Antipruritic Effects of the Fruits of Chaenomeles sinensis

Hisae OKU, Yoshimi UEDA, and Kyoko ISHIGURO*

Mikogawa Women’s University, School of Pharmaceutical Sciences; Koshien Kyuban-cho, Nishinomiya, 663–8179, Japan.

Received November 1, 2002; accepted April 14, 2003

A 35% EtOH extract of the fruits of Chaenomeles sinensis, long utilized as a folk medicine for cough, significantly inhibited the pruritogenic agent compound 48/80 (COM)-induced scratching behavior in mice. Antipruritic activity-guided fractionation and purification yielded active quercetin, apigenin, and catechin derivatives, which exhibited significant inhibitory effects on COM-induced scratching behavior. To the best of our knowledge, apigenin (5), apigenin 7-glucuronide (6), and apigenin 4’-methoxy-7-glucuronide (acacetin 7-glucuronide) (7) were isolated from the fruits of C. sinensis for the first time. The active fraction and these compounds also inhibited serotonin-, platelet activating factor-, and prostaglandin E₂-induced scratching behavior, but did not inhibit histamine-induced scratching behavior or locomotive behavior. This study also showed that the fruits of C. sinensis could be used to treat allergic itching sensation.

Key words antipruritic; Chaenomeles sinensis; apigenin; quercetin; catechin; antiallergy

In cases of allergic disease with chronic and severe pruritus, such as atopic dermatitis, it is very important not only to treat the allergy but also to inhibit scratching of the lesion. Therefore we previously1,2) searched for natural compounds with antipruritic activity using in vivo assay systems,3) which evaluate the inhibitory effect of pruritogenic agent-induced scratching behavior (avoidance behavior from itching sensation) in mice.

The result of our search revealed the antipruritic activity of the fruit of Chaenomeles sinensis. This fruit has been used in Chinese herbal medicine4) under the name of Motuka, which is utilized against spasmolysis and as an antitussive, antiflatulent, and diuretic agent. Motuka is also an antitussive and expectorant in folk remedies. The fruit of C. sinensis has been reported5,6) to contain flavonoids, triterpenes, and fragrant compounds. Antibacterial and antihemolytic activities, an inhibitory effect7) on histamine release from mast cells in vitro, and an inhibitory effect8) on hyaluronidase have been reported as pharmacological activities of this fruit. However, there has been no report of antipruritic activity. We report here the antipruritic effects of the fruit of C. sinensis and its active compounds.

MATERIALS AND METHODS

Plant Material  C. sinensis was grown in Tokushima, Japan, and the fruit was collected in October.

Reagents  The regents used were as follows: compound 48/80 (COM), Sigma Chemical; catechin (9) and epicatechin (10), Aldrich Chemical Co., Ltd.; platelet-activating factor (PAF), Avanti Polar Lipids, Inc; ketanserin tartrate, Research Biochemicals Inc.; rac-3-(N-n-octadecycarbamoloyloxy)-2-methoxypropyl 2-thiaizoliethyl phosphate (CV-3988), Wako Pure Chemical Industries, Ltd.; serotonin (5-HT) hydrochloride, Tokyo Kasei Kogyo Co., Ltd; prostaglandin E₂ (PGE₂), Funakoshi Co., Ltd; disodium cromoglycate (DSCG), Biomol Research Laboratories Inc.; diphenhydramine hydrochloride (DPH), histamine dihydrochloride and cimetidine, Nacalai Tesque.

Extraction and Isolation  The fruits of C. sinensis (dry wt. 1.65 kg) were subjected to extraction with 35% EtOH (101×2) over 3 d at room temperature and filtered. After removal of alcohol in vacuo, the water phase was successively extracted with AcOEt and n-BuOH. The 35% EtOH extract (6.1 g) for bioassay was obtained by the concentration of the part of filtration liquid (50 ml). The active AcOEt fraction (fr.) (3.3 g) was successively partitioned with CHCl₃ to a soluble fraction (70 mg) and an insoluble residue (CS) (2.7 g). The most active CS was subjected to silica gel chromatography using a CHCl₃–AcOEt–MeOH gradient system to give fr. I—VI. Fraction III (720 mg) was repeatedly subjected to silica gel column chromatography using a CHCl₃–AcOEt–MeOH gradient system to give fr. 1—VI. Fraction III (720 mg) was repeatedly subjected to silica gel column chromatography using a CHCl₃–AcOEt–MeOH gradient system, followed by Sephadex LH-20 with MeOH to give compounds 1 (3.4 mg), 5 (1.8 mg), and 8 (8.3 mg). Fraction IV (294 mg) was subjected to silica gel chromatography using a CHCl₃–MeOH gradient system, followed by Sephadex LH-20 with MeOH, and successive recrystallization from MeOH to obtain compounds 3 (46 mg) and 7 (2.5 mg). Fraction V (129 mg) was subjected to silica gel chromatography using an n-hexane–AcOEt gradient system, followed by Sephadex LH-20 with MeOH to give compound 6 (3.4 mg). Fraction VI (586 mg) was subjected to silica gel chromatography using a CHCl₃–MeOH gradient system followed by Sephadex LH-20 with MeOH to give compound 2 (0.6 mg).

Identification of Compounds  The structures of the purified compounds were identified by analysis of the spectroscopic data (UV, IR, MS, ¹H- and ¹³C-NMR) and by comparison with those previously described in the literature.⁹¹⁰)

Animals  Male ddY and ICR mice (SPF grade), 6 weeks old, were obtained from Japan SLC (Shizuoka, Japan) and housed at 24±2 °C and 60±5% relative humidity. Food and water were available ad libitum.

Assay for Antipruritic Activity against Compound 48/80-Induced Scratching Behavior  The antipruritic activity was measured using a previously reported¹¹) method examining the incidence of scratching. A dose of COM 3 mg/kg s.c., dissolved in saline, a degranulation agent of mast cells, was injected into the base of the neck on the back of ddY mice to provoke scratching behavior. Crude extracts and fractions (100 mg/kg) and compounds 1—5 and 8—10 (10 mg/kg), dissolved in water, were orally administered 1 h before injection with COM. The test agents, dissolved in saline, were administered using effective routes, time sched-
ules, and doses described in the literature. DSCG and CV-3988 10 mg/kg i.v. were injected 1 h before injection with COM. Cimetidine 10 mg/kg i.p. was injected 30 min before injection with COM. Ketanserin 10 mg/kg i.v. was injected 30 min before injection with COM. As a control, saline-treated or water-treated mice were injected with COM. The results were expressed as the percentage of scratching behavior of the control group. The incidence of scratching behavior on the whole body was counted for 20 min.

Assay of Antipruritic Activity against Chemical Mediator-Induced Scratching Behavior. The ddY mouse was used for experiments in the induction of scratching behavior by chemical mediators, except for histamine. In the case of histamine, the ICR mouse, a good responder for scratching behavior against histamine, was used because the histamine-induced scratching response could not be induced in the ddY mouse. Histamine 100 mg/kg s.c. was injected into the base of the neck on the back of ICR mice to provoke scratching behavior. DPH (10 mg/kg i.v.), as a positive control, was injected 30 min before injection with histamine. PAF (1 mg/kg s.c.), 5-HT (20 mg/kg s.c.), and PGE2 (0.2 mg/kg s.c.) were injected into the base of the neck on the back of ddY mice to provoke scratching behavior. CV-3988 (10 mg/kg i.v.) and ketanserin (10 mg/kg i.v.), as positive controls, were injected 30 min before injection with PAF and 5-HT. CS (100 or 200 mg/kg) and 1 and 5 (10 mg/kg) were orally administered 1 h before injection with a chemical mediator of the pruritogen. As a control, saline-treated or water-treated mice were injected with the chemical mediator of pruritogen. The incidence of scratching behavior on the whole body was counted for 20 min. In the case of histamine and PGE2, the incidence of scratching behavior at the injected site was counted for 20 min. The results were expressed as the percentage of scratching behavior of the control group.

Assay of Sedative Activity Using Locomotive Behavior Experiments. CS (200 mg/kg), 1 and 5 (10 mg/kg) and water (control group) were orally administered to 5 mice per group 1 h before the experiment. Diazepam 5 mg/kg s.c., as a positive control, was injected 30 min before measuring locomotion. The mice were put into an acrylic cage (30×36×17 cm) 5 min before the locomotion measurement. Lines were drawn on the bottom of the cage to divide it into nine rectangles. Locomotive activity was evaluated as the frequency at which a mouse crossed a line during the 20-min observation period.

Statistical Analysis. Each value represents the mean ± S.E. for 5 mice. The data were evaluated using Student’s t-test.

RESULTS

Isolated Compounds. Activity-guided fractionation using the COM-induced scratching model gave the most active part, the CS from the AcOEt fr. of 35% EtOH extract of the fruit of C. sinensis (Fig. 1). The CS was subjected to repeated chromatography on silica gel followed by Sephadex LH-20 chromatography and recrystallization to obtain compounds 1–3 and 5–8. These compounds were identified as quercetin (1), quercetin-3-galactoside (hyperin) (2), quercetin-3-rhamnoside (quercitrin) (3), apigenin (5), apigenin 7-glucuronide (6), apigenin 4’-methoxy-7-glucuronide (acacetin 7-glucuronide) (7), and protocatechuic acid (8) by direct comparison with authentic samples, and/or by comparison of the spectral data and physical properties with those of authentic sample and the values reported in the literature.

Antipruritic Effects of Extracts and Compounds of the Fruit of C. sinensis against COM-Induced Scratching Behavior. Figure 1 shows the inhibitory effects of the crude extract and fraction against COM-induced scratching behavior. A 35% EtOH extract, AcOEt fr., and CS all significantly inhibited COM-induced scratching behavior. The antipruritic effects of isolated compounds 1–3 and 5 and 8 from CS, and quercetin 3-glucoside (4), catechin (9), and epicatechin (10) were investigated. Compounds 4, 9, and 10 could not be isolated from CS in this study, but were previously isolated from the fruit of C. sinensis. Compounds 6 and 7 were not investigated, because the quantity was not sufficient. Figure 2 shows that 1, 3–5, and 8–10 all exhibited significant inhibitory activity against COM-induced scratching behavior in mice. Compounds 1 and 5 were the main components of CS determined using HPLC method (data not shown). Then the antipruritic mechanisms of CS, 1, and 5 were examined.
Effects of Agents against COM-Induced Scratching Behavior

Using this system, we determined the effects of several agents. As shown in Fig. 2, DPH (a histamine H1-antagonist), ketanserin (5-HT2A and 5-HT1c receptor inhibitor), and CV-3988 (a PAF-antagonistic agent), significantly inhibited the incidence of scratching. In contrast, cimetidine (a histamine H2-antagonist) did not affect scratching behavior. The antipruritic effect of DSCG was also not significant.

Antipruritic Effects against Chemical Mediator-Induced Scratching Behavior

Table 1 shows that the CS 100 and 200 mg/kg did not affect histamine-induced scratching behavior. CS, 1, and 5 all significantly inhibited serotonin- and PAF-induced scratching behavior as effectively as ketanserin and CV-3988, the positive controls. CS and 1 significantly reduced PGE2-induced scratching behavior, and 1 was suppressed it to levels in water-treated mice.

Inhibitory Effects against Locomotive Behavior

As shown in Fig. 3, CS 100 mg/kg, 1 and 5 10 mg/kg did not inhibit the locomotive behavior of mice. Diazepam, the positive control, significantly suppressed the locomotive behavior, however.

DISCUSSION

In the present experiment, we demonstrated that the 35% ethanol extract of the fruit of C. sinensis significantly inhibited COM-induced scratching behavior in mice (Fig. 1). Activity-guided fractionation and purification led to the most active fraction, the CS of the AcOEt fraction (Fig. 1). CS was repeatedly subjected to chromatography and recrystallization to obtain quercetin (1—3), apigenin (5—7), and benzoyl acid (8) derivatives. Several compounds of these derivatives exhibited significant inhibitory effects on COM-induced scratching behavior (Fig. 2). The activities were stronger than that of DSCG of the positive control. To our knowledge, this is the first report of the isolation of the apigenin derivatives (5—7) from the fruit of C. sinensis.

COM can induce scratching behavior in mice independent of histamine15,16 and 5-HT.17 CS, 1, and 5 inhibited 5-HT-induced scratching behavior (Table 1), although they did not inhibit histamine-induced scratching behavior. To our knowledge, there has been no report on the antagonistic effects of 1 and 5 on the 5-HT receptor. Other inhibitory mechanisms against 5-HT-induced scratching behavior are currently under investigation. The 5-HT2A receptor is involved not only in pain18 but also with itching sensation, since ketanserin,11 a 5-HT2A antagonistic agent, can inhibit COM-induced scratching (Fig. 2). In addition, Yamaguchi et al. reported that the itch-associated response was induced by intradermal serotonin through 5-HT3 receptors in mice.19 The analgesic effects of the fruit of C. sinensis also are under investigation. Further work is needed on the antagonistic effects of these compounds on the 5-HT2A receptor.

PAF is a major mediator of pruritus, as is histamine.20 Our findings suggest that PAF also acts as a primary mediator in this assay system, because CV-3988, a PAF-antagonist, inhibited COM-induced scratching behavior (Fig. 2). Inagaki et al. reported that a PAF receptor antagonist, Y-24180, did not affect COM-induced scratching behavior in BALB/c mice.17 The difference between these two results might be due to the difference in the strains of mice, as in the case of hista-
mine. The activity of PAF-degrading enzyme is known to be weak in atopic dermatitis patients. PAF-antagonistic agents may be useful in relieving the chronic pruritus of atopic dermatitis patients, for whom antihistaminic agents are not effective. CS, I, and 5 showed inhibitory effects against the pruritogenic action of PAF, because they inhibited PAF-induced scratching behavior (Table 1). Several quercetin and apigenin derivatives have also been reported to inhibit PAF-induced aggregation and hypersensitivity. Further work is needed on the antagonistic effects of these compounds on the PAF-receptor.

PGE₂ is known to affect the induction of and increase itchiness. On the other hand, PGE₂ itself does not produce itching in the ICR mouse. However, PGE₂ significantly evokes scratching behavior in the ddY mouse. In addition, CS and I inhibited PGE₂-induced scratching behavior.

The sedative activity of 1 and 5, such as a decrease in locomotor activity, has been reported when they are used at high doses (3—100 times of the present experiments) in mice. Drugs with sedative action may also inhibit scratching behavior. However, at the doses used in the present experiments, the locomotive activity of the mice was not reduced by CS, I, and 5 (Fig. 3). Therefore the sedative action of 1 and 5 may be not involved in the inhibitory mechanism of scratching behavior.

One mechanism of the antipruritic activity of CS is the inhibition of degranulation of mast cells, since derivatives of quercetin and catechin have previously been reported to display inhibitory effects against hexosaminidase release from RBL2H3 cells in vitro. However, CS and active compounds differ from DSCG, a classical antidegranulation agent. These are new types of antidegranulation agents, which have not only antidegranulation action but also antagonist action against serotonin, PAF, and PGE₂, which are potent pruritogens. The active compounds also control the synthesis of substances in the cytokine network, substances from arachidonic acid, and nitric oxide. These substances have been reported to be related to the induction and transmission of itch sensation. The results of our experiments suggest that the active compounds of CS may be useful in the treatment of allergic disease, which is evoked by various mechanisms. At present, the depressive effects of these compounds on pruritus and dermatitis in atopic dermatitis model NC mice are under investigation.

This study has shown that the fruits of C. sinensis, long utilized as a folk medicine for cough, could also be used to treat allergic itching sensation. To our knowledge, this is the first report of the antipruritic effects of the fruits of C. sinensis. The present results may aid the development of new agents for the treatment of allergic diseases with chronic and severe pruritus.

REFERENCES AND NOTES