Journal of Traditional Medicinen **11**, 206－213, 1994

Studies on the anti-inflammatory effects of the medicinal plant 'Saiko' (*Bupleurum falcatum* L.)

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(Received July 18, 1994. Accepted October 1, 1994.)

Abstract

Anti-inflammatory effects of the Chinese medicinal plant Saiko' and the four fragments from its decocted extract were examined by using the carrageenin air-pouch inflammation test in rats. We found that the decocted fluid of Saiko had only a mild anti-inflammatory effect. The oral administration of a suspension of the saponins at 162 mg/kg resulted in a significant decrease in the granulation tissue weight with malnutrition of the rats. The saponin fraction was not water-soluble at a pH level of 3.8. The pH level of the saponin suspension was then adjusted with NaOH to be the same as that of the Saiko decoction (pH 5.4). The suspension changed its character to become water-soluble. Using these water-soluble saponins, we could observe significant reductions in the weight of the granulation tissue at a very low dose, 6 mg/kg, without malnutrition of the rats. A test with syrupy residue resulted in the tendency to increase granulation tissue. Tests with either the acid fraction or neutral fraction revealed no significant results. These findings suggest that saponins were the main active component for the anti-inflammatory potency that Saiko possesses. The number of leukocytes counted in the exudates also decreaseed dose-dependently. We considered that saponins mainly affected the second phase of inflammation (migration of leukocytes).

Key words *Bupleurum falcatum* L., Saiko, anti-inflammatory effect, carrageenin air-pouch method.

Abbreviations Saiko (Chai-hu), 柴胡 : Sho-saiko-to (Xiao-Chai-Hu-Tang), 小柴胡湯 ; Jumi-haidoku-to (Shi-Wei-Bai-Du-Tang), 十味敗毒湯 ; Hochu-ekki-to (Bu-Zhong-Yi-Qi-Tang), 補中益気湯 ; Shigyaku-san (Si-Ni-San), 四逆散 ; Kami-shoyo-san (Jia-Wei-Xiao-Yao-San), 加味逍遥散.

Introduction

Saiko (the root of *Bupleurum falcatum* L.) is classified as a member of the Umbelliferae family and has been said to have anti-inflammatory, antipyretic, analgesic and anti-depressive action from ancient times. It is a main ingredient plant in a number of traditional Chinese prescriptions such as Sho-saiko-to in the treatment of chronic hepatitis, Hochu-ekki-to for chronic persistent infections, Jumi-haidoku-to in the treatment of skin abscesses, Shigyaku-san in the treatment of depressive state or mental stress, and Kami-shoyo-san used in the treatment of menopausal disorders.[1)]

Recently Saiko's pharmacological effects have been studied about the use for hepatic injuries,[2-5)] anti-inflammatory,[2,6,7)] anti-allergic,[8)] anti-peptic ulcer,[2,9)] anti-stress effect,[10)] steroid-like action,[11)] and plasma-cholesterol lowering action.[12)] However, it has not been sufficiently tested in detail in regards to its main active components which cause anti-inflammatory effects in plant fractions such as the saikosaponin fraction, neutral and acid fractions, or syrupy residue.

This investigation was undertaken to determine the existence of active compounds and their localiza-

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tion in fractions by using the carrageenin air-pouch inflammation test system in rats.

Materials and Methods

Animals : Male rats of the 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 Saiko decoction : Saiko supplied by Tochimoto-Tenkaido Co., Ltd. (Osaka, Japan) was used in this study. For the decoction we used a commercially available decoction apparatus from Tochimoto-Tenkaido which decocts drugs at a constant 90°C under semi-closed conditions with a top cover on the bottle. Seven, 14, 28 and 56 g of Saiko were boiled with 400 ml of water for 40 minutes, and added with water to exactly 300 ml after filtration. The decoction fluid was kept in a frozen state, and then melted at room temperature prior to use.

Chemicals : Fractions of Saiko were isolated as described by Shibata *et al.*[13] (Fig 1, Table I). Fractions in the decocted fluid from 7, 14, 28, and 56 g of Saiko were also extracted and measured in a similar manner (Table II). Carrageenin (Seakem #202 carrageenin) from Marine Colloid Inc. (N.Y., USA), and penicillin G and streptomycin from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) were used. Carrageenin was dissolved 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 purposes. A mass of saikosaponins was crushed and ground with a pestle in a mortar and suspended in distilled water. The suspension was then prepared at three different concentrations, 1.8, 5.4 and 16.2 mg/ml. The water-soluble saikosaponin solution was prepared by mixing

Table I Amount and gain of fractions isolated from 2 kg of Saiko.

Fractions	Amount (g)	Gain (%)
Syrupy residue	225.9	11.3
Saponin fraction	60.6	3.0
Neutral fraction	38.5	1.9
Acid fraction	16.0	0.8

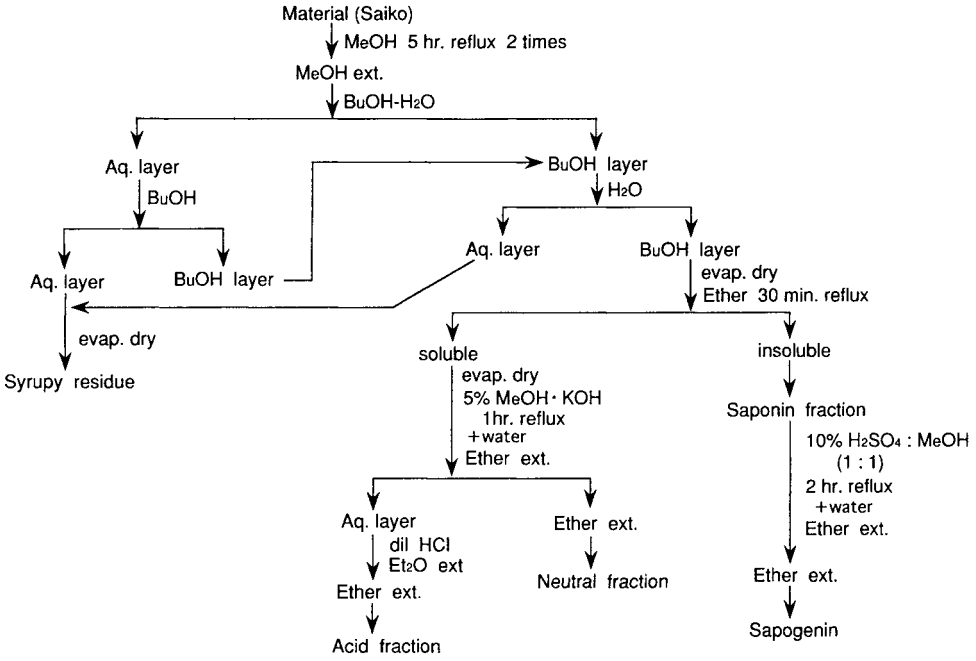

Fig. 1 Fractionation procedure

Table II Amount and gain of fractions isolated from the decocted fluid from 7, 14, 28, 56 g of Saiko.

Saiko (g/300 ml)	Amount (g) (Gain (%))			
	Syrupy residue	Saponin fraction	Neutral fraction	Acid fraction
7	2.09 (29.81)	0.06 (0.86)	0.01 (0.14)	0.01 (0.14)
14	3.80 (27.12)	0.10 (0.71)	0.02 (0.14)	0.01 (0.07)
28	6.65 (25.53)	0.24 (0.92)	0.03 (0.12)	0.01 (0.04)
56	13.78 (24.61)	0.39 (0.70)	0.03 (0.05)	0.01 (0.02)

5.4 mg/ml of saikosaponin suspension with a certain amount of 1 N NaOH solution so as to attain the same pH level (pH 5.5) as the decoction fluid (14 g/300 ml) of Saiko. At this pH level, the saikosaponin suspension became water-soluble and was thoroughly dissolved. A 5.4 mg/ml saikosaponin solution was then diluted to one-third or one-ninth with water in order to get three different concentrations, 5.4, 1.8 and 0.6 mg/ml. Each of syrupy residue, the neutral fraction and acid fraction was dissolved in water and adjusted to pH 5.5, with 1 N NaOH or 1 N HCl. Concentrations of the three fractions were 22.8, 0.12, 0.06 mg / kg respectively. We used 99.9 % ether from Nacalai Tesque Inc.(Kyoto, Japan) for anesthesia for surgical interventions.

Procedures of carrageenin air-pouch inflammation test : We used the modified carrageenin air-pouch inflammation test method which was used to assess potency of test drugs to inhibit growth of granulation tissue in the chronic proliferative phase.[14-16] In a total of 30 rats, an oval-shaped air-pouch was created on their backs by inserting 10 ml of air subcutaneouly under anesthesia. Twenty-four hours after the operation, 4 ml of 2 % carrageenin suspension, 40-45°C, was injected into the air-pouch. With the day of injection being designated as 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 5 rats. Two milliliters of the Saiko decoction was administered to the rats orally via a catheter once a day from day 4 to day 8. Similarly the 18, 54 and 162 mg/kg body weight of saikosaponin suspensions were administered orally. The 6, 18 and 54 mg/kg body weight saikosaponin solutions, 228 mg/kg body weight syrupy residue solution, 1.2 mg/kg body weight neutral fraction solution and 0.6 mg/kg body weight acid fraction solution were given in the same way. Normal saline of the same volume was given to the rats of the control group (Fig. 2). After 5 days of repeated administration, the rats were anesthetized in a closed box in which vaporized gas of 99.9 % ether was diluted into air. They were then decapitated for examination. The air-pouches were removed to allow measurement of the granulation tissue mass and the exudated fluid in the pouch. In the group treated with the saikosaponin solution, white blood cell count was also performed with a blood cell counter on the exudated fluid (Celltac MEK-4500, NIHON KOH-

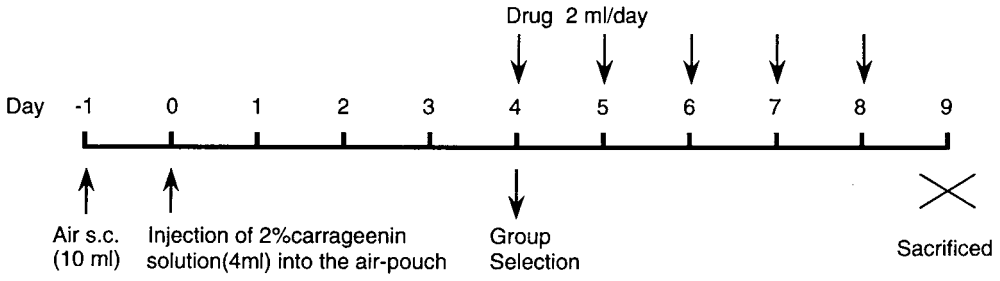

Fig. 2 Method of carrageenin air-pouch inflammation test in rats for measurement of anti-inflammatory effects of test drugs.

DEN, Tokyo). The body weight of the rats was measured on days 0 and 9 to determine if the experimental procedures had any effect on the growth rate or the nutrition of the animals.

Statistical analysis : In all of the above experiments, the results were expressed as mean±S.D., and were compared by the percent inhibition of the control values. Statistical analyses were done by using the 'ANOVA and Scheffe's F-test'.

Results

Carrageenin air-pouch inflammation test

The average value of granulation tissue weight treated with the decoction of 14 g of Saiko was 8.6 %, significantly less ($p<0.05$) than that of the control group. On the other hand, the values from treatment with the decoctions of 7, 28 and 56 g of Saiko were not statistically significant. In regard to the exudate treated with the decoction of Saiko, in no case was the average value of the exudate significantly smaller than that of the control group. The changes in body weight showed no significant differences in comparison with control (Table III). The average value of granulation tissue weight treated with 162 mg/kg of saikosaponin suspension was 9.8 %, significantly less ($p<0.05$) than that of the control group. In the other two cases treated with either 18 or 54 mg/kg, the values were not statistically less than that of control. In regard to the exudate, in none of the three cases was the average significantly smaller in comparison with that of the control group. In regard to the change of body weight of the rats during the saikosaponin suspension treatment, the group treated with 162 mg/kg showed a significant reduction ($p<0.01$), but the two other groups showed no significant difference, though the growth rate of the rats was dose-dependently depressed as doses increased (Table IV). The

Table III Anti-inflammatory effects of orally administered Saiko decoctions.

Group	Dose *(p.o.)* (g)	No. of rats	Granulation tissue weight (g) (mean±S.D.)	Volume of exudate (ml) (mean±S.D.)	Body weight change (g) (mean±S.D.)
Control (saline)	–	5	6.54±0.42 100%	42.0±5.3 100%	17.2±7.9 100%
Saiko	7	5	6.20±0.48 94.8%	38.8±3.7 92.4%	18.0±4.3 104.7%
	14	5	5.98±0.32* 91.4%	36.8±3.5 87.6%	17.0±7.6 98.8%
	28	5	6.12±0.46 93.6%	38.7±5.2 92.1%	11.6±6.8 67.4%
	56	5	6.24±0.54 95.4%	40.7±5.2 96.9%	13.4±9.0 77.9%

*$p<0.05$ vs. control

Table IV Anti-inflammatory effects of orally administered saikosaponin suspensions.

Group	Dose *(p.o.)* mg/kg	No. of rats	Granulation tissue weight (g) (mean±S.D.)	Volume of exudate (ml) (mean±S.D.)	Body weight change (g) (mean±S.D.)
Control (saline)	–	5	6.56±0.44 100%	37.9±6.4 100%	18.0±9.6 100%
Saikosaponin suspensions	18	5	6.62±0.50 100.9%	37.2±5.4 98.1%	16.2±9.8 90%
	54	5	6.32±0.65 96.3%	35.2±5.9 92.9%	12.8±5.7 71%
	162	5	5.92±0.38* 90.2%	32.0±4.5 84.5%	-0.8±4.9** −4.4%

*$p<0.05$ vs. control **$p<0.01$ vs. control

Table V Anti-inflammatory effects of orally administered saikosaponin solutions.

Group	Dose (*p.o.*) mg/kg	No. of rats	Granulation tissue weight (g) (mean±S.D.)	Volume of exudate (ml) (mean±S.D.)	Leukocytes in exudate ($\times 10^2/\mu l$) (mean±S.D.)	Body weight change (g) (mean±S.D.)
Control (saline)	–	5	6.64±0.46 100%	39.5±6.6 100%	52.6±10.5 100%	19.4±8.5 100%
Saikosaponin solutions	6	5	6.02±0.35* 90.7%	35.7±4.2 90.3%	39.4±7.3* 74.9%	16.6±6.6 85.6%
	18	5	5.88±0.32* 88.6%	32.5±6.7 82.2%	36.2±6.5* 68.8%	15.6±8.3 80.4%
	54	5	5.46±0.62** 82.2%	28.6±7.4* 72.3%	28.8±6.8** 54.8%	11.8±7.0 60.8%

*$p<0.05$ vs. control **$p<0.01$ vs. control

Table VI Anti-inflammatory effects of orally administered syrupy residue, acid fration and neutral fraction.

Group	Dose (*p.o.*) mg/kg	No. of rats	Granulation tissue weight (g) (mean±S.D.)	Volume of exudate (ml) (mean±S.D.)	Body weight change (g) (mean±S.D.)
Control (saline)	–	5	5.92±0.39 100%	32.5±7.2 100%	20.4±5.7 100%
Syrupy residue	228	5	6.24±0.54 105.4%	35.4±7.0 109.1%	15.2±9.0 74.5%
Neutral fraction	1.2	5	5.86±0.50 99.0%	31.4±6.9 96.7%	21.6±6.9 105.9%
Acid fraction	0.6	5	6.02±0.47 101.7%	34.6±6.3 106.5%	18.8±9.2 92.2%

averages of granulation tissue weight from the rats treated orally with the saikosaponin solutions of 6 mg/kg and 18 mg/kg were significantly less ($p<0.05$), 9.3 % and 11.4 % respectively, than the control value. Moreover, the value from the treatment with 54 mg/kg of saikosaponin solution was more markedly reduced, by 17.8 % ($p<0.01$), in comparison with the control value. As for the exudate, a significant reduction of 27.7 % was observed in the 54 mg/kg case ($p<0.05$). The number of leukocytes in the exudates decreased significantly in all three groups treated with 6, 18 and 54 mg / kg of saikosaponin solutions, with reduction rates of 25.1 %, 31.2 % ($p<0.05$) and 45.2 % ($p<0.01$), respectively (Table V).

In the experiments where rats were treated with 228 mg/kg of syrupy residue, 1.2 mg/kg of neutral fraction, or 0.6 mg/kg of acid fraction, the average values of the granulation tissue weights did not show any significant reduction by any of the three agents. On the contrary, the granulation tissue of the rats treated with syrupy residue showed a tendency to increase (increase rats 5.4%). The average values of all the exudates in the three groups were not significantly different in comparison with control. In addition, body weight changes showed no significant differences compared to control with any of the three agents (Table VI).

Discussion

Many studies have been reported on anti-inflammatory effects of medicinal plants and Kampo formulas,[17-19] for example, by using the adjuvant-induced arthritis method or the carrageenin paw - edema method. In this study we applied the carrageen air-pouch inflammation test system to identify and grade the anti - inflammatory effect of Saiko, *Bupleurum falcatum* L., because the system has the following advantages : 1) the system can produce multi-staged inflammatory reactions from acute to chronic phase,

2) the system can provide a sufficient amount of inflammatory tissue to allow the examination of the tissue biochemically in a relatively short period, usually less than a week, and 3) it is an efficient, easily reproducible system, and has many advantages as a pathological model in the study of proliferative inflammation.[20]

In this study, an experiment to assess the anti-inflammatory effect of the Saiko decoction showed that the inhibitory ratios by 7, 14, 28 and 56g of Saiko were not dose-dependent and, except for the 14 g dose, did not produce any significant effects. This result is complex because, as shown in Table II, at least two of the four fragments in Saiko decoction increased proportionally in amount as more Saiko was decocted in 300 ml water. The amounts of four frament in case of 14 g of Saiko were figured out to be 6 mg/kg of saponin, 228 mg/kg of syrupy residue, 1.2 mg/kg of neutral fraction and 0.6 mg / kg of acid fraction. Therefore, in the experiments to examine potency of each fragment, 228 mg/kg of syrupy residue, 1.2 mg/kg of neutral fraction and 0.6 mg/kg of acid fraction were used. In the experiments using the saikosaponin fraction and the syrupy residue, the results were quite opposite. That is, the saikosaponin fraction showed a significant anti-granulomatous effect, but the syrupy residue in contrast tended to facilitate the growth of granulation tissue mass. Therefore, in regard to the limited anti-inflammatory potency of Saiko decoction noticed in the present study, it is possible to simply consider that the presence of two such contradictory elements in the decoction are to blame. Some researchers have insisted that decline of the pH level of the decoction fluid, which is observed as Saiko is more concentratedly decocted, is the cause which makes Saiko appear less effective, because the decline of the pH level is capable of inducing the transformation of saikosaponin structures from active saikosaponin a, d, to inactive b_1, b_2.[21,22] Detailed analyses have been reported on the saikosaponin fraction for its anti-inflammatory or anti-granulomatous effects, and also on the syrupy residue concerning its analgesic effect, but there have been only a few reports on the other fractions.[2,7] The aim of this study, therefore, was to clarify in detail the anti-granulomatous effect that each fraction of Saiko may possess. The saikosaponin fraction is water-insoluble. Saikosaponin water suspension orally administered at 162 mg/kg for 5 days continuously showed a significant anti-granulomatous effect. However, it also brought about a significant ($p<0.01$) deterioration of the rat's growth rate during the treatment. From this, it would be difficult to conclude that saikosaponins in a water suspension have an anti-granulomatous action, as it would be much more likely that the poor granulation growth was a result of internal mal-conditions, such as poor nutrition, of the rats.

Takagi *et al.*[2] and Yamamoto *et al.*[6,7] have reported on the anti-granulomatous effect of orally administered saikosaponins. Takagi *et al.*,[2] by the granuloma pouch method, reported the positive effects of crude saponins which were water-insoluble, given to rats orally at 100 mg/kg/day in normal saline suspension orally for 7 days. Study of Yamamoto *et al.*[6,7] was performed with saikosaponins in normal saline suspension given by peroral administration and intra-muscular routes, and positive results were attained. The granuloma pouch and cotton pellet methods were used, and rats were given 50 mg/kg/day of the agent for 8 days. The peroral administration was found to be only one-tenth as effective, a result attributed to either poor absorption from the gut or inactivation of the agent in the gut.

In the present study, the further experiments using the thusly formed semitransparent saikosaponin fluid revealed the following results. The oral dose of 6 mg / kg, which caused a significant decrease in granulation tissue, was only 1/27 of the dose of saikosaponin suspension needed to achieve the same effect. Apart from its similar potency, the dose of 6 mg/kg of saikosaponin solution did not affect the rat growth rate. The oral dose of 6 mg/kg of saikosaponins used for the rat in this study is relatively close to the clinical oral dose for man, *i.e.*, the total amount of saikosaponins included in the fluid decocted from 14 g of Saiko is 100 mg (Table II). This fact is quite persuasive when it comes to explain that the amount of saikosaponins contained in a dose range of 4-14 g/day of Saiko can in pharmacological terms serve as a reasonable standard for the actual clinical dosage in medical practice.

As for the large dose of the saikosaponin suspen-

sion reported to be effective by Yamamoto *et al.*,[6,7] our present results seem to indicate that saikosaponins would have been gravely influenced and altered by a different approach in the manner and character of drug administration.

Inflammation is staged into three phases : the first phase features increased vascular permeability ; the second phase is represented by the migration of leukocytes ; proliferation of connective tissue is the hallmark of the third phase. Saikosaponins have been considered to exert their anti-inflammatory effect mainly on the third phase. But our present results indicate that they influence the second phase also, because both the number of leukocytes in the exudate and the weight of granulation tissue were depressed dose-dependently.

This would be the first study in which the anti-inflammatory effect of saikosaponins has manifested itself at a dose equivalent to the amount that is contained in Saiko decoctions presently used in clinical practice.

Acknowledgements

We express our gratitude to Mr. Arndt Gerz for his critical reading of this manuscript.

和文抄録

生薬「柴胡」とその成分の画分（サポニン画分，樹脂画分，中性画分，酸性画分）について，ラットのカラゲニン空気嚢炎症モデルを用いて経口投与による抗炎症効果を検討した。柴胡水煎液の抗炎症効果は軽度であり，用量依存性を認めなかった。サポニン画分は水に難溶性であり，その水懸濁液は pH 3.8 を示した。この懸濁液では 162 mg/kg で肉芽重量は有意に抑制されたが，ラットの体重も有意に抑制され，抗炎症効果を確定できなかった。次にこれを NaOH を用いて柴胡水煎液の pH である 5.5 に調整したところ水溶液となった。この水溶液では 6 mg/kg で体重の抑制を伴わずに，肉芽重量は有意に抑制された。また滲出液中の白血球数と肉芽重量はサポニンの用量に依存して抑制されたことから，サポニンは炎症第 2 期（白血球遊走）に有効であると考えられた。樹脂画分では肉芽重量の増加傾向が認められた。中性画分と酸性画分には抗炎症効果は認められなかった。以上の成績から柴胡の抗炎症効果をもたらす画分はサポニンに求められることが明らかとなった。

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