ANTI-ANAPHYLACTIC ACTIVITIES OF A NEW BENZOPYRANOPYRIDINE DERIVATIVE Y-12,141 IN RATS AND GUINEA PIGS*

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Abstract—The active anaphylactic bronchoconstriction of rats mediated by IgE-like antibody against DNP-Ascaris was inhibited by intravenous and intratracheal treatment with Y-12,141 in a dose-dependent manner. In both routes, the inhibitory effect of Y-12,141 on this response was more potent than that of disodium cromoglycate (DSCG). The oral administration of Y-12,141 also produced a similar inhibition of the response. The passive anaphylactic bronchoconstriction of guinea pigs mediated by IgG-like antibody against egg albumin was also prevented dose-dependently by treatment with Y-12,141 given intravenously, but not with DSCG. The present results suggest that Y-12,141 may be effective for the treatment of allergic bronchial asthma.

In a previous paper (1), we demonstrated that 9-chloro-5-oxo-7-(1H-tetrazol-5-yl)-5H-[1]benzopyrano[2,3-b]pyridine sodium salt pentahydrate (Y-12,141) potently inhibits the passive cutaneous anaphylaxis (PCA) and the release of histamine from mast cells in the PCA site and peritoneal cavities of rats, mediated by heat-labile homocytotropic antibodies (IgE-like). These findings suggest that Y-12,141 may also prevent bronchial anaphylaxis by inhibiting the release of allergic mediators, in a manner similar to disodium cromoglycate (DSCG).

The active anaphylactic bronchoconstriction in rats sensitized with several antigens such as egg albumin provides a model which is similar to human bronchial asthma immunologically and in the response to DSCG (2-6). In addition, the anaphylactic bronchoconstriction in guinea pigs is usually chosen for the experimental simulation of human allergic bronchial asthma (7). However, the anaphylactic bronchoconstriction in guinea pigs differs from human allergic asthma both in the immunoglobulins involved (8, 9) and in the response to DSCG (10). The present studies were designed to elucidate the effectiveness of Y-12,141 for prophylaxis in the bronchial anaphylaxis in both rats and guinea pigs.

MATERIALS AND METHODS

Animals: Rats, guinea pigs and rabbits were used. The animals were housed under ordinary conditions and allowed free access to food and water.

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Administration of drugs: Y-12,141, DSCG and other test drugs were dissolved or suspended in 0.85% (w/v) NaCl solution (saline) or 0.5% methylcellulose solution. Pressurized aerosols of Y-12,141 and DSCG were prepared in our laboratories. Particles of the drugs (less than 10 μ in diameter) were suspended in freon. Doses of Y-12,141 are expressed as the amount of sodium salt containing no pentahydrate.

Preparation of antigen: Extracts from Ascaris suum were prepared by the method of Strejan and Campbell (11). The conjugates of 2,4-dinitrophenyl sulfonic acid (200 mg, 2× recrystallized, Tokyo Kasei Co.) with the Ascaris protein (DNP-As) were obtained as the antigen, by the method of Eisen et al. (12).

Active bronchial anaphylaxis in rats: Male Donryu rats (200-250 g) were actively sensitized with DNP-As and Bordetella pertussis vaccine using the method of Tada and Okumura (13) and were used 25 to 30 days after the first immunization. The PCA titers in the rat for IgE-like antibody 8 and 25 days after the first immunization were 64–128 and 16–32, respectively. In the preliminary experiments, we found that the bronchial anaphylaxis could be elicited in all the rats sensitized actively by the challenge with 25 mg/kg of DNP-As 25 to 30 days after the first immunization.

Airway resistance was measured by the overflow technique of Konzett and Rössler (14), as modified for electrical recording via an air pressure transducer. The rats sensitized actively were anaesthetized with 1.5 g/kg of urethane given i.p. An endotracheal tube was inserted and connected to an air pump (Matsushita Denko Co. Ltd.) and exhaust relay of 68 strokes/min (Natume Seisakusho Co. Ltd.). The animals were artificially ventilated at a constant volume (3-4 ml). Tracheal pressure was recorded by means of a side-arm of the cannula connected to a pressure transducer (MFP-1, Nihon Kohden Co. Ltd.). The resting pressure of the airway for a control period was recorded for 5 min to ensure consistency, and to produce a tracing of 15 to 20 mm height and maximum pressure (obtained by clamping off the trachea) 90 to 100 mm height. The test drug was given i.v. as solution in saline through a cannulated jugular vein, was inhaled via a branch of the inserted tracheal cannula using the 4 cm length polyethylene tube attached to the aerosol spray, or was administered p.o. as suspension in 0.5% methylcellulose 1, 3 or 120 min before the antigenic challenge, respectively. The animals were challenged i.v. with 25 mg/kg of DNP-As through the cannulated vein. The resultant increase in tracheal pressure, termed as active bronchial anaphylaxis, was recorded for 7 min and used as a measure of bronchoconstriction. Active bronchial anaphylaxis was shown as a percent of the maximal pressure obtained by clamping off the trachea. The inhibitory effect of drugs was given by the following formula:

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\% \text{ inhibition} = 100 - \frac{\% \text{ peak bronchoconstriction in the test group}}{\% \text{ peak bronchoconstriction in the control group}} \times 100
\]

Passive bronchial anaphylaxis in guinea pigs: Male rabbits (3–3.5 kg) were immunized 4 times weekly with 25 mg/kg of egg albumin given i.m. (Sigma chemical Co.) emulsified with an equal volume of Freund's complete adjuvant. Antisera were obtained 7 days after the last injection. The titer of the antiserum in the interfacial ring test used to detect antibody
was 1:128. Male Hartley guinea pigs (300-400 g) were passively sensitized by an i.v. administration of the antiserum (0.5 ml/kg). Twenty-four hours later airway resistance was measured using the same method described above. The animals were challenged with 2 mg/kg of egg albumin given i.v. just after the intravenous treatment with test drugs in saline. The resulting increase of airway resistance was recorded for 10 min.

Release of histamine and slow reacting substance of anaphylaxis (SRS-A) from the sensitized lung of guinea pigs: Male Hartley guinea pigs were sensitized by the i.p. and s.c. administrations of 100 mg egg albumin prepared as 10% solution in saline. Three weeks later, the animals were killed by a blow on the head, and blood in the lungs was removed by perfusion with 5 ml of Tyrode’s solution, via the pulmonary artery. Tyrode’s solution had the following composition (g/l): NaCl 8.0, KCl 0.2, NaHCO₃ 1.0, NaH₂PO₄ 0.05, CaCl₂ 0.2, MgCl₂ 0.1, glucose 1.0, and was aerated with 95% O₂:5% CO₂ mixture at room temperature. The isolated lung tissue was dissected from the trachea and major bronchioles, and then finely cut using a tissue chopper. The portion (1 g) of the lung tissue was pre-incubated for 5 min at 37°C in 5 ml of Tyrode’s solution with or without drugs. To the mixture 0.05 ml of 1% egg albumin was added and incubation was carried out for a further 15 min at 37°C. After the centrifugation, 0.1 ml of 4 N perchloric acid was added to the supernatant (1.0 ml) and the preparation was stored −20°C until use in spectrofluorimetrical assay for histamine (1). Histamine contents in the lung were 4.6–5.2 μg/g. The remainder of the supernatant was stored at −20°C until assay for SRS-A. We then determined the amount of SRS-A released from the isolated guinea pig ileum in the presence of 10⁻⁶ M mepyramine malate and 5×10⁻⁷ M atropine sulfate. Y-12,141 (0.1 mM) failed to antagonize SRS-A by simultaneous addition of Y-12,141 and SRS-A. Addition of the agent to the organ bath 10 min before the agonist resulted in inhibition (24.5±3.1% at 0.03 mM and 49.3±3.3% at 0.1 mM) of contractions of the ileum. Results are shown as the percent inhibition ± SE in comparison with the control.

RESULTS

Active bronchial anaphylaxis in rats: Y-12,141 (0.1 mg/kg) or DSCG (0.25 mg/kg) was administered i.v. to the rat 1, 2, 5 or 30 min before or 1 min after the challenge with the antigen, at the time intervals indicated in Fig. 1. Y-12,141 given 1, 2 or 5 min before the antigen significantly inhibited the bronchoconstriction. This agent, however, failed to inhibit this response when given 1 min after the antigen. DSCG tested as the positive control significantly inhibited the bronchoconstriction when given 2 min before the antigen.

Figure 2 shows that Y-12,141 inhibited the bronchoconstriction and delayed the peak time dose-dependently when given i.v. 1 min before challenge with the antigen. The significant difference from the control in inhibition of the peak bronchoconstriction was found when Y-12,141 was given as a pretreatment in a dose of 0.1 mg/kg or more (Table 1). DSCG also inhibited the response. This table shows that isoproterenol and theophylline also significantly inhibited the bronchoconstriction. Cyproheptadine partially prevented the response in a dose of 0.025 mg/kg. A 4-fold increase in the dose (0.1 mg/kg) of cyproheptadine
FIG. 1. Inhibitory effect of Y-12,141 and DSCG given i.v. on active anaphylactic bronchoconstriction of rats sensitized with DNP-Ascaris and Bordetella pertussis vaccine. Each group of rats was treated with 0.1 mg/kg of Y-12,141 (○) or 0.25 mg/kg of DSCG (●). **P<0.01 (significantly different from the control).

FIG. 2. Inhibitory effect of Y-12,141 given i.v. on active anaphylactic bronchoconstriction in rats sensitized with DNP-Ascaris and Bordetella pertussis vaccine. Each test solution was given 1 min before the antigenic challenge. Results are shown as the mean ± SE from: vehicle control (—○—, N=9); Y-12,141 treated (N=7), 0.05 mg/kg (—●—), 0.1 mg/kg (—△—) and 0.25 mg/kg (—□—).

did not further reduce this response. A large dose of atropine also significantly inhibited the response. Figure 3 shows that dexamethasone given p.o. 16–18 hr before the antigenic challenge resulted in inhibition of the bronchoconstriction, in a dose-dependent manner. The ED50 (the dose required to reduce the response of the control group by 50%) was about 0.8 mg/kg. Both Y-12,141 and DSCG given i.v. 30 min before the antigen also
TABLE 1. Inhibitory effect of Y-12,141 and anti-asthmatic drugs on active anaphylactic bronchoconstriction in rats sensitized with DNP-Ascaris and Bordetella pertussis vaccine

| Compound          | Dose (mg/kg) | No. of rats | % Inhibition±SE |
|-------------------|--------------|-------------|-----------------|
| Y-12,141          | 0.05         | 8           | 17.5±12.5       |
|                   | 0.10         | 9           | 43.1±10.2*      |
|                   | 0.25         | 7           | 65.7±9.5**      |
|                   | 1.0          | 6           | 68.7±13.9**     |
| DSCG              | 0.25         | 5           | 34.8±14.9       |
|                   | 1.0          | 6           | 58.7±10.9**     |
|                   | 3.0          | 6           | 91.3±7.5**      |
| Isoproterenol     | 0.01         | 6           | 45.2±7.6*       |
| hydrochloride     | 0.1          | 6           | 45.7±8.6*       |
| Theophylline      | 25           | 6           | 42.6±4.1**      |
| ethylendiamine    | 50           | 7           | 78.8±7.1**      |
| Atropine sulfate  | 1.0          | 7           | 20.0±6.2        |
|                   | 3.0          | 7           | 61.4±9.0**      |
| Cyproheptadine    | 0.025        | 6           | 34.8±9.4        |
|                   | 0.05         | 6           | 42.0±14.4*      |
|                   | 0.10         | 5           | 42.6±2.8*       |
| Mepyramine malate | 1            | 6           | 21.5±11.6       |

Each group of rats was given Y-12,141 i.v. and anti-asthmatic drugs 1 min before the antigenic challenge. *P<0.05; **P<0.01 (significantly different from the control).

**FIG. 3.** Inhibitory effect of Y-12,141, DSCG and dexamethasone on active anaphylactic bronchoconstriction in rats sensitized with DNP-Ascaris and Bordetella pertussis vaccine. Each group of rats was given Y-12,141 (○) or DSCG (●) i.v. 30 min before the antigenic challenge, and orally with dexamethasone (■) 16-18 hr before the antigenic challenge. *P<0.05; **P<0.01 (significantly different from the control).
FIG. 4. Inhibitory effect of Y-12,141 given topically on active anaphylactic bronchoconstriction in rats sensitized with DNP-Ascaris and Bordetella pertussis vaccine. Each test agent was inhaled, by the method shown in the text. Results are shown as the mean±SE from: vehicle control (—□—, N=17); Y-12,141 inhaled (N=8), 0.74 (0.64-0.81) µg/rat (—○—), 1.14 (0.96-1.36) µg/rat (—△—), 5.4 (3.5-7.5) µg/rat (—□—) and 37.7 (32.3-51.4) µg/rat (—■—).

FIG. 5. Inhibitory effect of DSCG given topically on active anaphylactic bronchoconstriction in rats sensitized with DNP-Ascaris and Bordetella pertussis vaccine. Each test agent was inhaled by the method shown in the text. Results are shown as the mean±SE from: vehicle control (—□—, N=17); DSCG treated (N=8), 11 (9.4-12.0) µg/rat (—○—), 43 (33.9-53.0) µg/rat (—△—) and 109 (80-136) µg/rat (—■—).
inhibited the bronchoconstriction in a dose-dependent manner, the ED50 being 0.4 mg/kg for Y-12,141 and 15 mg/kg for DSCG (Fig. 3). Therefore, Y-12,141 was about 37 times as potent as DSCG.

Figures 4 and 5 show the time course of anaphylactic bronchoconstriction after inhalation of Y-12,141 and DSCG with aerosol. Y-12,141 inhibited this response in doses of 1.14 to 37.7 μg/rat (P<0.01, Fig. 4). The treatment with DSCG in a dose of 43 μg/rat or more also resulted in a significant inhibition (P<0.01, Fig. 5). Therefore, Y-12,141 inhaled was over 10 times as potent as DSCG. In addition, Y-12,141 given p.o. in a dose of 100 mg/kg, 2 hr before the challenge produced a significant inhibition (53.3±9.1%, P<0.05) of the response (Fig. 6). In this experiment, 1 mg/kg of DSCG given i.v. produced a 59.1±6.1% inhibition (P<0.01).

**Passive bronchial anaphylaxis in guinea pigs:** Figure 7 shows that 1 to 5 mg/kg of Y-12,141 given i.v. inhibited the bronchoconstriction of guinea pigs, in a dose-dependent manner. In contrast, DSCG (250 mg/kg) did not affect the initial bronchial anaphylaxis, but did inhibit slightly the subsequent protracted bronchoconstriction (Fig. 8). The same result was obtained by the treatment with 1 mg/kg of Y-12,141. In this experiment, mepyramine (1 mg/kg) completely inhibited the bronchial anaphylaxis.

**Release of histamine and slow reacting substance of anaphylaxis (SRS-A) from the sensitized lung of guinea pigs in vitro:** The antigenic challenge against the sensitized lung

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**Fig. 6.** Inhibitory effect of Y-12,141 given orally on active anaphylactic bronchoconstriction in rats sensitized with DNP-Ascaris and Bordetella pertussis vaccine. Y-12,141 was given 2 hr before the antigenic challenge, and DSCG given i.v. 1 min before the antigenic challenge. Results are shown as the mean±SE from: vehicle control (—○—, N=5); Y-12,141 treated, 100 mg/kg (—●—, N=7) and DSCG treated, 1 mg/kg (—△—, N=7).
resulted in the release of histamine (1.64 μg/g lung) and SRS-A (197 units/g lung, 1 unit = 5 ng histamine in bioassay) during a 15 min incubation. The release of both histamine and

**Fig. 7.** Inhibitory effect of Y-12,141 on passive anaphylactic bronchoconstriction in guinea pigs sensitized with antiserum of rabbit against egg albumin. Y-12,141 was given i.v. immediately before the antigenic challenge. Results are shown as the mean ± SE from: vehicle control (–□–, N=10); Y-12,141 treated (N=7), 1 mg/kg (–●–), 2.5 mg/kg (–△–) and 5 mg/kg (–□–).

**Fig. 8.** Inhibitory effect of mepyramine and DSCG on passive anaphylactic bronchoconstriction in guinea pigs sensitized with antiserum of rabbit against egg albumin. Each test solution was given i.v. immediately before the antigenic challenge. Results are shown as the mean ± SE from: vehicle control (–○–, N=10); DSCG treated, 250 mg/kg (–●–, N=7) and mepyramine treated, 1 mg/kg (–△–, N=7).
SRS-A was significantly inhibited by Y-12,141 at concentrations of 0.001 to 0.1 mM DSCG, however, did not inhibit the release at a concentration of 1 mM (Table 2).

### DISCUSSION

Y-12,141 was shown to inhibit the bronchoconstriction mediated by the IgE-like antibody in rats and by IgG-like antibody in guinea pigs and also to inhibit the release of histamine and SRS-A.

The bronchoconstriction in rats sensitized actively by the method of Tada and Okumura (13) could be elicited in response to the challenge with DNP-As as the antigen. In this test, DSCG (1 mg/kg) given i.v. was confirmed to inhibit the bronchoconstriction (Table 1). This result is consistent with the data showing that DSCG is effective in doses of 0.5 to 3 mg/kg (2, 3). In our experiment, isoproterenol, theophylline and dexamethasone which are clinically effective anti-asthmatic drugs also prevented the bronchoconstriction in rats. Similar results have already been reported by Stotland and Share (5). Church and Miller (15) reported that dexamethasone (5 mg/kg) produces a highly significant inhibition of anaphylactic bronchoconstriction when given to rats 12 or 24 hr before challenge. In our experiments, dexamethasone produced a dose-related inhibition when given 16-18 hr before the challenge (Fig. 3). Antagonists of serotonin and SRS-A, such as methysergide or FPL 55712 partially inhibit bronchoconstriction induced in rats (2, 5, 16). In the present experiment, a partial inhibition of the bronchoconstriction in rats occurred following treatment with cyproheptadine. An N-isopropyl acid ester of atropine (Sch 1000) causes a significant bronchodilation in asthmatic subjects (17). Immunologic release of histamine and SRS-A from human lung tissue can be enhanced by stimulation with cholinergic agents (acetyl-
choline or carbachol) *in vitro*, and this release is inhibited by atropine (18). Acetylcholine causes a release of histamine from rat mast cells (19). We found herein that a large dose of atropine inhibited the bronchoconstriction induced in rats. Therefore, it is suggested that the active anaphylactic bronchoconstriction in rats is one of the useful models for evaluation of anti-asthmatic drugs.

Figure 2 shows that Y-12,141 is an inhibitor of the bronchial anaphylaxis in rats. The inhibitory activity of Y-12,141 is longer-lasting and more potent than that of DSCG, as shown in Figs. 1 and 3 and Table 1. Y-12,141, however, did not act as an antagonist to either histamine or serotonin (1), and failed to prevent the anaphylaxis when given after challenge with the antigen (Fig. 1). In addition, the dose of this agent required to inhibit the bronchial anaphylaxis (i.v. route) is the same as that required to inhibit the PCA and histamine release mediated by the IgE-like antibody (1). Therefore, inhibition of the bronchial anaphylaxis seen with this agent may be due to the block of release of allergic mediators.

Topical treatment of Y-12,141 to the rat lung also resulted in inhibition of the bronchoconstriction in a dose of 1 μg/rat or over (Fig. 4). In addition, the effect of Y-12,141 on the bronchoconstriction in rats was further tested by the oral route and compared with findings seen with DSCG given i.v.. Here, Y-12,141 was administered to rats 2 hr before the antigenic challenge, as the rats were anaesthetized for an operation 1 hr before the challenge. The results obtained suggest that Y-12,141 is effective on the bronchoconstriction, even with oral treatment (Fig. 6).

Further studies were carried out to test the effect of Y-12,141 and DSCG on the bronchoconstriction of guinea pigs sensitized passively. This model has often been used for the study of allergy related to asthma. Mepyramine inhibited completely the bronchoconstriction of guinea pigs provoked by a heat-stable antibody against egg albumin (Fig. 8), although this drug was inactive in rats, at the same dose. Therefore, histamine is suggested to be a major chemical mediator for induction of the bronchoconstriction of guinea pigs sensitized passively. The bronchoconstriction of guinea pigs was inhibited by the treatment with Y-12,141 (Fig. 7), but not even with a large dose of DSCG (Fig. 8). Y-12,141 (5 mg/kg, i.v.), however, did not inhibit the bronchoconstriction of guinea pigs induced by histamine and serotonin. These findings suggest that Y-12,141 may inhibit the release of allergic mediators from the lung of guinea pigs induced by a heat stable antibody against albumin. In fact, Y-12,141 was found to inhibit the release of histamine and SRS-A from the lung of guinea pigs sensitized actively (Table 2). In addition, we found that Y-12,141 did not inhibit the antigen-antibody reaction (20). In these respects, Y-12,141 is different from DSCG and mepyramine.

In conclusion, Y-12,141 was found to inhibit the bronchial anaphylaxis in the two experimental models, and to be more potent than DSCG by topical, intravenous and oral treatments. Therefore, this agent may be effective for the treatment of bronchial asthma.
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