Antipruritic Effects of *Sophora flavescens* on Acute and Chronic Itch-Related Responses in Mice

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To find new antipruritic herbal medicines for pruritus, we screened the methanol extracts of seven herbal medicines which have been used to treat dermatologic diseases, testing them on mouse models of acute and chronic itch. When administrated perorally (p.o.) at a dose of 200 mg/kg, methanol extracts of *Sophora flavescens* and *Cnidium monnieri*, but not the others, significantly inhibited a serotonin (5-HT)-induced itch-related response (scratching) and the spontaneous scratching of NC mice, a mouse model of atopic dermatitis. The inhibitory effect of *Sophora flavescens* was stronger than that of *Cnidium monnieri*. The methanol extract from *Sophora flavescens* (50—200 mg/kg) inhibited 5-HT-induced scratching in a dose-dependent manner, without any effects on the locomotor activity. These results suggest that *Sophora flavescens* and its constituents widely affect acute and chronic pruritus, and are possible as new antipruritic agents.

Key words herbal medicine; itch; atopic dermatitis; NC mice; serotonin

Pruritus is a dominant symptom of many dermatologic disorders (e.g., atopic dermatitis, contact dermatitis and urticaria), and is a frequent manifestation of several systemic diseases (e.g., chronic renal failure and cholestasis). Anti-histaminic and anti-allergic drugs are generally prescribed for cutaneous pruritus. However, histamine is not considered to be the major pruritogen in atopic dermatitis,1—3) because histamine H₁ receptor antagonists do not generally depress itch and scratch in patients. Thus, the mechanism of itch in atopic dermatitis is still unclear, and effective treatment is not established.

Kampo formulas (traditional Chinese and Japanese herbal medicinal mixtures) are widely used in the clinical treatment of allergic and inflammatory dermatitis in Japan. In some cases, dermatitis improves after Kampo treatment, but the pharmacological characteristics and mechanisms of their action are obscure. Recently, 33 herbal medicines were screened for inhibitory effects on substance P (SP)-induced scratching in mice.4) However, herbal medicines have not been screened for inhibitory effects on chronic itch-related responses.

Previously, we established 5-HT-induced scratching in mice as an acute itch model.5) 5-HT is pruritogenic in humans, and is suggested to be involved in some pruritic diseases.6—8) We have shown that 5-HT elicits scratching of the treated skin by the hind paws in mice, and that the 5-HT-induced scratching has features similar to that of human itching.5) The mechanisms of 5-HT-induced scratching may be different from those of SP- or histamine-induced scratching.9)

NC mice are claimed to be a model of atopic dermatitis.10) When maintained for a long time in a conventional environment, the NC mouse spontaneously and frequently scratches its face, ears and rostral part of the back by the hind paws.11) We have shown that the spontaneous scratching is thought to be a model of itch-related response in atopic dermatitis.12)

The purpose of the present study is to find new antipruritic herbal medicines that are also effective in treating chronic itch. We screened seven herbal medicines which have been used to treat dermatologic diseases, testing them on mouse models of itch.

MATERIALS AND METHODS

**Plant Materials** The root of *Angelica acutiloba* and the root of *Saposhnikovia divaricata* were provided by the Toyama Medicinal Plant Research Center (Kamiichi, Japan). The fruits of *Cnidium monnieri*, the root of *Cnidium officinale*, the fruits of *Forsythia suspensa*, the aerial part of *Schizonepeta tenuifolia* and the root of *Sophora flavescens* were purchased from Enjyudo (Toyama, Japan). Fifty grams of crude drugs were extracted three times with methanol (300 ml once, 200 ml twice) for 3 h. The decoctions were evaporated under reduced pressure and dried.

**Animals** Male ICR mice (Japan SLC, Ltd., Shizuoka, Japan) at 4 to 6 weeks of age were used. NC/Jic mice (male, 4—7 months old) were kept for a long time under conventional conditions to induce chronic itch-related responses, as described.12) They were housed under controlled temperature (22—26 °C) and light (lights on from 06:00 to 18:00). Food and water were freely available. The experimental procedures for mice were conducted in accordance with guidelines for the care and use of experimental animals from the Japanese Association for Laboratory Animals Science.

**Administration of Materials** Methanol extracts were dissolved or suspended in 5% Arabic gum and administrated p.o. 1 h before the start of behavioral observation. Serotonin hydrochloride (Sigma, St. Louis, U.S.A.) was dissolved in physiological saline at a dose of 100 nmol/50 μl. Terfenadine (Sigma, St. Louis, U.S.A.) was dissolved in saline and administered intraperitoneally (i.p.) 1 h before the beginning of behavioral observation.

**Behavioral Observation** Scratching behavior was observed as described.13) Briefly, the behaviors of the mice were videotaped, with all experimenters kept out of the observation room. The playback of the video served for counting scratching. When 5-HT was injected, the scratching of the
skin around the injected site by the hind paws was counted. In the case of spontaneous scratching of NC mice, the scratching of any regions of the body by the hind paws was counted. The locomotor activity was measured for 1 h using a locomotion measurement system (SCANET MV-10, Toyo Sangyo Co., Ltd., Toyama, Japan), from 1 h after extract administration.

**RESULT**

We examined whether methanol extracts of seven herbal medicines would reduce scratching in the mice models of acute and chronic itching. When injected intradermally into the rostral back, 5-HT (100 nmol/site) elicited scratching of the skin around the injected site by the hind paws. Methanol extracts of herbal medicines (*Angelica acutiloba*, *Cnidium monnieri*, *Cnidium officinale*, *Forsythia suspensa*, *Saponshnikovia divaricata*, *Schizonepeta tenuifolia* and *Sophora flavescens*) were administrated at a p.o. dose of 200 mg/kg.

**DISCUSSION**

In the present study, we screened methanol extracts of seven herbal medicines using mouse models of acute and chronic itch to find antipruritic herbal medicines. *Sophora flavescens* and *Cnidium monnieri* reduced 5-HT-induced scratching in ICR mice and spontaneous scratching in NC mice. The other herbal medicines tested, *Angelica acutiloba*, *Cnidium officinale*, *Forsythia suspensa*, *Saponshnikovia divaricata*, and *Schizonepeta tenuifolia* were without effects. In addition, *Sophora flavescens* did not inhibit locomotor activity at 200 mg/kg, a dose which was effective against 5-HT-induced scratching. Therefore, the scratch-inhibiting effect of this extract may be due to its inhibition of the itching sensation, rather than to sedation.

Recently, it was reported that methanol extracts of *Cnidium monnieri*, *Forsythia suspensa* and *Schizonepeta tenuifolia* inhibited SP-induce scratching. Neurokinin-1 (NK1) tachykinin receptors and leukotriene B4 are involved in the itch-related response induced by SP. Scratching induced by SP and histamine, but not by 5-HT, was suppressed by emedastine difumarate, anti-histaminic and anti-allergic drugs. These results suggest that the mechanism of 5-HT-induced scratching is different from that of SP- and histamine-induced scratching. The antipruritic effects of methanol extracts of *Forsythia suspensa* and *Schizonepeta tenuifolia* might be specific to SP, but *Sophora flavescens* and *Cnidium monnieri* might produce the effects through different mechanisms.

We have shown that 5-HT induces an itch-related response in mice, and that the action is mediated at least partly by cutaneous 5-HT3 receptors. However, although 5-HT induces the scratching in NC mouse without spontaneous scratching, 5-HT antagonist does not inhibit the spontaneous scratching of NC mice. Therefore, 5-HT may not be involved in the spontaneous scratching of NC mice. Pruritus of NC mice may be elicited via mechanisms different from 5-HT induced itch in peripherals.

When NC mice are kept for a long time in a conventional laboratory, the scratching in NC mouse without spontaneous scratching, 5-HT antagonist does not inhibit the spontaneous scratching of NC mice. Therefore, 5-HT may not be involved in the spontaneous scratching of NC mice. Pruritus of NC mice may be elicited via mechanisms different from 5-HT induced itch in peripherals.
environment, skin lesions develop, and both the plasma concentration of total immunoglobulin E (IgE) and the invasion of inflammatory cells into the lesions are markedly increased.\(^{10}\) These mice markedly show spontaneous scratching. In the present experiments, terfenadine (10 mg/kg) with anti-histaminic and anti-allergic action did not inhibit spontaneous scratching of NC mice. Terfenadine at the same dose significantly inhibited scratching induced by histamine.\(^{15}\) Taking into account these findings, the present results suggest that Sophora flavescens and Cnidium monnieri produce antipruritic action through non-histamine systems.

Sophora flavescens and Cnidium monnieri may have wide influence on acute and chronic pruritus, because these medicines significantly inhibited not only 5-HT-induced scratching but also the spontaneous scratching of NC mice. The inhibitory effect of Sophora flavescens was stronger than that of Cnidium monnieri. A dry root of Sophora flavescens has been used in Kampo formula for the treatment of eczema, skin disease, bacillary dysentery, jaundice and fever. Pharmacological studies have revealed antipyretic, antiulcer, anti-inflammatory, antitumor and antinociceptive activities for the crude extracts or isolated constituents from Sophora flavescens and other Sophora (subfamily Papilionaceae in the family Leguminosae) plants.\(^{16—20}\) However, the antipruritic effect of Sophora flavescens has never been studied. In the present study, we found a strong antipruritic effect of Sophora flavescens on acute and chronic itch. We are now trying to isolate active constituents from the methanol extract of Sophora flavescens, and are studying the antipruritic mechanisms.

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REFERENCES AND NOTES