Inhibitory Effects of Methanol Extracts of Herbal Medicines on Substance P-Induced Itch-Scratch Response

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In a search for new anti-pruritic drugs we screened methanol extracts of 33 herbal medicines which have been used for cutaneous diseases for their antipruritic activity using substance P (SP) as a pruritogen in mice. When administered perorally 30 min before SP injection, methanol extracts of 6 of these herbal medicines, the root of *Scrophularia ningpoensis* HEMS., the root of *Patrinia villosa* (THUNB.) JUSS, the fruit of *Forsythia suspensa* VAHL, the rhizome of *Cimicifuga dahurica* (TURCZ.) MAXIM., the aerial part of *Schizonepeta tenuifolia* BRIQ. and the fruit of *Cnidium monnieri* (L.) CUSS, inhibited SP-induced itch-scratch response at a dose of 200 mg/kg without affecting locomotor activity. Dose dependence of these 6 extracts (50—500 mg/kg) was investigated and all of them inhibited SP-induced itch-scratch response, with extracts from *Scrophularia ningpoensis*, *Schizonepeta tenuifolia* and *Cnidium monnieri* showing particularly significant inhibition. The results suggest that these 6 methanol extracts have inhibitory activity against SP-induced itching.

Key words substance P; itch-scratch response; herbal medicine; mouse

Itching is the most common symptom of a cutaneous disease (e.g., atopic dermatitis, contact dermatitis and urticaria) and accompanies several systemic disorders (e.g., chronic renal failure and cholestasis). This unpleasant sensation stimulates scratching of the lesioned skin thus worsening the lesions. Although anti-histamine and anti-allergy drugs are generally prescribed for cutaneous pruritus, they do not allay a severe itching sensation.^{1,2} Corticosteroids are also used as external medicines to relieve cutaneous pruritus, however, they are thought to produce an antipruritic effect as a result of their anti-inflammatory action and are therefore not applied to pruritus unless inflammation is present. Thus, the development of novel anti-pruritic drugs is necessary.

There are many herbal medicines which have been applied to cutaneous diseases and several Kampo formulae (the traditional Chinese and Japanese herbal medicines) are prescribed for dermatitis. In some cases they ameliorate the dermatitis, but their underlying mechanisms are not understood and scientific experiments devoted to the anti-pruritic effects of herbal medicines have been few and far between.

The purpose of this study was to find herbal medicines with antipruritic activity. We have found that substance P (SP), one of the most potent pruritogenic endogenous peptides in human subjects,³ elicits scratching of the treated skin by the hind paws in the mouse⁴ and that SP-induced scratching in mice has features like those of human itching.⁵ Therefore, we screened for herbal medicines with inhibiting activity to an SP-induced itch-scratch response in mice.

MATERIALS AND METHODS

Preparation of Methanol Extracts of Herbal Medicines

Herbal medicines used in this study are listed in Table 1; all were purchased from Tochimoto Tenkaido Co., Ltd. (Osaka, Japan). Voucher specimens of each sample were deposited in the Museum of Materia Medica, Research Center for Ethnomedicines. Fifty grams of each herbal medicine was extracted with 300 ml of methanol under the reflux condition for 3 h. The decoction was evaporated under a reduced pres-

sure and freeze-dried. These extracts were dissolved in 5% arabic gum immediately before use.

Animals Male ICR mice (Japan SLC, Ltd., Shizuoka, Japan) 5 to 11 weeks of age were used. They were housed under controlled temperature (23—25 °C) and light (lights on from 08:00 to 20:00). Food and water were freely available.

Behavioral Observation Before behavioral recording, the mice (4 animals per observation) were put into an acrylic cage (26×18×30 cm) composed of four cells (13×9×30 cm) for 1 h for acclimation. Methanol extract (100 μ l) of herbal medicine was administered perorally (*p.o.*) and 30 min later SP (Peptide Institute, Minoh, Japan) was injected intradermally (*i.d.*) in 50 μ l physiological saline into the rostral part of the back.⁵ Immediately after *i.d.* injection of SP, the mice were put back to the same cell and their behaviors were videotaped using an 8-mm video camera for 20 min, with any experimenter outside the animals' room. Play back of the video allowed counting of the scratching behavior. Mice generally scratched several times with the hind paws for about 1 s and a series of these movements was counted as one bout of scratching.⁴ The degree of inhibition of scratching was calculated based on the number of scratches per 20 min with the average number of the control group being 100%. The locomotor activity was measured for 20 min by a locomotor activity sensor (Neuroscience Inc., Tokyo, Japan) from 30 min after extract administration.

Data Analysis Data were analyzed by Student's *t*-test or one-way analysis of variance and *post hoc* Dunnett's test; *p*<0.05 was considered as significant. The means of data are presented together with S.E.

RESULTS

When injected *i.d.* into the rostral back, SP (100 nmol/site) elicited scratching of the skin around the injected site by the hind paws. The effect of SP reached maximum at 100 nmol/site, peaked in the initial 10-min period and had almost subsided by 20 min after the injection.⁵ Therefore, SP (100 nmol/site)-induced scratching was counted for 20 min

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Table 1. Effects of Methanol Extracts of Herbal Medicines on SP-Induced Itch-Scratch Response in Mice

Scientific name	Part used	Scratches ^{a)} (% of control)
<i>Tribulus terrestris</i> L.	fruit	87.1±17.9
<i>Kochia scoparia</i> SCHRAD.	fruit	119.2±24.5
<i>Artemisia capillaris</i> THUNB.	young shoot	180.1±39.7
<i>Coix lochryma-jobi</i> L. var. <i>ma-yuan</i> STAPP.	seed	80.7±21.6
<i>Polygonum multiflorum</i> THUNB.	caulis	134.9±24.1
<i>Eucommia ulmoides</i> OLIV.	bark	121.6±16.9
<i>Prunus jamasakura</i> SIEB. ex KOIDZ.	bark	175.9±35.3
<i>Scrophularia ningpoensis</i> HEMSLE.	root	63.6±15.8
<i>Gentiana scabra</i> BUNGE.	root, rhizome	86.8±32.9
<i>Houttuynia cordata</i> THUNB.	whole plant	149.4±12.9
<i>Lonicera japonica</i> THUNB.	stem, branch	129.4±10.7
<i>Patrinia villosa</i> (THUNB.) JUSS.	root	53.2±18.4 ^{b)}
<i>Pulsatilla chinensis</i> (BUNGE) REGEL.	root	97.2±13.0
<i>Dictamnus dasycarpus</i> TURCZ.	root bark	134.1±20.7
<i>Forsythia suspensa</i> VAHL.	fruit	55.7±14.6 ^{b)}
<i>Artemisia annua</i> L.	aerial part	131.1±23.8
<i>Cimicifuga dahurica</i> (TURCZ.) MAXIM.	rhizome	60.3±10.8
<i>Cryptotympan atrata</i> FABR.	slough shed	109.8±15.5
<i>Angelica dahurica</i> BENTH. et HOOK.	root	125.7±18.5
<i>Schizonepeta tenuifolia</i> BRIQ.	aerial part	42.3±8.5 ^{b)}
<i>Perilla frutescens</i> (L.) BRITTON var. <i>crispa</i> (THUNB.) DECEN.	leaf	124.9±17.4
<i>Saposhnikovia divaricata</i> (TURCZ.) SCHISCHK.	root	100.7±15.2
<i>Cnidium monnieri</i> (L.) CUSS.	fruit	67.2±20.3
<i>Polistes mandarinus</i> SAUSSURE, etc.	nest	90.7±11.4
<i>Lappula echinata</i> GILIB.	fruit	111.9±15.2
<i>Cynanchum paniculatum</i> (BUNGE) KITAG.	root, rhizome	138.0±25.5
<i>Erythrina indica</i> L.	bark	96.6±13.2
<i>Bombyx mori</i> L.	face	82.6±16.9
<i>Pinus sensiflora</i> SIEB. et ZUCC.	leaf	125.4±14.0
<i>Quercus acutissima</i> CARRUTHERS.	bark	88.5±16.2
<i>Bombyx mori</i> L.	dead-worm infected by <i>Batrytis bassiana</i> BALS.	96.1±16.9
<i>Serratula chinensis</i> S. MOORE	root	120.3±20.0
<i>Speranskia tuberculata</i> (BUNGE) BAILLON.	whole plant	87.6±15.7

a) The number of scratchings of vehicle was 76.1±13.7 per 20 min (n=48). b) p<0.05 compared with vehicle control.

after i.d. injection. Among methanol extracts of 33 herbal medicines examined, 6 extracts from the root of *Scrophularia ningpoensis* HEMSLE., the root of *Patrinia villosa* (THUNB.) JUSS, the fruit of *Forsythia suspensa* VAHL, the rhizome of *Cimicifuga dahurica* (TURCZ.) MAXIM., the aerial part of *Schizonepeta tenuifolia* BRIQ. and the fruit of *Cnidium monnieri* (L.) CUSS inhibited SP-induced itch-scratch response to less than 70% of control at a p.o. dose of 200 mg/kg (Table 1); the inhibitory effects of *Patrinia villosa*, *Forsythia suspensa* and *Schizonepeta tenuifolia* were particularly significant.

These 6 methanol extracts did not significantly inhibit locomotor activity at a p.o. dose of 200 mg/kg, and *Scrophularia ningpoensis* extract, in fact, tended to increase locomotor activity (Fig. 1).

Investigation of the dose-dependency of inhibitory action of the 6 extracts on SP-induced itch-scratch response (Fig. 2) showed that inhibition by the extract of *Scrophularia ningpoensis* at p.o. doses of 200 and 500 mg/kg was significant (Fig. 2A). The extract of *Schizonepeta tenuifolia* at p.o. doses of 50, 200 and 500 mg/kg dose-dependently inhibited the response (Fig. 2E), while that of *Cnidium monnieri* (50—500 mg/kg, p.o.) also dose-dependently inhibited the response, with almost complete inhibition at a dose of 500 mg/kg (Fig. 2F).

Fig. 1. Effects of Methanol Extracts on Locomotor Activity in Mice

Methanol extracts of the root of *Scrophularia ningpoensis* (A), the root of *Patrinia villosa* (B), the fruit of *Forsythia suspensa* (C), the rhizome of *Cimicifuga dahurica* (D), the aerial part of *Schizonepeta tenuifolia* (E) and the fruit of *Cnidium monnieri* (F) were p.o. administered at a dose of 200 mg/kg. Thirty minutes after the administration, the number of locomotions was counted for 20 min and the area under the curve (AUC) was calculated. Each value represents the mean±S.E. (n=8). * p<0.05 when compared with control.

DISCUSSION

In the present experiments, from among 33 herbal medicines, methanol extracts from the root of *Scrophularia ningpoensis*, the root of *Patrinia villosa*, the fruit of *Forsythia suspensa*, the rhizoma of *Cimicifuga dahurica*, the aerial part of *Schizonepeta tenuifolia* and the fruit of *Cnidium monnieri* inhibited SP-induced scratching response in mice. Extracts of *Scrophularia ningpoensis*, *Schizonepeta tenuifolia* and *Cnidium monnieri* showed clear dose-dependent inhibition of the scratching. None of the 6 inhibited locomotor ac-

Fig. 2. Dose-Response Relationship of Inhibiting Action of Methanol Extracts on SP-Induced Itch-Scratch Response in Mice

The methanol extracts of *Scrophularia ningpoensis* (A), *Patrinia villosa* (B), *Forsythia suspensa* (C), *Cimicifuga dahurica* (D), *Schizonepeta tenuifolia* (E) and *Cnidium monnieri* (F) were administered p.o. at doses of 50, 200 and 500 mg/kg 30 min before i.d. injection of SP (100 nmol/site). Immediately after the SP injection, the number of scratchings was measured for 20 min. V on the x-axis means vehicle administration (control). Each value represents the mean $\pm$ S.E. ($n=8$). * $p<0.05$ compared with control.

tivity at 200 mg/kg, a dose which was effective against SP-induced scratching. Therefore, the scratch-inhibiting action of these extracts may be due to their inhibition of the itching sensation and/or scratching reflex, rather than to sedation or depression of general functions of the central nervous system. Since all extracts were administered 30 min before the test, we cannot exclude the possibility that some methanol extracts other than the 6 effective ones might inhibit SP-induced scratching with different timing of the pretreatment. However, as 17 extracts increased rather than decreased the scratching and the other 10 had almost no effect, the inhibitory action of these extracts if any may not be strong.

Repeated pretreatment with capsaicin, which alleviated human itching, also significantly inhibits SP-induced scratching in mice.⁵⁾ SP-induced scratching is markedly inhibited by the opioid antagonist naloxone,⁵⁾ which ameliorates the itching sensation and scratching by patients with pruritic disease. SP is claimed to be involved in inflammatory responses, but the onset of the scratching behavior was immediate after SP injection, and no eczema or rash was seen within 20 min after injection in the mouse. These findings taken together suggest that SP-induced scratching is an itch response, and is not due to cutaneous inflammation. Thus, we suggest that the methanol extracts of the 6 herbal medicines referred to above inhibit the SP-induced itch sensation in the mouse. The water extracts of *Patrinia villosa*, *Schizonepeta tenuifolia* and *Cnidium monnieri*, but not those of *Scrophularia ningpoensis*, *Forsythia suspensa* or *Cimicifuga dahurica*, also significantly inhibited SP-induced itch-scratch response (unpublished observation). Thus, the anti-pruritic constituents of the first three may be extracted into both water and methanol.

Forsythia suspensa, *Schizonepeta tenuifolia* and *Cimicifuga dahurica* are included in several Kampo formulae

(e.g., Keigai-rengyo-to, Bofu-Tsusho-san, Chizuso-ippo, Shoma-kakkon-to) which are clinically prescribed for cutaneous diseases such as atopic dermatitis. An ether fraction of a methanol extract of *Cimicifuga dahurica*⁶⁾ and a 70% methanol extract of *Forsythia suspensa*⁷⁾ inhibit carrageenan-induced edema in rats. Total coumarins in the fruit of *Cnidium monnieri* show a protective effect against histamine-induced bronchial asthma in guinea pigs.⁸⁾ These herbal medicines are thought to inhibit inflammation, but our results suggest that they have direct anti-pruritic potencies.

The mechanism of inhibiting actions of the herbal medicines on SP-induced itch-scratch response are unknown. However, the response by SP of the mouse is mediated at least partly by cutaneous NK₁ receptors⁵⁾; mast cell-histamine systems are not involved.^{4,5)} SP was reported to be increased in the lesioned skin of patients with pruritic dermatitis, such as atopic dermatitis^{9–11)} and psoriasis.^{12–14)} These findings suggest that SP in the skin is involved in some pruritic diseases. We need to investigate whether the sites of anti-pruritic effects of the methanol extracts are the skin or the central nervous system. To determine whether the anti-pruritic effects of methanol extracts of the herbal medicines are specific to SP, we are studying the inhibitory actions of the extracts on the itch-scratch response to serotonin¹⁵⁾ and histamine¹⁶⁾ of the mouse. We are also now trying to isolate active constituents from these methanol extracts.

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