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Studies on the anti-inflammatory effects of the Chinese crude drug 'Shoma '(Cimicifugae Rhizoma)

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Abstract

The Chinese medicinal plant 'Shoma' (Cimicifugae Rhizoma) available in the Japanese market consists of botanically two different rootstocks, i.e., *Cimicifuga dahurica* MAXIM and *Cimicifuga heracleifolia* KOMAROV. By using the carrageenin air-pouch inflammation test system in rats, we found *C.heracleifolia* to be a more potent anti-inflammatory agent than *C. dahurica*. From biochemical measurements, *C.heracleifolia* was found to contain 20% more isoferulic acid (IFA) than *C.dahurica*. Because IFA is not water-soluble, the pH of the IFA suspension (pH4.07) was adjusted with NaOH to be the same as the Shoma decoction (pH5.4). Through this shifting of pH, IFA changed its character to a water-soluble salt form. The amount of IFA salt in the *C.heracleifolia* decoction found to be an effective dose for inhibiting the growth of granulation tissue was 4.8 mg/kg. The results suggest that the IFA salt in the decoction was one of the main active components based on the following : 1) a 20 mg/kg dose of IFA salt presented the equivalent potency of inhibition, and 2) IFA was found in the serum of rats after the oral administration of *C. heracleifolia* decoction.

Key words Cimicifugae Rhizoma, Shoma, *Cimicifuga dahurica* MAXIM, *Cimicifuga heracleifolia* KOMAROV, isoferulic acid, ferulic acid, caffeic acid, carrageenin air-pouch method.

Abbreviations IFA, isoferulic acid ; FA, ferulic acid ; CA, caffeic acid ; Shoma, Cimicifugae Rhizoma, 升麻 ; *C.dahurica, Cimicifuga dahurica* MAXIM, 北升麻 ; *C.heracleifolia, Cimicifuga heracleifolia* KOMAROV, 関升麻 ; CMC, carboxymethyl cellulose sodium salt ; Hochu-ekki-to (Bu-Zhong-Yi-Qi-Tang), 補中益気湯 ; Otsuji-to (Yi-Zi-Tang), 乙字湯 ; Shini-seihai-to (Xin-Yi-Qing-Fei-Tang), 辛夷清肺湯 ; Shoma-kakkon-to (Sheng-Ma-Ge-Gen-Tang), 升麻葛根湯.

Introduction

Shoma (Cimicifugae Rhizoma) is classified as a member of the Ranunculaceae family. The rhizome is a main ingredient in a number of traditional Chinese prescriptions such as Shoma-kakkon-to for the treatment of the common cold, Shini-seihai-to used in the treatment of chronic sinusitis, Otsuji-to for hemorrhoid treatment and Hochu-ekki-to for chronic persistent infections.[1]

However, its pharmacological effects have previously not been adequately studied,[2,3] and have only been described in a few reports in terms of its anti-pyretic, analgesic and anti-edematous effects.[4,5]

On the other hand, in the Japanese market, two botanically different kinds of plant have been dealt with equally as Shoma, i.e., *Cimicifuga dahurica* MAXIM (*C. dahurica*) and *Cimicifuga heracleifolia* KOMAROV (*C. heracleifolia*). In daily medical practice, these two plants have been used interchangeably without either strict distinction or recognition.

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This investigation was undertaken by using the carrageenin air-pouch inflammation method in rats to clarify possible differences in anti-inflammatory efficacy between *C. dahurica* and *C. heracleifolia,* as well as to evaluate the efficacy of phenylpropanoids in the Shoma decoction which have been considered as anti-inflammatory agents of Shoma.[6-8]

Materials and Methods

Animals: Male rats of Sprague-Dawley strain, 6 weeks old and weighing 180-210 g raised by Japan SLC Co.,Ltd. (Hamamatsu, Japan) were used in this study.

Preparation of Cimicifugae Rhizoma decoction: Cimicifugae Rhizoma imported from China and supplied by Tochimoto-Tenkaido Co.,Ltd. (Osaka, Japan) and Uchida Wakan-Yaku Co.,Ltd. (Tokyo, Japan) was used in this study.

After being delivered to our laboratory, we performed separating procedures, for example, *C. dahurica* and *C.heracleifolia* in their crude states were separated from each other on the basis of their appearance to the naked eye.

For the decoction we used a special apparatus from Tochimoto-Tenkaido which decocts drugs at a constant 90°C under semi-closed conditions with a top cover on the bottle. Forty five grams of *C.dahurica* or *C.heracleifolia* was boiled with 400 ml water for 40 minutes, and then 300 ml of decocted fluid was obtained after filtration. The pH levels of decoctions of *C.dahurica and C. heracleifolia* were both shown to be 5.4 when they were measured at room temperature one hour after being decocted. The decoction fluid was kept in a frozen state, and then melted at room temperature prior to use.

Chemicals: Isoferulic acid (IFA) and ferulic acid (FA) from Carl Roth GmbH (Karlsruhe, Germany), caffeic acid (CA) from Tokyo Kasei Kogyo Co.,Ltd. (Tokyo, Japan), carrageenin (Seakem #202 carrageenin) from Marine Colloid Inc. (N.Y., USA), carboxymethyl cellulose sodium salt (CMC) from Nacalai Tesque, Inc. (Kyoto, Japan). and penicillin G and streptomycin from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) were used.

Carrageenin was suspended in 0.9% NaCl for 2 % (w/v) carrageenin suspension. This suspension was sterilized by autoclaving at 110°C for 15 minutes, and after cooling at 40-45°C, penicillin G and streptomycin were each added to the carrageenin suspension at a concentration of 0.1 mg/ml for bactericidal purpose.

One gram of IFA, FA or CA, all of which are water-insoluble, was prepared in suspensions of 100 ml of 0.5 % CMC fluid (10 mg/ml).

A water-soluble IFA solution was prepared by mixing IFA suspension with certain amounts of 1-N NaOH solution so as to attain the same pH level (pH : 5.4) and the same concentration (0.48 mg/ml) as the decocted solution of *C.heracleifolia.* At this pH level, the IFA suspension becomes water-soluble and is thoroughly dissolved, because the IFA salt is most likely formed during the titration process.

About a four-fold concentration solution, 2mg/ml of IFA, was also prepared by the same procedure.

For intraperitoneal administration, the IFA solution was prepared by mixing IFA with a sterile phosphate-buffered saline (PBS) solution, and was matched to a pH level of 7.4. The solution was then prepared at three different kinds of concentrations, 0.12 mg/ml, 0.48 mg/ml and 1.92 mg/ml. We used 99.9 % ether from Nacalai Tesque. Inc.(Kyoto, Japan) for anesthesia during this procedure.

Measurement of phenylpropanoids: The concentrations of IFA, FA and CA in the decoctions of the two kinds of Cimicifugae Rhizoma were determined by HPLC (Shimadzu : LC-9A). HPLC was carried out by using a YMC-Pack AP-302 column, 150×4.6 mm I.D. (YMC Co.,Ltd. Kyoto, Japan), eluted with a linear gradient of 11-29 % acetonitrile containing 0.1 % trifluoroacetic acid for 30 minutes at a flow rate of 1.0 ml/min. UV detector for HPLC was used at 260 or 280 nm. The measurements were done on each of the eight decoctions of both rhizomes.

Procedures of carrageenin air-pouch inflammation test: We used a modified carrageenin air-pouch inflammation test method, slightly changed

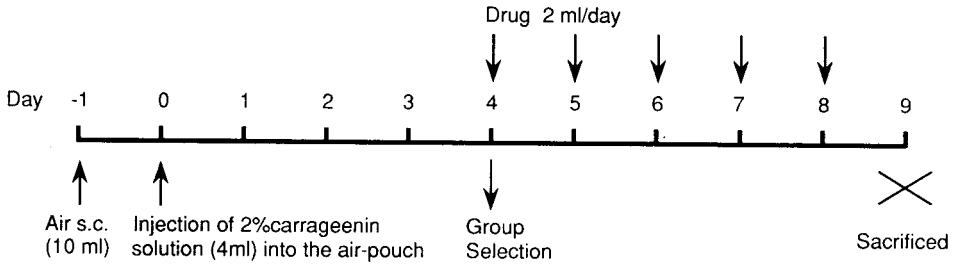

Fig. 1 Method of carrageenin air-pouch inflammation test in rats for measurement of anti-inflammatory effects of test drugs.

from Tsurufuji's original,[9-11] which has been used to assess the potency of test drugs to inhibit the growth of granulation tissue in the chronic proliferative phase. An air-pouch with an oval shape was created on the rat's back (total 30 rats) by inserting 10 ml of air subcutaneously under anesthesia with ether. Twenty four hours after the operation, four ml of 2 % carrageenin suspension, 40-45°C, was injected into the air-pouch. With the day of injection being designated day 0, those rats with a large air-pouch on day 4 were selected and divided into control and test groups for further experiments. Each group consisted of 6 rats. Two ml of the test drugs was administered to the rats orally via catheter or intraperitoneally once a day from day 4 to day 8. A normal saline solution of the same volume was given to the rats of the control group (Fig. 1).

After 5 days of continuous administration, the rats were anesthetized in a closed box where vaporized gas of 99.9 % ether was diluted into the air. They were decapitated for examination. Then the air-pouches were removed so as to allow for measurement of the granulation tissue mass and the exudated fluid in the pouch.

Measurement of serum concentration of IFA : The measurement to detect serum IFA levels was done in three groups, each group consisting of 30 rats : one group given orally 3 ml of *C.heracleifolia* decoction, one group orally 1.4 mg of IFA in a 3 ml suspension, one group orally 1.4 mg of IFA salt in a 3 ml solution (IFA salt dose equivalent to that included in 3 ml of *C.heracleifolia* decoction), and one group intraperitoneally 1.4 mg of IFA salt in a 3 ml solution. Thirty minutes, 1 hour, 2 hours, 3 hours, 6 hours and 12 hours later, the rats were killed under ether anesthesia by decapitation and the blood samples were collected in centrifuged tubes from packs of 5 rats in each group.

All blood samples were centrifuged at 800×g for 10 minutes, and then ultrafiltered under high pressure with 'Air-press-30' (TOSOH Co.,Ltd. Tokyo, Japan) to eliminate any proteins with molecules larger than 30,000. The remainder of the serum was then used for measuring IFA by HPLC.

Statistical analysis : In all of the above experiments, the results were expressed as mean±S.D., and compared by the percent inhibition of control values. Statistical analyses were done by using the 'two sample *t*-test'. The results of the blood samples were then expressed as mean±S.E.M..

Results

Concentrations of three phenylpropanoids in the decoctions of C.dahurica and C.heracleifolia

The average amounts of IFA, FA and CA contained in *C.dahurica* were 398 μg/ml, 71 μg/ml and 60 μg/ml, respectively. Those in *C. heracleifolia* were 477 μg/ml, 74 μg/ml and 57 μg/ml, respectively.

The average values of IFA were the largest in both decoctions and amounted to 5 times as much as those of FA or CA. *C.heracleifolia* contained more IFA than the *C.dahurica* group by 20% (Table I).

Carrageenin air-pouch inflammation test

The average value of granulation tissue weight treated with *C.heracleifolia* was 9.6%, sig-

Table I The concentrations of components in the decoctions of *Cimicifuga dahurica* and *Cimicifuga heracleifolia*.

decoction	Concentration (μg/ml)		(n=8) (mean±S.D.)
	isoferulic acid	ferulic acid	caffeic acid
C.dahurica	398±16	71±11	60±8
C.heracleifolia	477±37*	74±9	57±13

*$p<0.01$ *vs. C.dahurica*

nificantly less ($p<0.05$) than that of the control group, while the value treated with *C.dahurica* was smaller by 7.1 % than that of the control group, which was not statistically significant. No significant difference was noted between the two values, one treated with *C.dahurica* and the other with *C.heracleifolia* in granulation tissue weight. In regard to the exudate, the averages of exudate in the rats treated with either *C.dahurica* or *C. heracleifolia* were both smaller than that of the control group, by 13.0 % and 16.5 %, respectively, but were not significantly smaller (Table II).

The averages of the granulation tissue weights and exudate obtained after the oral administration of 100 mg/kg of IFA, FA or CA resulted in no significant difference in the three groups in comparison with that of the control group (Table III).

Table II Anti-inflammatory effects of orally administered *Cimicifuga dahurica* and *Cimicifuga heracleifolia*.

Group	No. of rats	Granulation tissue weight (g) (mean±S.D.)	Volume of exudate (ml) (mean±S.D.)
Control (saline)	6	6.17±0.40 100 %	30.5±5.3 100 %
C.dahurica	6	5.73±0.44 92.9 %	26.5±3.3 87.0 %
C.heracleifolia	6	5.58±0.34* 90.4 %	25.5±4.3 83.5 %

*$p<0.05$ *vs.* control

Table III Anti-inflammatory effects of orally administered isoferulic acid (IFA), ferulic acid (FA), caffeic acid (CA).

Group	Dose (*p.o.*) mg/kg	No. of rats	Granulation tissue weight (g) (mean±S.D.)	Volume of exudate (ml) (mean±S.D.)
Control (saline)	–	6	5.81±0.41 100 %	30.4±6.1 100 %
IFA	100	6	5.55±0.43 95.5 %	29.0±4.6 95.4 %
FA	100	6	5.52±0.36 95.0 %	27.8±4.1 91.4 %
CA	100	6	5.93±0.51 102.1 %	31.3±6.5 103.1 %

The averages of granulation tissue weights obtained after the oral administration of IFA salt, 4.8mg/kg or 20 mg/kg, showed an insignificant 6.3 % reduction and a significant 8.5 % decrease ($p<0.05$), respectively. Similarly, the averages of exudate resulted in an insignificant 10.3 % reduction and a significant 14.3 % decrease ($p<0.05$), respectively (Table IV).

The averages of granulation tissue weights obtained after intraperitoneal administration of IFA salt, 4.8 mg/kg or 19.2 mg/kg, showed significant reductions ($p < 0.05$), 8.4 % and 10.8 %, respectively. Similarly, the averages of exudate resulted in significant reductions ($p<0.05$), 14.6 % and 15.8% at doses of 4.8 mg/kg and 19.2 mg/kg, respectively (Table V).

No rats which were subjected to decapitation for measurement had any loss of body weight or suffered from any adverse effects from the test drugs during the whole procedure.

Serum concentration of IFA

Three sets of chronological alterations of the serum contents of IFA after drug administrations are shown in Fig. 2.

In the case of the *C.heracleifolia* decoction being orally administered, the average values of the serum level of IFA were 200 ng/ml at 30 minutes and 85.4 ng/ml at 1 hour. When the suspension of IFA was used, the average values of the serum level of IFA were 244 ng/ml at 30 minutes and 47 ng/ml at 1 hour. As for the oral administration of IFA salt, the average values of serum IFA were 308 ng/ml at 30 minutes, 479 ng/ml at 1 hour. When the suspension of IFA was administered orally, the value of serum level of IFA at 1 hour was very low, only 1/10 of that

Table IV Anti-inflammatory effects of orally administered salt of isoferulic acid (IFA salt).

Group	Dose (*p.o.*) mg/kg	No. of rats	Granulation tissue weight (g) (mean±S.D.)	Volume of exudate (ml) (mean±S.D.)
Control (saline)	–	6	5.92±0.38 100 %	29.8±3.8 100 %
IFA salt	4.8	6	5.55±0.34 93.7 %	26.7±3.0 89.7 %
	20	6	5.42±0.32* 91.5 %	25.5±3.5* 85.7 %

*$p<0.05$ *vs.* control

Table V Anti-inflammatory effects of intraperitoneally administered IFA salt.

Group	Dose (*i.p.*) mg/kg	No. of rats	Granulation tissue weight (g) (mean±S.D.)	Volume of exudate (ml) (mean±S.D.)
Control (saline)	–	6	6.68±0.44 100 %	27.4±4.7 100 %
IFA salt	1.2	6	6.33±0.42 94.8 %	24.0±3.8 95.4 %
	4.8	6	6.12±0.35* 91.6 %	23.4±4.3* 85.4 %
	19.2	6	5.96±0.34* 89.2 %	23.1±3.2* 84.2 %

*$p<0.05$ *vs.* control

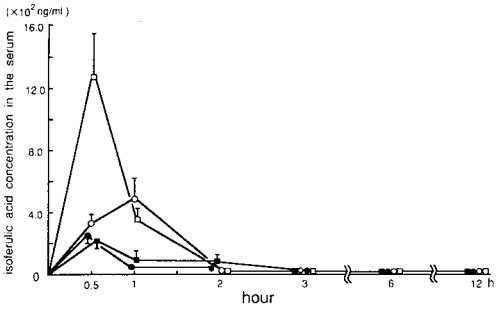

Fig. 2 Chronological changes of serum isoferulic acid concentrations in rats treated with 1) oral administration of decoction of *C. heracleifolia* (— ■ —), 2) oral administration of salt of isoferulic acid (— ○ —), 3) oral administration of suspension of isoferulic acid (— ● —), and 4) intraperitoneal administration of salt of isoferulic acid (— □ —).

Each point represents mean ± S.E.M. obtained from five rats.

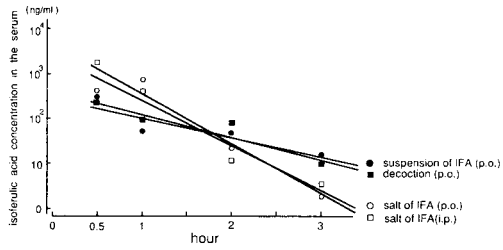

Fig. 3 The three regression lines show the relationship between time and logarithmic values of isoferulic acid concentrations presented in Fig. 2.

obtained with oral administration of IFA salt. After the intraperitoneal administration of IFA salt, the average values were 1264 ng/ml at 30 minutes and 361 ng/ml at 1 hour. In all cases only traces of, or no serum IFA, was observed after 3 hours.

The following regression lines were obtained concerning the four groups between time and logarithmic values of serum IFA (Fig. 3):

Oral administration of *C.heracleifolia* : log Y = −0.45X + 2.52 (r=0.946)

Oral administration of IFA : log Y = −0.36X + 2.20 (r=0.874)

Oral administration of IFA salt : log Y = −0.96X + 3.27 (r=0.967)

Intraperitoneal administration of IFA salt : log Y = −1.08X + 3.56 (r=0.981)

Discussion

Many studeis have been raported on the anti-inflammatory effects of medicinal plants and Kampo formulas, as for example, by the adjuvant-induced arthritis method or the carrageenin paw-edema method.[12-15]

In this study we applied the carrageenin air-pouch inflammation test system to identify and grade the anti-inflammatory effect of Shoma, Cimicifugae Rhizoma, because the system has the following advanteges:

1) The test system can produce multi-staged inflammatory reactions from acute to chronic phase.

2) The system can provide sufficient amounts of inflammatory tissue to allow the examination of the tissue bio-chemically in a relatively short period, usually less than a week.

3) It ts an efficient, well reproducible system, that has many advantages as a pathological model in the study of proliferative inflammation.[16]

The experiment aimed to observe the anti-inflammatory potency of two kinds of Shoma, *C. dahurica* and *C.heracleifolia,* and revealed the following results:

1) decoctions of both rhizomes worked to decrease the amount of exudated fluid in the pouch, 2) as for the weight granulation tissue as a parameter to assess chronic inflammation, the rats treated with *C. dahurica* decoction showed only an insignificant inhibition, but the rats treated with *C.heracleifolia* showed a significant decrease when compared with results of the control group.

These results suggest that *C.heracleifolia* should be a more potent drug than *C. dahurica* at least in terms of its anti-inflammatory aspect. In order to identify the factors which make *C.heracleifolia* more effective, we tried to measure the amounts of three phenylpropanoids, IFA, FA and CA, contained in *C.heracleifolia* and *C.dahurica*

decoctions, because they were all reported to be active chemicals in both plants.[4,5] We could not find any differences between them in regard to either FA or CA, but, *C.heracleifolia* proved to contain more IFA than *C.dahurica* by 20 %. This result implies that the high efficacy of *C.heracleifolia* could be ascribed to the high contents of IFA found in its decoction.

In the next experiment, to ascertain if the above hypothesis was correct, we tried to give the rats extreme amounts of phenylpropanoids orally, in the form of suspensions of 100 mg/kg of IFA, FA and CA. However, none of the three agents proved to be positive, even if the dose of 100 mg/kg was about 21×, 136× and 175× as much as those included in 2 ml of *C.heracleifolia* decoction.

Shibata *et al.*[4,5] reported that one of the pharmacological actions of *C.dahurica* was due to the presence of IFA, citing that 1-2 g/kg showed a significant anti-edematous or analgesic action. We thought it rather difficult to apply their theory here because the amount of IFA included in the decoction, 4.8 mg/kg was extremely small in comparison with the amounts they used in their study. While proceeding with this study, we noticed interesting features of the decoction that would help explain the large differences in dosage between Shibata's study and ours. That is, the decoction fluid was transparent and not cloudy at all, being contrary to the fact that IFA usually stays cloudy in the CMC fluid due to its insolubility in water. Further, the pH level of IFA in suspension when measured was found to be 4.07, and that of the decoction 5.4.

By titrating the pH level of the suspension fluid in an alkaline direction with 1-N NaOH, we found that we could obtain a transparent fluid of IFA at a pH level of 5.4. This was probably due to the formation of salt between the carboxylic acid of IFA and NaOH, as the salt of IFA is considered water-soluble. Accordingly, we tried to administer the thusly formed clear IFA solution, which we called an IFA salt solution, to rats at dosages similar to, or up to about four-fold the amount included in the *C.heracleifolia* decoction.

The results of the oral administration tests showed that 4.8 mg/kg of IFA salt, which is equivalent to the IFA salt included in the decoction, decreased both granulation tissue and exudate fluid, and 20 mg/kg caused a significant decrease in both parameters. As regards to the low efficacy of IFA in suspension, the very low serum level of IFA as 1/10 at 1 hour in comparison with the case of IFA salt can be considred as a main reason. Intraperitoneal administration trials of IFA salt at 4.8 mg/kg and 19.2 mg/kg also resulted in the same significant effects.

We also proved the presence of IFA in rat blood after orlly administering the *C.heracleifolia* decoction.

This would be the first study to show that the anti-inflammatory effects of the decoction of Shoma is possibly mediated by the soluble salt form of IFA in the decoction, and not by the insoluble form of IFA in suspension which Shibata *et al.* used in their study.[4,5]

Another interesting fact that we should consider is the differences of serum concentrations of IFA found to exist between cases of oral dosing of the *C.heracleifolia* decoction and the oral administration of IFA salt. The serum levels of IFA after the oral administration of *C.heracleifolia* decoction were 2/3 at 30 minutes and only 1/6 at 1 hour of the levels which we obtained after the oral administration of the IFA salt solution.

In the experiment with oral administration of the decoction of Shoma, the elimination rate of serum IFA from blood was found to be much lower than that of the case of IFA salt. It is possible to consider that such a low elimination rate could make the decoction of Shoma more effective with a relatively low serum level. The possible presence of other effective components in the Shoma decoction should also be considered.

Acknowledgements

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和文抄録

中国産の生薬・升麻は植物学的に基源の異なる北升麻と関升麻が混在して日本市場に流通している。

いずれの升麻が抗炎症効果に優れているかをラットのカラゲニン空気嚢炎症実験を用いて検討したところ，関升麻が北升麻に比べて優れていた。二種の升麻の成分含有量を測定したところ，関升麻のイソフェルラ酸（IFA）が北升麻に比べて20％多く含まれていた。IFAは水に難溶性で，その水懸濁液はpH 4.07を示したが，これをNaOHを用いて升麻水煎液のpHである5.4に調整したところ，完全に溶解した。このことはIFAが水に溶解性の塩の形に変化したものと考えられた。肉芽組織増殖の抑制に有効な関升麻の水煎液のIFAの量は4.8 mg/kgであった。IFAの塩類の20 mg/kgの経口投与によって関升麻の水煎液にほぼ匹敵する肉芽組織の抑制がみられたこと，さらにラットに関升麻の水煎液を経口投与した血中にIFAが認められたことから，升麻水煎液中のIFAの塩類が升麻の抗炎症効果の主要活性成分の一つであることが明らかとなった。

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