior laryngeal nerves.

Application of compound 48/80: Inhalations of compound 48/80 and histamine were made for 10 min at a volume of about 3 ml with the use of an ultrasonic nebulizer (Nihon Kohden, TUR-3000) introduced into the respiratory circuit.

Close intraarterial infusion of compound 48/80 was made into the perfused bronchial artery through the rubber tubing at a rate of 0.08 ml/min using an infusion pump (Natsume, KN-202). Before the infusion of compound 48/80, histamine (1, 3 and 10 µg/body) was injected closely into the perfused bronchial artery at a volume of 0.1 ml in 5 sec to examine the reactivity to histamine. The animals that did not respond to histamine even at a dose of 10 µg were not used in the experiment.

Determination of airway constriction: In anesthetized and immobilized dogs, the bronchomotor tone was measured by a modification of the Konzett-Rossler method (4). 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 (Nihon Kohden, MFP-IT) and an integrator (Nihon Kohden, RFJ-5) as an index of change in airway resistance.

Drugs: The drugs used were compound 48/80 (Sigma), trans-4-guanidinomethylcyclohexanecarboxylic acid p-tert-butylphenyl ester hydrochloride (NCO-650) (Nippon Chemiphar), disodium cromoglycate (Fujiwasa), tranilast (synthesized by Nippon Boehringer Ingelheim), histamine dihydrochloride (Wako Pure Chemical), and chlorpheniramine maleate (Tokyo Kasei). All these drugs were dissolved in saline when used for intraarterial administration, and they were suspended in 5% arabic gum solution when used for intraduodenal (i.d.) administration, at the time of usage. The doses of NCO-650 and disodium cromoglycate were expressed as their respective salts. The doses of the other drugs were expressed as the respective bases. In the experiments for i.d. administration, the animals were fasted overnight before use and drugs were administered through a tube intubated from the stomach wall.

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

Effect of inhalation of compound 48/80: Inhalation of compound 48/80 in concentrations of 0.05–1.0% caused no effects on ventilation overflow, systemic blood pressure and heart rate in all of the three dogs tested. On the other hand, histamine in concentrations of 0.00125 and 0.0025% caused a marked increase in ventilation overflow in the same dogs.

Effect of bronchial arterial infusion of compound 48/80: Compound 48/80 was infused closely into the bronchial artery at a dose of 0.1 or 0.2 mg/min for 10 min. The increase in ventilation overflow induced by compound 48/80 at a dose of 0.1 mg/min was weak and variable. The treatment with compound 48/80 at a dose of 0.2 mg/min caused a marked increase in ventilation overflow. Typical responses to compound 48/80 at a dose of 0.2 mg/min are shown in Fig. 1. Ventilation overflow began to increase in 7-8 min after the start of infusion, and the increase reached for at least 30 min following the end of infusion. Perfusion pressure began to decrease immediately after the start of infusion of compound 48/80. A slight to moderate decrease in systemic blood pressure and a slight decrease in heart rate were also observed.

Time course of the change in ventilation overflow induced by close intraarterial infusion of compound 48/80 is shown in Fig. 2. The maximal changes in ventilation overflow at doses of 0.1 and 0.2 mg/min were 7.7±4.6 ml (N=7) and 17.7±1.2 ml (N=9), respectively.

Changes in perfusion pressure and systemic blood pressure are shown in Fig. 3. The mean basal blood flow of the perfused artery was 2.2±0.6 ml/min (N=13). Decreases in perfusion pressure and systemic blood pressure at 0.1 mg/min were 17.8±7.6 mmHg and 12.6±2.7 mmHg, respectively, and at 0.2 mg/min, 29.5±4.9 mmHg and 36.9±7.9 mmHg, respectively. The dogs that did not respond to an intraarterial injection even at a dose of 10 μg did not respond to compound 48/80.

Effect of vagotomy on the compound 48/80-induced bronchoconstriction: The effect of vagotomy on the change in ventilation overflow induced by intraarterial infusion of compound 48/80 at a dose of 0.2 mg/min is shown in Fig. 4. The maximal increase in ventilation overflow in the vagotomized group was 7.5±5.6 ml (N=8) as compared with the control value of 17.7±1.2 ml (N=9). The

![Fig. 1. Responses of the canine bronchial musculature and vasculature to the intraarterial (i.a.) infusion of compound 48/80 in a dose of 0.2 mg/min for 10 min. HR: heart rate, BP: systemic blood pressure, PP: perfusion pressure, and VO: ventilation overflow as an index of airway resistance.](image-url)
Fig. 2. Time course of the change in ventilation overflow induced by intraarterial (i.a.) infusion of compound 48/80 (0.1 and 0.2 mg/min for 10 min). Each point represents the mean with S.E. Statistical differences were performed using the paired t-test. *: P<0.05, **: P<0.01 and ***: P<0.001 vs. zero level.

Fig. 3. Changes in perfusion pressure (PP) and systemic blood pressure (SBP) induced by intraarterial (i.a.) infusion of compound 48/80 (0.1 and 0.2 mg/min for 10 min). **: P<0.01 and ***: P<0.001 vs. zero level.

inhibition ratio to the maximal increase was 58% and statistically significant. The decreases in perfusion pressure and systemic blood pressure caused by compound 48/80 were also inhibited (Fig. 5). Surgical vagotomy by itself caused no change in ventilation overflow, perfusion pressure and systemic blood pressure.

Effect of chlorpheniramine on the compound 48/80-induced bronchoconstriction: Effect of chlorpheniramine on the bronchoconstriction induced by intraarterial infusion of compound 48/80 at a dose of 0.2 mg/min is shown in Fig. 4. Chlorpheniramine at a dose of 10 mg/kg, i.d., administered 60 min prior to the intraarterial infusion of compound 48/80 almost abolished the increase in ventilation overflow induced by compound 48/80. The inhibition ratio to the maximal increase in ventilation overflow was 88%. The decrease in perfusion pressure induced by compound 48/80 also almost disappeared, and decreases in systemic blood pressure and heart rate were moderately inhibited (Fig. 5).

Effects of mast cell stabilizers on the com-
Compound 48/80-induced bronchoconstriction:
Effects of disodium cromoglycate, tranilast and NCO-650, a new antiallergic drug, on the bronchoconstriction induced by intraarterial infusion of compound 48/80 at a dose of 0.2 mg/min are shown in Fig. 6. Inhalation of 1% solution along with 5 mg/kg, i.v., of disodium cromoglycate, and i.d. administrations of tranilast at a dose of 300 mg/kg and NCO-650 at a dose of 100 mg/kg, significantly inhibited the increase in ventilation overflow. The inhibition ratios to the maximal increase in ventilation overflow in the control group were 59, 42 and 64%, respectively. Effects of disodium cromoglycate, tranilast and NCO-650 on the decreases in perfusion pressure and systemic blood pressure induced by compound 48/80 are shown in Fig. 7. The decreases in perfusion pressure and systemic blood pressure were significantly inhibited by these drugs.

NCO-650 at a dose of 100 mg/kg, i.d., which significantly inhibited the compound 48/80-induced bronchoconstriction did not inhibit the increase in ventilation overflow induced by inhalation of histamine for 10 min in concentrations of 0.005–0.01%; the maximal increase in the histamine alone response was 15.0±4.5 ml, and that in the response after NCO-650 treatment was 14.3±2.7 ml (N=4).

Discussion
A typical 'histamine liberator', compound
48/80 directly affects the membrane of mast cells and releases histamine without causing irreversible damages to the cells (5–7). Compound 48/80 has been widely used in in vitro experiments as an agent to release chemical mediators such as histamine, serotonin (8) and SRS (9) from mast cells, to screen antiallergic agents and to study the mechanism of release of chemical mediators from mast cells. However there have been few reports on the application of compound 48/80 to in vivo studies of the respiratory tract system.

In the attempt to develop an in vivo bronchial asthma model using compound 48/80 inhalation, inhalation of compound 48/80 was at first made, because bronchial asthma attacks are usually provoked by inhalations of allergens, dust, cold air and so on in humans. Compound 48/80, however, induced no bronchoconstriction even in high concentrations up to a 1.0% solution. Russi et al. (10) reported that a 5% solution of compound 48/80 caused an increase in airway resistance in sheep. Their experiments were done under non-anesthesia and spontaneous respiration, and compound 48/80 was inhaled until airway resistance increased. Since osmotic pressure would rise with an increase in the concentration of compound 48/80, a nonspecific irritant effect might occur when such a high concentration is inhaled.

Inhaled agents have to pass many tissues to reach interstitial mast cells and to cause effects. Compound 48/80 is a synthetic polyamine produced by the condensation of N-methyl-p-methoxyphenethylamine and formaldehyde (11). Since its mean molecular weight is about 1300, it may be too massive to penetrate into tissue layers. This could be a possible reason for the finding that no bronchoconstriction occurred by inhalation of compound 48/80 in the present study.

In the second experiment, we infused compound 48/80 directly into the bronchial artery using the right bronchial artery perfusion (BAP) method. Compound 48/80 at a dose of 0.2 mg/min produced a remarkable asthma-like bronchoconstriction in all the animals tested. The bronchoconstrictive response had a characteristic pattern that a 5–7 min delay time existed following the start of infusion until the bronchoconstriction began to occur. Administrations of histamine, acetylcholine and Ascaris suum antigen into the bronchial artery provoked a rapid increase in ventilation overflow immediately after administrations of these agents (3, 12). In in vitro experiments using rat peritoneal mast cells, addition of compound 48/80 results in an immediate release of histamine (13–15). Therefore, low permeability of compound 48/80 into tissues may also be concerned with the delay of the onset of response in this case.

The fact that an intraarterial infusion of compound 48/80 caused a remarkable bronchoconstriction, in contrast with the case of inhalation, may possibly result from an easier accessibility to interstitial mast cells when the compound is given into the bronchial artery. Another possibility is that it may be attributed to mast cell heterogeneity. It is now generally accepted that mast cells from different locations in the body are functionally heterogeneous, and mucosal mast cells and connective tissue mast cells differ in their responsiveness to compound 48/80 (16–18). In the canine bronchial lavage cells, Patterson et al. (19) demonstrated the presence of two types of cells with granules which have the staining characteristics of mast cells or basophils. It is possible to consider that the inhaled compound 48/80 may react with mucosal mast cells located on the surface of airways, but mucosal mast cells would not respond to compound 48/80.

A decrease in perfusion pressure which indicates a dilatation of the bronchial arterial bed was observed from the beginning of infusion of compound 48/80. A number of mast cells are located in blood vessels. It is, therefore, suggested that compound 48/80 can easily approach to many mast cells around and in blood vessels to release chemical mediators.

The decrease in systemic blood pressure produced by an intraarterial infusion of compound 48/80 may be explained as compound 48/80 stealing into the systemic circulation through bronchial arterial bed to result in a release of chemical mediators including histo-
mine from mast cells in the whole body. Throughout the experiments, compound 48/80 was given only once to an individual animal, since the compound 48/80-induced bronchoconstriction did not recover to the basal level, and tachyphylaxis was predicted.

It is reported that the vagal reflex is involved in the regulation of airway muscular tone through the sensory receptors such as irritant receptors, stretch receptors and C-fiber endings (20-22). Allergic asthma in humans (23) and type I allergic asthma in dogs induced by Ascaris antigen (24) are partially inhibited by atropine. The compound 48/80-induced bronchoconstriction was inhibited 58% by surgical vagotomy in the present study. It is thus suggested that the vagal reflex is involved in the compound 48/80-induced bronchoconstriction, too, probably via histamine action. Decreases in perfusion pressure and systemic blood pressure were also inhibited by surgical vagotomy. The mechanism of the inhibitory effect is not clear. The vagal reflex may also be responsible for part of the vascular reactions.

The compound 48/80-induced bronchoconstriction was almost abolished by chlorpheniramine. Histamine-induced bronchoconstriction in dogs is abolished or strongly inhibited by histamine H1-receptor antagonists (25, 26), whereas it is not effected by an H2-receptor antagonist, cimetidine (25). That is, the histamine-induced bronchoconstriction in dogs is mainly through H1-receptors. Leff et al. (2) reported that administration of compound 48/80 into the cranial thyroid arteries in dogs caused an increase in tracheal tension, a decrease in histamine content of the upper trachea and an increase in blood histamine concentration. The dose of chlorpheniramine they used was 5 mg/kg, i.v. They confirmed that this dose caused a specific antagonism against histamine without influencing the response to acetylcholine. In our study, 10 mg/kg, i.d., of chlorpheniramine was used. Taking consideration of the difference of administration routes, the dose we used would also inhibit histamine specifically.

Compound 48/80 releases arachidonic acid as well as histamine from rat peritoneal mast cells in vitro (27). However, the chief mediator which was involved in the compound 48/80-induced bronchoconstriction in the present study would be histamine, because 1) the response was almost abolished by chlorpheniramine. 2) the dogs that were poorly sensitive to a close intraarterial injection of histamine did not respond to compound 48/80 and 3) dogs are substantially insensitive against leukotriienes (28).

Ascaris antigen-induced asthma in dogs is quite different from compound 48/80-induced asthma in which H1-receptor antagonists are only slightly effective (26). In these two asthma models, mast cells are considered to be involved. So, different mediators are probably released from mast cells between the two responses. It is worthwhile to investigate the mechanisms by which different chemical mediators are released from mast cells of the airway tissues depending on the type of stimulus.

In the present study, disodium cromoglycate, tranilast and NCO-850 significantly inhibited the compound 48/80-induced bronchoconstriction at doses comparable with those which inhibit Ascaris antigen-induced asthma (29), indicating the effectiveness of mast cell stabilizers on the bronchial asthma model using compound 48/80 in dogs. Compound 48/80 is often used in vitro for screening antiasthma agents. However, mast cells used in such cases are usually from tissues other than the airway system. As mentioned above, mast cells from different tissues are heterogeneous. Additionally, complex participations of some nervous systems and hormones have to be taken into consideration in bronchial asthma. Therefore, the in vivo asthmatic model using compound 48/80 is considered to be quite useful as a screening model for anti-allergic agents.

While the present model has various advantages, there would be a discrepancy in the chemical mediators involved in the responses between allergy-induced asthmas and compound 48/80-induced bronchial asthma model will, nevertheless, contribute to the study on antiallergic drugs and airway mast cells.

References
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