

Inhibitory Effect of Shimotsu-to, a Traditional Chinese Herbal Prescription, on Acute Inflammation in Rats and Guinea Pigs

Katsuya Sakuma¹, Izumi Kaji¹, Masahiko Ogihara² and Katsumi Yamamoto²

¹Research Laboratories, Hollywood Cosmetic Co., Ltd., 66-1, 3-chome, Chofu-shi, Tokyo 182, Japan and

²Biochemical Pharmacology Group, Faculty of Pharmaceutical Sciences, Josai University, 1-1, Keyakidai, Saitama 350-02, Japan

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We examined the effect of topical application of Shimotsu-to, a traditional Chinese herbal prescription, on carrageenin-induced edema in rats and ultraviolet radiation-induced erythema in guinea pigs. Shimotsu-to (5% in water) markedly suppressed an acute edema of rat hindpaw induced by 1% carrageenin, and was more effective than any other single crude drug component of Shimotsu-to. Topical treatment with this prescription also inhibited ultraviolet erythema on the back skin of guinea pigs (a human sunburn model). These results suggest the therapeutic effect on acute inflammation by topical application of Shimotsu-to.

Key words : Shimotsu-to, Traditional herbal prescription, Antiinflammatory effect

INTRODUCTION

Herbal extracts have been used as traditional remedies for thousands of years in Japan, China and other Asian countries. The traditional herbal medicine (Kampo reagent) is usually orally administered to humans for treating various diseases (Ogaki, 1995; Yasukawa *et al.*, 1995).

Shimotsu-to is a herbal medicine consisting of crude extract from four medicinal herbs according to a traditional prescription (Matsuda, 1978). It is usually administered orally to treat dermal diseases (Tokada *et al.*, 1987). Topical application, however, is special application for skin disease, and the therapeutic potentials of this medicine applied topically has not been studied.

In this report, we evaluate the effect of topical application of Shimotsu-to to both carrageenin-induced edema in rats and ultraviolet-induced erythema in guinea pigs.

MATERIALS AND METHODS

Animals

Male Wistar rats (100 g in body weight) were housed under conditions of 25±1°C and 12-h light-dark

cycle (from 6 a.m. to 6 p.m.), fed a commercial diet (Oriental Yeast Co., Japan) and allowed tap water *ad libitum* before the experiments. Female Hartley guinea pigs (Tokyo Experimental Animal Co., Tokyo, Japan), 5 weeks of age and weighing 250-300 g were used. They were kept in an animal room (25±1°C) with humidity (55±5%), fed a commercial diet and allowed tap water *ad libitum*.

Rat hindpaw edema

Edemas on the right hindpaw of the animals were induced by subcutaneous injection of carrageenin (0.5 ml/rat) prepared as 1% suspension in saline (Winter *et al.*, 1962). The hindpaw of control rats was administered saline alone (0.05 ml/rat). The volume injected to the hindpaw was measured by immersing the limb in a modified adapt-volume meter (Natsume Co., Tokyo). The limb was immersed to an ink mark marked on the skin previously. The hindpaw was then immersed in 5% Shimotsu-to solution kept at 37°C for 30 min after the indicated time. Edema ratio was calculated according to the following formula:

$$\text{Edema ratio (\%)} = (V_c - V_t) \div V_c \times 100$$

where V_c is the volume of control hindpaw and V_t is the volume of carrageenin-treated hindpaw the inhibition ratio (%) is a function of the edema ratio:

$$\text{Inhibition ratio (\%)} = (E_c - E_t) \div E_c \times 100$$

where E_c is the edema ratio of control hindpaw and E_t

Correspondence to: Katsuya Sakuma, Research Laboratories, Hollywood Cosmetic Co., Ltd., 66-1, 3-chome, Chofu-shi, Tokyo 182, Japan

is the edema ratio of carrageenin-treated hindpaw.

Ultraviolet erythema in guinea pigs

Hair of guinea pig back skin was cut by an electric shaver, the skin was covered with a cloth with two openings (25 mm × 15 mm). The openings was irradiated with ultraviolet (UV) light using an UV lamp (L4887, Toshiba Co., Japan) for 10 min, positioned 10 cm above the back skin. Five percent Shimotsu-to solution was applied topically to the skin 10 min after UV-irradiation. The degree of erythema in the UV-irradiated area was evaluated by four categories and scored as reported by Tsurumi (Tsurumi, 1982).

Extraction of traditional Chinese herbal medicine

Shimotsu-to consists of crude mixtures from four medicinal herbs: Jio (*Rehmanniae Radix*, 4 g), Senkyu (*Nidii Rhizoma*, 4 g), Toki (*Angelicae Radix*, 4 g), and Shakutyaku (*Peoniae Radix*, 4 g). Mixture of 4 herbs were extracted by refluxing with distilled water (0.5 l) for 1 h. The Shimotsu-to solution was then cooled at room temperature, filtered through a paper filter, and the pH was measured at 25°C. Aqueous extract of Shimotsu-to was then evaporated to dryness. Each herb was also extracted by refluxing with distilled water (0.5 l) for 1 h and was evaporated to dryness. In all case, powdered extracts were dissolved in distilled water to prepare 5% (w/v) suspension.

Drugs

The crude drugs were obtained from Uchida Wa-kan-yaku Co., Tokyo; carrageenin and hydrocortisone 21-acetate were purchased from sigma Co. (St. Louis, MO). Other reagents were of analytical grade.

Statistical analysis

Statistical analysis was carried out by Student's paired T-test. P value less than 0.05 was regarded as statistically significant.

RESULTS AND DISCUSSION

Table I shows yield content of aqueous extracts of Shimotsu-to and the crude drug components of this prescription. The pH (at 25°C) of the filtrate is also shown.

Table II shows the inhibitory effect of Shimotsu-to on the swelling of rat hindpaw induced by 1% carrageenin, an extract of *Chondrus*. Peak edema induced by carrageenin developed with the first 3 to 4 hr and was significantly inhibited by topical treatment of rat hindpaw with 5% Shimotsu-to solution. Each crude drug component of Shimotsu-to also inhibited

Table I. Yield content of aqueous extract of shimotsu-to and crude drug components of its herbal prescription

Extract (Family name)	Yield of extract (g/ daily dose)	pH of the extract solution (25°C)	Extract amount from 1 g of crude Drug (mg)
Shimotsu-to	7.98	5.2	499
<i>Angelicae Radix</i> (Umbelliferae)	12.0	5.4	480
<i>Paeoniae Radix</i> (Paeoniaceae)	9.33	5.3	373
<i>Cnidii Rhizoma</i> (Umbelliferae)	9.07	5.4	363
<i>Rehmanniae Radix</i> (Scrophulariaceae)	17.4	4.6	696

the edema, but the activity was weaker than the combined solution. Intraperitoneal administration of hydrocortisone 21-acetate (5 mg/kg) 30 min before carrageenin injection showed similar effects as 5% Shimotsu-to (Table II). Oral administration of Shimotsu-to at 200 mg/kg (lie, daily dose in man) had no acute antiinflammatory effects on the carrageenin-induced rat hindpaw edema (not shown).

Table III shows the effect of 5% Shimotsu-to and its crude drug components on an acute erythema induced by ultraviolet irradiation on the back shin of guinea pigs. The herbal medicine was applied topically to the surface of the back shin for 4 hr or 24 hr after ultraviolet irradiation; the erythema developed rapidly and reached a maximum within 30 min. Shimotsu-to significantly inhibited ultraviolet-induced erythema in the back skin of guinea pigs. but crude drug components alone were not effective in reducing the condition (Table III). Intraperitoneal injection of hydrocortisone 21-acetate 30 min before UV-radiation significantly inhibited UV-erythema in guinea pigs (Table III). Oral administration of Shimotsu-to at 50 and 200 mg/kg (lie, daily dose in man) had no acute antiinflammatory effects on the erythema induced by UV-irradiation (not shown).

A traditional Chinese herbal prescription (Kampo preparation) is usually administered orally to treat various diseases in human. Since topical therapy is especially appropriate for diseases of the skin, it can be very useful if such a prescription could be used as a topical medication. We examined here whether Shimotsu-to, one of traditional prescription, inhibited the acute inflammation induced both by the phlogistic agent, carrageenin, and ultraviolet irradiation in animals.

As shown in Table II, Shimotsu-to showed significant anti-inflammatory effect in rat hindpaw edema. This activity was greater than those of the each crude component prescribed, but the reason of results is not clear yet. The Shimotsu-to prescription (a com-

Table II. Effects of topical application of shimotsu-to and its crude drug components on the swelling of rat hindpaw induced by 1% carrageenin

Extract ^{a)}	4 Hour ^{b)}		6 Hour ^{b)}	
	Mean ± S.E. (%) ^{c)}	Inhibition ratio (%) ^{d)}	Mean ± S.E. (%) ^{c)}	Inhibition ratio (%) ^{d)}
Shimotsu-to	68.4 ± 18.4	31.6*	28.3 ± 15.7	55.6*
Angelicae radix	83.9 ± 7.9	16.1	44.6 ± 14.4	34.3
Paeoniae radix	98.4 ± 11.7	1.60	91.1 ± 13.5	-1.22
Cnidii rhizoma	86.5 ± 16.7	13.5	76.9 ± 13.7	11.1
Rehmanniae radix	73.3 ± 30.0	26.7	62.2 ± 21.2	28.3
Hydrocortisone acetate (5.0 mg/kg, i.p.)	58.0 ± 12.3	42.0*	20.5 ± 7.8	40.2*

Each value represents the mean ± S.E.M. (n=6).

Significantly different from control group at: *p<0.05

^{a)}5(w/v) %

^{b)}Times shown are 4 and 6 hours after carrageenin treatment.

^{c)}Increase rate for measuring before carrageenin treatment

^{d)}Increase ratio to control group with time

Table III. Effects of shimotsu-to and its crude drug components on erythema induced by ultraviolet irradiation in the back skin of guinea pigs

Extract ^{a)}	4 Hour ^{b)}		6 Hour ^{b)}	
	Mean ± S.E. (%) ^{c)}	Inhibition ratio (%) ^{d)}	Mean ± S.E. (%) ^{c)}	Inhibition ratio (%) ^{d)}
Shimotsu-to	2.05 ± 0.29	31.9**	2.50 ± 0.29	31.9**
Angelicae radix	3.75 ± 0.25	-2.18	2.75 ± 0.25	25.1*
Paeoniae radix	3.25 ± 0.48	11.4	3.25 ± 0.48	11.4
Cnidii rhizoma	3.25 ± 0.48	11.4	3.75 ± 0.25	-2.18
Rehmanniae radix	2.67 ± 0.67	27.2	3.33 ± 0.67	9.26
Hydrocortisone acetate (5.0 mg/kg, i.p.)	2.07 ± 0.59	32.1**	2.66 ± 0.21	33.6**

Each value represents the mean ± S.E.M. (n=6)

Significantly different from control group at: *p<0.05, **p<0.01

^{a)}5(w/v) %

^{b)}Times shown are 4 and 6 hours after ultraviolet treatment.

^{c)}Inhibition ratio to control group with time

ination of four medicinal herbs) showed inhibitory effects on erythema induced by ultraviolet irradiation in the dorsal skin in guinea pigs (a human sunburn model, Table III). In this case, the full prescription was also more effective than each one of the crude components alone. Since inhibition of prostaglandin synthesis and antiinflammatory effects are well correlated in the UV-induced erythema model (Black *et al.*, 1977), it seems likely that Shimotsu-to also inhibited the acute inflammation through a reduction of prostaglandin synthesis in guinea pigs.

To our understanding, this is the first report that topical application of Shimotsu-to significantly inhibited both carrageenin-induced edema in rats and ultraviolet-irradiation-induced erythema in guinea pigs. These results also suggest that topical application of the prescription may be beneficial in the treatment of acute skin inflammation in human. Further studies are necessary to clarify the contribution of the crude drugs in this traditional prescription and to discover their precise mechanism of action.

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