Effects of FR50948, a New Orally Active Antiallergic Agent, in Experimental Allergic Models

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Abstract—The antiallergic activity of sodium 10-(2,3-dimethyl pentanamido)-4-oxo-4H-pyrimido-[1,2-C] quinazoline-3-carboxylate•(hydrate (FR50948) was studied and compared with the activities of sodium cromoglycate (SCG) and lodoxamide. FR50948 had inhibitory effects on type I and type III allergic reactions, but not on type II and IV allergic reactions. FR50948 also had weak inhibitory effects on inflammation (carrageenin paw edema and adjuvant arthritis) and SRS release from rat neutrophils, but no antagonistic effects to histamine and serotonin. The inhibitory effect of FR50948 on IgE-mediated type I allergic reactions was essentially the same as those of SCG and lodoxamide, because FR50948 inhibited the histamine release from rat peritoneal mast cells and had cross tachyphylaxis with SCG in the rat PCA test. However, FR50948, like lodoxamide, had a stronger activity than SCG and was effective by the oral route, unlike SCG which was effective only by the parenteral route. Furthermore, the inhibitory effects of FR50948 on type III reactions and inflammatory reactions were much more potent than those of SCG and equal to those of lodoxamide, and the effect on IgG-mediated PCA was stronger than that of either reference drug. These results suggest that FR50948 will be beneficial in clinical use.

Bronchial asthma is characterized by increased responsiveness of the trachea and bronchi to a variety of stimuli and is manifested by a widespread narrowing of the airways that is reversible spontaneously or as a result of therapy. β-Agonists and theophyllines, as bronchodilators, and SCG and glucocorticoid, as prophylactic drugs, have been used for the treatment of asthma. Among these drugs, SCG is a useful drug for mild and moderate asthma because it shows the effect prophylactically and has few side effects (1). The major disadvantage of SCG is that it must be administered parenterally. Therefore, the development of an orally (p.o.) active SCG type drug is probably imminent.

The main therapeutic activity of SCG has been believed to be its inhibition of type I allergic reactions (2). Since type I allergic reactions are considered to be the main triggers of asthmatic reactions, such an agent must block the asthmatic response. Recent observations, however, confirm that inhibition of the type I allergic reaction is not the sole mechanism of its action. Namely, SCG blocks asthmatic bronchoconstriction in which mast cells may not be involved (3-5), and many other compounds with potent inhibitory effects on the type I reaction in rats have been shown to have no clinical effects (6-8). Since the exact mechanism for the antiasthmatic action of SCG is not clear, it is important to determine the effects of the SCG-like drugs on allergic and inflammatory models other than type I allergic reactions for developing new SCG type drugs. The present experiments were carried out to evaluate the effects of FR50948 on type I to IV allergic reactions and inflammatory models and to compare these effects with those of SCG and
lodoxamide.

Materials and Methods

FR50948, with a molecular weight of 403.97, is a faintly yellowish white non-hygroscopic crystalline powder, and it is very slightly soluble in water and slightly soluble in methanol. The LD50 values by oral and subcutaneous administration of this compound were >3200 mg/kg for male and female mice and rats. SCG was kindly provided by Fisons Co., Ltd., and lodoxamide was synthesized in the Laboratory of Fujisawa Pharmaceutical Co., Ltd. For p.o. use, all the drugs were dissolved or suspended in 0.5% methylcellulose (MC). For intravenous (i.v.) use, the drugs were dissolved in 0.1% MC saline. For the in vitro studies, FR50948 was dissolved in dimethyl sulfoxide and diluted with reaction medium. SCG and lodoxamide were dissolved in water and diluted with reaction medium.

1. Type I allergic reaction

1) Passive cutaneous anaphylaxis (PCA) in rats: Male Sprague-Dawley strain rats aged 8 weeks were used in groups of 5. The rats were sensitized intradermally on their backs with 0.1 ml of diluted homologous anti-egg albumin (EA) serum containing IgE (1:64) or IgG (1:128), and challenged i.v. 48 hr after IgE or 4 hr after IgG sensitization with 1 ml of saline containing EA (5 mg) and Evans blue (5 mg). One hr later, the animals were killed and the skin was removed. The severity of PCA was assessed by measuring the mean diameter of the blueing spot. When administered i.v., the drugs were given just before challenge and when administered p.o., 15 min before challenge. In the IgE-mediated PCA reaction, the effect of larger doses of FR50948 or SCG, given 30 min before antigen, on the inhibitory activity of each drug, given simultaneously with antigen, was observed to study the presence of tachyphylaxis by themselves and cross tachyphylaxis between the two compounds.

2) Anaphylactic bronchoconstriction in rats: Four or five male Sprague-Dawley strain rats aged 6 weeks, were used in each group. Adrenalectomized rats were sensitized i.v. with 1 ml of rat reaginic antiserum raised against EA (1:128). Two days after sensitization, the rats were anesthetized with pentobarbital (25 mg/kg, i.p.) and challenged with EA solution (10 mg/animal, i.v.). Airway resistance to expiration imposed by an artificial respiration was measured by the method of Konzett and Rossler (9). The i.v. drugs were given just before challenge and the p.o. drugs, 15 min before challenge.

2) Systemic anaphylaxis in guinea pigs: Male Hartley strain guinea pigs weighing 310–350 g were used in groups of 5. Systemic anaphylaxis was induced by the method described previously (10). Briefly, the animals were sensitized passively with rabbit anti-serum raised against EA and challenged with an aerosolized EA solution 24 hr after sensitization. When the animals survived for 2 hr after challenge, they were considered to be protected from anaphylactic asthma. SCG was given i.v. 5 min before challenge, and FR50948 and lodoxamide were given p.o. 15 min before challenge.

4) Histamine release from peritoneal mast cells in rats: Male Sprague-Dawley strain rats weighing 400–450 g were used to provide mast cells harvested from the peritoneal cavity. The cells were sensitized for 30 min at 37°C with homologous reagin anti-EA serum (PCA titer 1:256) and suspended at 10^6 cells/ml in medium (154 mM NaCl, 2.7 mM KCl, 0.9 mM CaCl_2, 1 mM MgCl_2, 10 mM tris-acetate, 1% normal rat serum (pH 7.4)) after washing. One ml of cell suspension was preincubated for 5 min at 30°C and challenged with 0.5 ml of 12 μg/ml EA solution in duplicate tubes. The drugs, 0.5 ml, were added 5 sec before challenge. Five min after the challenge, the reactions were terminated by cooling the tubes in ice, and the suspensions were centrifuged at 1500 rpm for 10 min at 4°C. The concentration of histamine in the supernatant was assessed by Shore's method (11).

2. Type II allergic reaction

1) Forssman-induced bronchoconstriction in guinea pigs: Four or five male Hartley strain guinea pigs weighing 380–510 g were used in each group. The animals were anesthetized with 25 mg/kg pentobarbital, i.p., and airway resistance was measured by the method of Konzett and Rossler as mentioned above. The animals were ventilated with a respirator at
60 stroke/min and 5 ml/stroke. Forssman antiserum (0.1 ml) was injected i.v. to induce bronchoconstriction. The drugs were given i.v. 5 min before challenge.

3 Type III allergic reaction

1) Reversed passive cutaneous anaphylactic reaction (RPCAR) in rats: Four or five male Sprague-Dawley strain rats aged 8 weeks were used in each group. Reaction was induced by the method described previously (12). One ml of saline containing EA and Evans blue given i.v.; and 5 min later, partially purified homologous serum containing anti-EA IgG was injected intradermally to the back of each animal. Thirty min later, the animals were sacrificed and the skin was removed. Evans blue was extracted from each sensitized spot with detergent (2% solution of RBS25) and acetone (1:4). SCG (i.v.) was given 10 min and FR50948 and lodoxamide (p.o.) given 15 min before challenge.

2) Arthus type paw edema in rats: Male Sprague-Dawley strain rats aged 6 weeks were used in groups of 5. Paw edema was induced by i.v. injection of EA solution and injection of homologous anti-EA antiserum into the subplantar region of the right hind paw according to the method described previously (12). The i.v. drugs were given 10 min before challenge, and the p.o. drugs were given 15 min before challenge.

4. Type IV allergic reaction

1) Contact hypersensitivity in mice: Male ICR strain mice aged 8 weeks were used in groups of 10. The animals were sensitized by application of 7% picryl chloride ethanol solution to the shaved abdominal skin twice at an interval of 1 week. One week after the second sensitization, the animals were challenged with 1% picryl chloride olive oil solution applied to both ears. Increase in thickness of the ears 24 hr after challenge was an indication of hypersensitivity. The drugs were given i.v. (SCG) or p.o. (FR50948 and lodoxamide) 1 hr before challenge.

5. Inflammatory models

1) Carrageenin paw edema in rats: Male Sprague-Dawley strain rats aged 6 weeks were used in groups of 5. Lambda-carrageenin (0.1 ml of 1% solution) was injected subcutaneously (s.c.) under the plantar surface of the right hind paw. Paw volume was measured with a plethysmometer just before and 3 hr after carrageenin injection. SCG (i.v.) was given 10 min before and FR50948 and lodoxamide (p.o.) were given 15 min before carrageenin injection.

2) Adjuvant arthritis in rats: Female Sprague-Dawley strain rats aged 8 weeks were used in groups of 10. Mycobacterium butyricum (0.5 mg, Aoyama B strain) suspended in 0.05 ml liquid paraffin was injected s.c. into the subplantar region of the right hind paw. Paw volume was measured with a plethysmometer just before and 23 days after adjuvant. The drugs were given s.c. (SCG) or p.o. (FR50948 and lodoxamide) once a day for 23 days.

6. Chemical mediator induced cutaneous reaction

Male Sprague-Dawley strain rats aged 8 weeks were used in groups of 5. An intradermal injection of physiological saline containing histamine (5 μg) and serotonin (1.25 μg) into the shaved back of the rats was followed by an i.v. injection of Evans blue (5 mg) in 1 ml of saline. One hr later, the animals were bled to death and the skin was removed. Evans blue was extracted as described above. SCG (i.v.) was given 10 min before and FR50948 and lodoxamide (p.o.) were given 15 min before injection of the chemical mediators.

7. SRS release from neutrophils of rats

Male Sprague-Dawley strain rats aged 6 weeks and male Hartley strain guinea pigs weighing 520-670 g were used. Eighteen hr after an i.p. injection of 0.1% glycogen, neutrophils were collected from the peritoneal washings of rats. The cells, suspended at a concentration of 1×10⁷ cells/ml in Tyrode’s solution, were incubated for 15 min with indomethacin (10 μg/ml), arachidonic acid (25 μg/ml) and the test drugs and then challenged by calcium ionophore (A23187, 1 μg/ml) in triplicate tubes. After 10 min, the reaction was terminated by centrifugation at 4°C. Samples of the supernatant (100 μl) were bioassayed in a superfused guinea pig ileum in the presence of mepyramine (2×10⁻⁷ g/ml), atropine (2×10⁻⁷ g/ml) and methysergide (2×10⁻⁷ g/ml). To test the antagonistic activity of FR50948 and lodoxamide, both drugs were added to the supernatant of the
control samples and applied to the ileum.

8. Statistical analysis

Statistical significance was assessed by Student's t-test, and ED50 values were calculated by the probit method.

Results

1. Type I allergic reaction

1) PCA reaction in rats: The i.v. injections of FR50948, SCG and lodoxamide produced a dose-dependent inhibition of IgE-mediated PCA reactions. The potency of FR50948 was much greater than that of SCG and nearly equal to that of lodoxamide (Fig. 1). ED50 values for FR50948, SCG and lodoxamide were 0.0078, 0.81 and 0.0079 mg/kg, respectively.

FR50948 and lodoxamide were equally ac-
tive p.o., but p.o. SCG had no inhibitory effect (Fig. 1). ED50 values for these two drugs p.o. were 0.44 and 1.64 mg/kg, respectively.

In the IgG-mediated PCA reaction, i.v. SCG and lodoxamide were equally inhibitory, but the inhibition was still less than 50% even at the highest dose of 10 mg/kg (Fig. 2). In contrast, FR50948 caused marked inhibition with an ED50 value of 0.12 mg/kg; and when given p.o., the drug was a more effective inhibitor than lodoxamide with an ED50 value of 7.3 mg/kg. Inhibition by lodoxamide was less than 50% even at a dose of 100 mg/kg (Fig. 2).

SCG showed tachyphylaxis in the inhibitory activity on IgE-mediated PCA. FR50948 also showed the tachyphylaxis by itself and exhibited cross tachyphylaxis with SCG (Table 1).

2) Anaphylactic bronchoconstriction in rats: As shown in Fig. 3, i.v. injections of FR50948, SCG or lodoxamide produced dose-dependent inhibitions of anaphylactic bronchoconstriction, and the ED50 values for these agents were 0.02, 0.23 and 0.01 mg/kg, respectively. FR50948 and lodoxamide produced similar levels of inhibition p.o., with ED50 values of 2.5 mg/kg and 4.5 mg/kg, respectively (Fig. 3).

3) Systemic anaphylaxis in guinea pigs: Neither p.o. doses of FR50948 or lodoxamide (both 100 mg/kg) nor i.v. injection of SCG at 32 mg/kg had any effect on systemic anaphylaxis in guinea pigs (data not shown).

4) Histamine release from peritoneal mast cells in rats: As shown in Fig. 4, FR50948, SCG and lodoxamide caused a dose-dependent inhibition of histamine release from the mast cells. The IC50 values for FR50948, SCG and lodoxamide were $1.6 \times 10^{-8}$, $1.1 \times 10^{-6}$ and $5.8 \times 10^{-9}$ g/ml, respectively.

2. Type II allergic reaction

1) Forssman-induced bronchoconstriction in guinea pigs: Forssman antiserum induced biphasic constriction of the airways in guinea pigs. None of the drugs inhibited either phase of bronchoconstriction even at 10 mg/kg, i.v. (data not shown).

3. Type III allergic reaction

1) RPCAR in rats: i.v. SCG and p.o. FR50948 and lodoxamide all dose-dependently inhibited the cutaneous reaction. ED50 values for these agents were 15, 89 and 16 mg/kg, respectively (Fig. 5).

2) Arthus type paw edema in rats: Both
Table 1. Cross tachyphylaxis between FR50948 and SCG in IgE-mediated PCA test in rats

| Dose (mg/kg, i.v.) | Diameter of bluing spot (mean±S.E., mm) | Inhibition (%) | Dose (mg/kg, i.v.) | Diameter of bluing spot (mean±S.E., mm) | Inhibition (%) |
|-------------------|----------------------------------------|----------------|-------------------|----------------------------------------|----------------|
| SCG               | (Control)                              | 11.0±0.4       | —                 | (Control)                              | 11.1±0.3       |
| 20                |                                        | 9.5±0.3        | 13.6              |                                        | 7.9±0.7**      | 28.2          |
| SCG 3.2           |                                        | 0.0±0.0**      | 100.0             | SCG 3.2                                | 0.0±0.0**      | 100.0         |
| 20                | SCG 3.2                                | 3.0±1.2**#     | 72.7              | SCG 3.2                                | 6.4±0.7**##    | 42.3          |
| (Control)         | 10.2±0.4                               | —              | (Control)         | 10.2±0.4                               | —              |
| 20                |                                        | 8.9±0.9        | 12.7              |                                        | 6.8±0.9**      | 33.3          |
| FR60948 0.1       |                                        | 1.4±0.9**      | 86.3              | FR50948 0.1                            | 1.4±0.9**      | 86.3          |
| 20                | FR50948 0.1                            | 5.9±1.5*##     | 42.2              | FR50948 0.1                            | 6.0±1.7*##     | 41.2          |

*SCG or FR50948 was given 30 min before the antigen. **SCG or FR50948 was given simultaneously with the antigen. Statistical significance as compared with the control group: *P<0.05, **P<0.01. Statistical significance as compared with each non-pretreated group: #P<0.05, ##P<0.01.
10 and 100 mg/kg of p.o. FR50948 caused significant inhibition of edema 1 and 3 hr after challenge, and lodoxamide was a similarly effective inhibitor. SCG (100 mg/kg, i.v.) inhibited the reaction, but only 1 hr after challenge (Table 2).

4. Type IV allergic reaction

1) Contact hypersensitivity in mice: SCG (100 mg/kg, i.v.) produced only a slight inhibition, and p.o. doses of FR50948 and lodoxamide (100 mg/kg) had no inhibitory effect on contact hypersensitivity in mice (Table 3).

5. Inflammatory models

1) Carrageenin paw edema in rats: SCG (10 mg/kg, i.v.), FR50948 (100 mg, p.o.) and lodoxamide (100 mg/kg p.o.) all produced slight inhibitions (Table 4).

2) Adjuvant arthritis in rats: Both s.c. SCG (100 mg/kg) and p.o. FR50948 (1 mg/kg) were inhibitory. Lodoxamide was ineffective even in a dose of 100 mg/kg, p.o. (Table 5).

6. Chemical mediator-induced cutaneous reaction

Neither i.v. SCG (1–10 mg/kg) nor p.o. FR50948 (1–100 mg/kg) nor p.o. lodoxamide (1–100 mg/kg) had any inhibitory effect on skin reactions induced by histamine and serotonin (data not shown).

7. SRS release from neutrophils of rats

Both FR50948 (3.2 x 10^{-7}–1 x 10^{-5} g/ml) and lodoxamide (1 x 10^{-6}–1 x 10^{-5} g/ml) inhibited SRS release, with IC30 values of 1 x 10^{-6} and 7.7 x 10^{-6} g/ml, respectively, but SCG had no apparent effect even at...
3.2×10⁻⁵ g/ml. The IC₃₀ of phenidone, a typical lipoxygenase inhibitor, was 5.3×10⁻⁷ g/ml (Table 6). Neither FR50948 nor lodoxamide had any antagonistic effect at receptors levels up to 1×10⁻⁵ g/ml.

**Table 2. Effects of FR50948, SCG and lodoxamide on Arthus type paw edema in rats**

| Drug     | Dose (mg/kg) | Number of animals | Increase of paw volume (ml) \(1\) hr | Inhibition (%) \(1\) hr | \(3\) hr | Inhibition (%) \(3\) hr |
|----------|--------------|-------------------|-------------------------------------|------------------------|---------|------------------------|
|          |              |                   |                                     |                        |         |                        |
| FR50948  | 0            | 5                 | 0.90±0.03                           | 0.94±0.02              |          |                        |
| (p.o.)   | 1            | 5                 | 0.82±0.04                           | 0.84±0.05              | 8.9     | 10.6                   |
|          | 10           | 5                 | 0.72±0.04**                         | 0.80±0.04*             | 20.0    | 14.9                   |
|          | 100          | 5                 | 0.66±0.04**                         | 0.76±0.04**            | 26.7    | 19.1                   |
| SCG      | 0            | 5                 | 0.96±0.05                           | 0.90±0.04              |          |                        |
| (i.v.)   | 1            | 5                 | 0.92±0.07                           | 0.92±0.05              | 4.2     | 0                      |
|          | 10           | 5                 | 0.86±0.05                           | 0.92±0.06              | 8.3     | 0                      |
|          | 100          | 5                 | 0.66±0.04**                         | 0.80±0.04              | 28.2    | 11.1                   |
| Lodoxamide| 0           | 5                 | 0.90±0.03                           | 0.94±0.02              |          |                        |
| (p.o.)   | 1            | 5                 | 0.88±0.02                           | 0.90±0.05              | 2.2     | 4.3                    |
|          | 10           | 5                 | 0.88±0.02                           | 0.88±0.05              | 4.4     | 8.5                    |
|          | 100          | 5                 | 0.66±0.06**                         | 0.70±0.06**            | 26.7    | 25.6                   |

Paw edema was induced by the i.v. injection of antigen (EA) and s.c. injection of IgG-rich homologous antiserum to the subplantar region of the right hind paw. Paw volume was measured 1 and 3 hr after challenge. SCG was given 10 min before the challenge, and FR50948 and lodoxamide were given 15 min before the challenge. * Each value indicates the mean±S.E. Statistical significance as compared to the control: *P<0.05, **P<0.01.

**Table 3. Effects of FR50948, SCG and lodoxamide on contact hypersensitivity in mice**

| Drug     | Dose (mg/kg) | Number of animals | Increase of ear thickness \(×10⁻³\) cm | Inhibition (%) |
|----------|--------------|-------------------|----------------------------------------|----------------|
|          |              |                   |                                        |                |
| FR50948  | 0            | 10                | 24.2±3.8                               |                |
| (p.o.)   | 1            | 10                | 22.6±4.6                               | 6.6            |
|          | 10           | 10                | 26.5±2.9                               | 0              |
|          | 100          | 10                | 21.4±2.8                               | 11.6           |
| SCG      | 0            | 10                | 27.1±3.2                               |                |
| (i.v.)   | 1            | 10                | 23.0±2.8                               | 15.1           |
|          | 10           | 10                | 21.6±3.0                               | 20.3           |
|          | 100          | 10                | 16.8±1.9                               | 38.0           |
| Lodoxamide| 0           | 10                | 16.2±2.8                               |                |
| (p.o.)   | 1            | 10                | 15.8±1.9                               | 2.5            |
|          | 10           | 10                | 16.9±2.4                               | 0              |
|          | 100          | 10                | 17.8±1.5                               | 9.9            |

Mice were sensitized with 7% picryl chloride ethanol solution on the abdominal skin twice at an interval of 1 week and challenged with 1% picryl chloride olive oil solution to both ears 1 week after the second sensitization. Hypersensitivity was assessed by increase of ear thickness 24 hr after challenge. Drugs were given 1 hr before challenge. * Each value indicates the mean±S.E. Statistical significance as compared to the control: *P<0.05.

Discussion

FR50948 was a much more effective inhibitor of IgE-mediated type I allergic reactions than SCG, and it was effective even when given orally to rats. The effects of FR-
50948 were stronger or similar to those of lodoxamide, another orally active SCG-like compound. It has been shown in studies on rats that the inhibitory activity of SCG on type I reactions is a result of the inhibition of release of mediators from the mast cells (13). FR50948 also had a much greater inhibitory effect than SCG on mediator release from the mast cells of rats, and furthermore, FR50948 exhibited tachyphylaxis in the PCA test and also showed cross tachyphylaxis with SCG. These results suggest that FR50948 and SCG share the same mechanism of action.

### Table 4. Effects of FR50948, SCG and lodoxamide on carrageenin paw edema in rats

| Drug       | Dose (mg/kg) | Number of animals | Increase of paw volume (ml) | Inhibition (%) |
|------------|--------------|-------------------|-----------------------------|----------------|
|            |              |                   | Injected paw | Non-injected paw | Injected paw | Non-injected paw |
| FR50948    |              | 5                 | 0.90±0.04     | 0.96±0.05     | 0.88±0.05   | 0.68±0.04   | 24.4 |
| (p.o.)     | 1            | 5                 | 0.86±0.04     | 0.82±0.04     | 0.74±0.02   | 0.82±0.09  | 4.7  |
|            | 10           | 5                 | 0.84±0.02     | 0.78±0.05     | 0.72±0.05   | 20.0       |
| SCG        |              | 5                 | 0.90±0.04     | 0.82±0.04     | 0.74±0.02   | 4.7        |
| (i.v.)     | 1            | 5                 | 0.84±0.02     | 0.78±0.05     | 0.72±0.05   | 20.0       |
|            | 10           | 5                 | 0.84±0.02     | 0.78±0.05     | 0.72±0.05   | 20.0       |
| Lodoxamide |              | 5                 | 0.90±0.04     | 0.82±0.04     | 0.74±0.02   | 4.7        |
| (p.o.)     | 1            | 5                 | 0.84±0.02     | 0.78±0.05     | 0.72±0.05   | 20.0       |
|            | 10           | 5                 | 0.84±0.02     | 0.78±0.05     | 0.72±0.05   | 20.0       |

Carrageenin solution (1%, 0.1 ml) was injected s.c. into the subplantar region of the right hind paw. Paw volume was measured 3 hr after carrageenin. SCG was given 10 min before carrageenin, and FR50948 and lodoxamide were given 15 min before carrageenin. * Each value indicates the mean±S.E. Statistical significance as compared to the control: *P<0.05, **P<0.01.

### Table 5. Effects of FR50948, SCG and lodoxamide on adjuvant arthritis in rats

| Drug       | Dose (mg/kg) | Number of animals | Increase of paw volume (ml) | Inhibition (%) |
|------------|--------------|-------------------|-----------------------------|----------------|
|            |              |                   | Injected paw | Non-injected paw | Injected paw | Non-injected paw |
| FR50948    | 0            | 10                | 2.43±0.22     | 1.14±0.24     | 1.6          | 0               |
| (p.o.)     | 0.1          | 10                | 2.38±0.19     | 1.16±0.99     | 1.6          | 0               |
|            | 0.32         | 9                 | 2.39±0.20     | 0.99±0.16     | 1.6          | 13.2            |
|            | 1.0          | 10                | 1.70±0.15*    | 0.63±0.16     | 30.0         | 44.7            |
|            | 3.2          | 10                | 1.89±0.18     | 0.68±0.13     | 22.2         | 40.4            |
| SCG        |              | 10                | 2.62±0.20     | 1.03±0.15     | 28.2         | 9.7             |
| (s.c.)     | 1            | 10                | 1.88±0.30     | 0.93±0.22     | 10.3         | 18.4            |
|            | 100          | 9                 | 1.91±0.20*    | 0.52±0.13*    | 27.1         | 49.5            |
| Lodoxamide |              | 10                | 2.51±0.21     | 1.20±0.14     | 6.4          | 0               |
| (p.o.)     |              | 10                | 2.35±0.31     | 1.22±0.33     | 2.8          | 26.7            |
|            |              | 10                | 2.44±0.29     | 0.88±0.19     | 9.2          | 7.5             |

Arthritis was induced by s.c. injection of *Mycobacterium butyricum* (0.5 mg, Aoyama B strain) into the subplantar region of the right hind paw. Drugs were given once a day for 23 days. * Each value indicates the mean±S.E. Statistical significance as compared to the control: *P<0.05, **P<0.01.
Lodoxamide also partially inhibited IgG-mediated PCA. FR50948, however, was a powerful inhibitor of the IgG-mediated PCA reaction in rats. The clinical significance of the inhibitory activity of the drugs on this reaction is still unclear, but it has been reported that IgG is involved in the pathogenesis of asthma, and the reaction mediated by IgG was not suppressed by SCG (16, 17). Therefore, we assume that FR50948 will be beneficial for the treatment of asthma.

| Drug       | Conc. (g/ml) | Contraction of ileum (g) | Inhibition (%) |
|------------|-------------|--------------------------|----------------|
| FR50948    | 0           | 1.38±0.02                | —              |
|            | 0.1         | 1.24±0.08                | 10.1           |
|            | 0.32        | 0.99±0.04**              | 28.3           |
|            | 1           | 0.94±0.06**              | 31.9           |
|            | 3.2         | 0.88±0.05**              | 36.2           |
|            | 10          | 0.98±0.02**              | 29.0           |

| Lodoxamide | 0           | 1.14±0.04                | —              |
|            | 1           | 1.01±0.01*               | 11.7           |
|            | 3.2         | 0.84±0.02*               | 26.3           |
|            | 10          | 0.82±0.03*               | 28.3           |

| SCG        | 0           | 1.14±0.04                | —              |
|            | 0.32        | 1.20±0.07                | —              |
|            | 1           | 1.21±0.02                | —              |
|            | 3.2         | 1.20±0.02                | —              |
|            | 10          | 1.14±0.03                | —              |
|            | 32          | 1.00±0.03*               | 12.5           |

| Phenidone  | 0           | 1.31±0.07                | —              |
|            | 0.1         | 1.04±0.02*               | 20.5           |
|            | 1           | 0.68±0.03**              | 48.1           |
|            | 10          | 0.14±0.01**              | 89.3           |

Table 6. Effects of FR50948, SCG, lodoxamide and phenidone on SRS release from neutrophils of rats

Neutrophils were stimulated by calcium ionophore (A23187, 1 μg/ml) in the presence of indomethacin (10 μg/ml) and arachidonic acid (25 μg/ml). SRS was bioassayed in a superfused guinea pig ileum in the presence of mepyramine, atropine and methysergide (all at 2×10⁻⁷ g/ml). Each value indicates the mean±S.E. of 3 experiments. Statistical significance as compared to the control: *P<0.05. **P<0.01.

Lodoxamide also partially inhibited IgG-mediated PCA. FR50948, however, was a powerful inhibitor of the IgG-mediated PCA reaction in rats. The clinical significance of the inhibitory activity of the drugs on this reaction is still unclear, but it has been reported that IgG is involved in the pathogenesis of asthma, and the reaction mediated by IgG was not suppressed by SCG (16, 17). Therefore, we assume that FR50948 will be beneficial for the treatment of asthma.

There is no doubt that mediator release from the mast cells is the trigger of the asthmatic reaction and that SCG inhibits this reaction effectively. However, the antiasthmatic activity of SCG is not solely dependent upon its inhibitory effect on type I reactions. We know this for several reasons. First, the inhibitory effect of SCG on bronchoconstriction is induced by mechanisms which are not related to mast cell activation (3–5). Second, many antiallergic drugs which inhibit type I allergic reactions in rats have no therapeutic effect on asthma (6–8). Furthermore, other mechanisms of SCG activity have been reported as follows: inhibition of excitation of 'C' fibre endings in the dog lung (18), inhibition of PAF-induced cutaneous reaction in human (19), and inhibition of type III allergic reactions in rats (20). However, many of the details remain to be clarified.

As shown above, SCG is reported to have inhibitory effects on type III allergic reactions (20); however, in our study, the effects of SCG were minimal, while both FR50948 and lodoxamide were effective. The type III allergic reaction or complement activation is reported to be the primary cause of asthma in some patients (21, 22). It has also been demonstrated in rats that the type III reaction is inhibited by glucocorticoids, but not by acidic nonsteroidal antiinflammatory drugs (23). These observations are consistent with the observations on the clinical effects of these antiinflammatory drugs on asthma (24, 25). Furthermore, other prophylactic
antiasthmatic drugs such as gold (26) and tiaramide (27, 28) all exhibited inhibitory effects on type III reactions in animal models (12, 29). Therefore, the effects on the type III reaction may play an important role in the treatment of asthma and may be predictive of clinical benefits. The effects of FR50948 on type III allergic reactions are stronger and longer-lasting than those of SCG and suggest a clinical benefit of FR50948 for asthma. Since the anti-complement effect was not demonstrated in the in vitro study (data not shown), the mechanism of inhibition of type III reactions by these drugs is not clear. In the present experiments, FR50948 and lodoxamide had an inhibitory effect on SRS release from rat neutrophils, and the importance of leukotrienes in the development of the type III reaction has been reported (30). Therefore, it is suggested that the inhibitory effects of both FR50948 and lodoxamide on the type III reaction are related at least in part to their inhibitory effect on SRS release.

The present study also indicated that FR50948, SCG and lodoxamide had slight inhibitory effects on carrageenin paw edema, and this inhibition might be explained by the role of histamine in the first phase of the edema reported by DiRosa et al. (31). FR50948 and SCG also had inhibitory effects on adjuvant arthritis. Mast cells were reported to be involved in the pathogenesis of rheumatoid arthritis (32) and in adjuvant arthritis (33), but the inhibitory mechanism of FR50948 and SCG on adjuvant arthritis was not clear because lodoxamide showed no inhibition. Although the inhibitory mechanism of the drugs on carrageenin paw edema and adjuvant arthritis is uncertain, it is believed that inflammation in the airways plays an important role in the pathogenesis of asthma (34, 35), and the antiinflammatory effects of these compounds are undoubtedly involved in such suppression.

In this study, FR50948 was shown to have inhibitory effects on both type I and type III allergic reactions and to have an antiinflammatory action. FR50948 was much more effective than SCG. Many compounds of this type have disappeared because of their inefficacy in clinical trials. Naturally, FR50948 must be clinically evaluated before it can be called an effective asthmatic agent; however, the present results suggest that FR50948 should be beneficial in the treatment of asthma.

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