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ANTI-INFLAMMATORY EFFECT OF SAIKOGENIN A

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Abstract—Saikogenin A, an anti-inflammatory drug, is present in the crude extract of a Chinese herbal plant called Tsai-Fu. Saikogenin A was less effective in adrenalectomized rats than in normal rats in reducing the carrageenin-induced edema. Serum corticosterone and ACTH were increased in the saikogenin A-treated rats, supporting the view that stimulation of hypothalamopituitary–adrenal system is responsible for the anti-inflammatory effect of saikogenin A. This is further supported by the findings that saikogenin A did not affect the spontaneous release of corticosterone but it facilitated the ACTH-induced release. In addition, cyclic AMP in isolated pituitary and adrenal glands was increased by saikogenin A. A role for cyclic AMP as the second messenger is thus considered. Otherwise, the direct action of saikogenin A on the process of inflammation cannot be ruled out because saikogenin A also functioned in the adrenalectomized rats and it inhibited the release of histamine induced by compound 48/80. Reduction of the vascular permeability was also observed in the saikogenin A-treated rats. These results suggest that the anti-inflammatory action of saikogenin A are due to an increase in corticosterone caused by the release of ACTH and a direct effect on the process of inflammation.

In Chinese herbal medicine, Tsai-Fu is generally used as anti-inflammatory drug [1]. Dry root of *Bupleurum falcatum* L. (Umbelliferae) is named as Tsai-Fu. Several active compounds have been purified in Japan and they are named as saikogenin, the saponin compounds derived from Tsai-Fu [2, 3]. Kato *et al.* have confirmed the anti-inflammatory actions of Tsai-Fu, named as Saiko in Japanese [4, 5]. However, the mechanisms of action of Tsai-Fu remained obscure.

In the present study, we report the effect of saikogenin A (Fig. 1) on experimental inflammation and investigate the mechanisms of action in relation to endogenous adrenocorticosteroids.

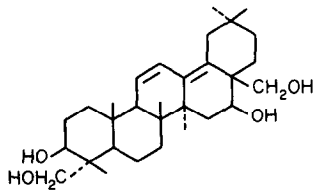

Fig. 1. The chemical structure of saikogenin A.

MATERIALS AND METHODS

Animals. Wistar rats of both sexes, weighing 230–250 g, were divided at random into two groups. One group served as control. The rats of the other group were treated with subcutaneous injections of drugs in the abdominal wall. Rats were housed two per cage under a 12 hr light–12 hr dark regimen, with lights on between 0700 and 1900 hr. Food and water were available *ad libitum*. Some animals of the two groups were adrenalectomized under ether-induced anaesthesia, by dorsal approach, and the control group was treated with a sham operation. The operated animals were employed 96 hr later.

Detection of anti-inflammatory effects. Foot volumes were measured by a water plethysmometer (MK-500, Muromachi, Japan) immediately before the injection of lambda carrageenin (0.1 ml; 2%) in the plantar region of the right hind paw, and at different intervals thereafter. The swelling was calculated as a mean percentage increase in the volume of the injected paw compared to the initial control value.

Detection of vascular permeability. According to the method of Whittle [6], 0.25% Evan blue was injected intravenously (0.5 ml/0.1 kg) followed with the intraperitoneal (i.p.) injection of acetic acid (0.25 ml; 0.6%). After 30 min, the rats were decapitated and the abdominal area was opened for the collection of dyes in peritoneal cavity through wash with Locke-Linger solution. The absorption of 10 ml of the collected dye was measured with a u.v. spectrophotometer (Gilford 260, Ohio, U.S.A.) at 610 nm. The rats were treated with the test drugs 30 min before the injection of acetic acid.

Measurement of histamine release. The mast cell was prepared according to the method of Atkinson and his colleagues [7] and the amount of histamine was determined by the method of Anton and Sayre [8]. The release of histamine was induced by the treatment of compound 48/80 (0.5 g/ml) and the inhibitory rate was calculated as

$$\text{Inhibition} = \frac{H_1 - H_2}{H_1},$$

Where H_1 is the amount of histamine released by compound 48/80 and H_2 is the amount of histamine released by 48/80 in the presence of the tested drug or vehicle.

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Determination of ACTH and cyclic AMP. Trunk blood was collected in cold heparin-containing tubes and centrifuged (1000 *g*; 10 min; 4°). The plasma was decanted and assayed directly for ACTH through the immunoradiometric method, described by Dallman *et al.* [9]. The contents of cyclic AMP were determined by cAMP-RIA kits purchased from Yamasa Shyoyu (Choshi, Japan), according to the method of Steiner *et al.* [10]. In addition, blood and adrenal perfused corticosterones were assayed by the method of Zenker *et al.* [22].

Chemicals. Carrageenin, corticosterone, compound 48/80 and indomethacin were purchased from Sigma Chemical Co. (St. Louis, MO). Histamine diphosphate was from Wako Chemical Co. (Osaka, Japan). $ACTH^{1-24}$ and aminopyrine were obtained from Daiichi Pharmaceutics Co. (Tokyo, Japan). Saikogenin A was extracted from the dry root of *Bupleurum falcatum* L. (Umbelliferae) and acidified from saikogenin F according to the report of Shibata *et al.* [2]. Chromatographic analysis (TLC) and other determinations (melting point test and atomic absorption spectrophotometry) indicated that this purification procedure yielded the homogenous substance. Crystal of saikogenin A was dissolved in 85% ethanol and diluted in 0.9% saline. Vehicle solutions were prepared in the same manner but no addition of saikogenin A. Result of the vehicle-treated alone was taken as control. All the other reagents were of analytical grade. Dilution of the drug solutions was made up fresh daily in 0.9% saline.

Results were expressed as mean ± S.E.M. Student's *t*-test was used for the statistical evaluation and $P < 0.01$ was the limit for significant differences.

RESULTS

Effect of saikogenin A on the edema

Edema induced by lambda carrageenin can be measured by the degree of paw swelling. Edema is increased gradually following the injection of lambda carrageenin and reached a plateau after 6 hr. Pre-treatment with i.p. injection of saikogenin A (80 mg/kg) at 10 min prior to the administration of lambda carrageenin produced a 27.4 ± 1.7% (N = 6) reduction of paw swelling, as compared with the vehicle-treated samples. Reduction became more marked (74.6 ± 3.1%, N = 6) when it was injected at 20 min prior to the administration. It reached a maximal effect (98.7 ± 2.8%, N = 6) when injected 30 min earlier. There was no difference ($P > 0.01$) between the reduction of paw swelling obtained from that treated 30 min earlier and that injected 60 min earlier (100%, N = 6). Thus, in order to make the drug more effective, pre-treatment with i.p. of saikogenin A was carried out at 30 min prior to the administration of chemical substances.

In the 30 min pre-treatment with i.p. injection,saikogenin A produced an antagonistic effect on the lambda carrageenin-induced edema in a dose-dependent manner. The obtained inhibitory effects were 17.8 ± 2.4% (N = 8) for 20 mg/kg, 39.6 ± 1.7% (N = 8) for 40 mg/kg, 52.4 ± 2.9% (N = 8) in 50 mg/kg, 76.4 ± 3.1% (N = 8) in 75 mg/kg and 98.7 ± 2.4% (N = 6) in 95 mg/kg, respectively, when the reduction induced in 0.1 g/kg was taken as 100% (N = 8). For the ED_{50}, 50 mg/kg of saikogenin A was then employed in the present study.

Saikogenin A inhibits the edema induced by carrageenin by about 48% in normal rats but only by about 26% in adrenalectomized rats (Fig. 2), as compared to the vehicle-treated animals.

In addition, edema is reduced by 11% with aspirin (200 mg/kg, i.p.) (89.7 ± 4.7%, N = 6) and by 46% with aminopyrine (200 mg/kg, i.p.) (54.7 ± 3.6%, N = 6). The results indicated that saikogenin A produced a similar anti-inflammatory effect as aminopyrine and a more marked effect than aspirin.

Effect of saikogenin A on vascular permeability

Leakage of the intravenous injected dye into peritoneal cavity of rats was determined. At 30 min

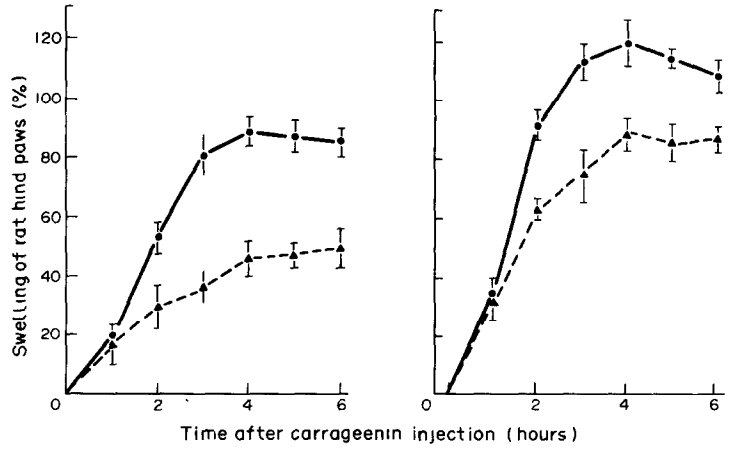

Fig. 2. Effect of saikogenin A on the carrageenin-induced paw edema in sham operated (left) and adrenalectomized (right) rats. Paw swelling is given as percentage increased in comparison to the initial volume. Solid line indicates the response of the vehicle-treated group and broken line represents the response of the saikogenin A-treated group. Saikogenin A (50 mg/kg i.p.) was administered 30 min prior to the application of lambda carrageenin. Each point is the mean ± S.E. of eight observations.

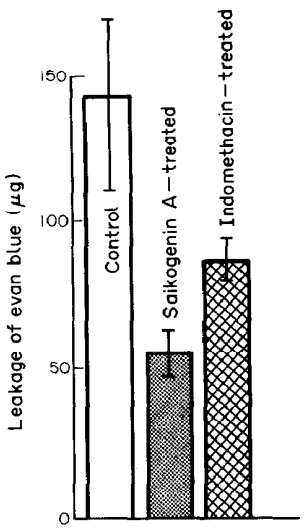

Fig. 3. Effect of saikogenin A on the leakage of Evan blue in rats. Evan blue (0.25%) was intravenously injected and it was perfused into the peritoneal cavity by intraperitoneal injection of 0.6% acetic acid (as control). Saikogenin A (50 mg/kg) and indomethacin (10 mg/kg) were each administered at 30 min prior to the injection of acetic acid. Vertical bar means the mean ± S.E. of eight experiments. All the differences were significant at least at $P < 0.05$.

after treatment with Evan blue, i.p. injection of saikogenin A (50 mg/kg) caused a leakage of Evan blue (18.9 ± 2.9 g, N = 6) into the peritoneal cavity. Vehicle saline injected in the same manner also caused a leakage of 17.4 ± 3.1 g (N = 6) of Evan blue. Since the difference was statistically insignificant ($P > 0.01$), the possibility of counter-irritation by saikogenin A can be ruled out.

On the other hand, i.p. injection of 0.6% acetic acid caused a statistically higher leakage of Evan blue into peritoneal cavity (143.2 ± 17.4 g, N = 8). Prior (30 min) treatment with indomethacin (10 mg/kg, i.p.) reduced the leakage of dye to 87.4 ± 7.2 g (N = 8), a 39% inhibition. When saikogenin A (50 mg/kg) was injected (i.p.) 30 min earlier, the leakage of dye was reduced to only 55.9 ± 7.6 g (N = 8), a 61% inhibition. But there was no reduction (138.6 ± 13.7 g, N = 8) in the vehicle-treated group. Thus, saikogenin A had a significant inhibitory effect on vascular permeability ($P < 0.01$).

Histamine release and saikogenin A

Release of histamine from mast cells *in vitro* induced by compound 48/80 (0.5 g/ml) was determined. Mast cells were prepared from peritoneal cavity of rats according to Atkinson *et al.* [7] and were incubated at 37° with compound 48/80 for 10 min. The released histamine was measured by the spectrofluorometric assay, described by Anton and Sayre [8].

Saikogenin A and indomethacin were treated 5 min prior to the incubation of compound 48/80. As shown in Fig. 4, in comparison with the vehicle-treated group, saikogenin A produced an inhibitory effect on the release of histamine in a dose-dependent manner. Indomethacin produced a similar effect at higher concentration.

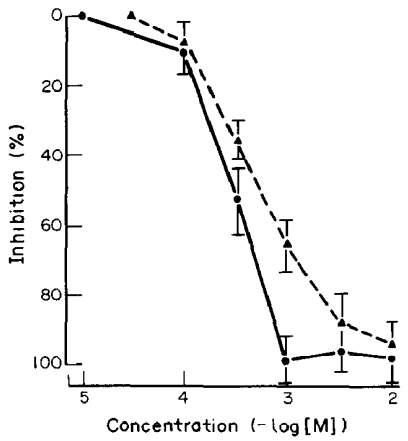

Fig. 4. Effects of saikogenin A (solid line) and indomethacin (broken line) on the release of histamine induced by compound 48/80 *in vitro*. Mast cells prepared from the peritoneal cavity of rats were incubated with compound 48/80 (0.5 μg/ml) at 37° for 10 min and the released histamine was measured by spectrofluorometric assay.

Saikogenin A and corticosterone

The concentration of corticosterone was elevated by the treatment of saikogenin A (50 mg/kg, i.p.). Adrenal corticosterone increased gradually with time and reached a maximal response at 3 hr following saikogenin A treatment (Fig. 5) whereas the peak of corticosterone in serum was observed at 2 hr later (Fig. 5). However, the weight of adrenal gland in saikogenin A-treated rats (at 3 hr later) was 15.4 ± 1.4 mg/100 g (N = 5) which was not markedly different from the non-treated animals (14.8 ± 1.8 mg/100 g, N = 5) ($P > 0.01$).

The release of corticosterone is spontaneous in the rats, no corticosterone can be detected at 4 days after the adrenalectomized operation. When the slice of adrenal gland is perfused in chamber with Locke-Linger solution, saikogenin A did not affect the spontaneous release of corticosterone *in vitro* (Table 1). The concentration of corticosterone was

Table 1. Effect of saikogenin A on the release of corticosterone from adrenal slice *in vitro*

	Corticosterone (ng/100 mg tissue/hr)
Non-treatment	4.16 ± 0.06 (N = 8)
Saikogenin A (0.5 mg/ml)	4.39 ± 0.93 (N = 8)
(0.8 mg/ml)	4.51 ± 0.76 (N = 8)
ACTH (10 ng/ml)	18.73 ± 2.41 (N = 8)
Vehicle + ACTH (10 ng/ml)	17.98 ± 1.98 (N = 8)
Saikogenin A (0.5 mg/ml) + ACTH (10 ng/ml)	25.61 ± 1.66* (N = 8)
Saikogenin A (0.8 mg/ml) + ACTH (10 ng/ml)	38.79 ± 2.36* (N = 8)

Each value represents the mean ± S.E. and N indicates the number of experiments. Non-treatment represents the spontaneous release and * indicates that the value is significantly different from the sample treated with ACTH ($P < 0.01$). The vehicle was injected in the same volume as that of 0.8 mg/ml saikogenin A.

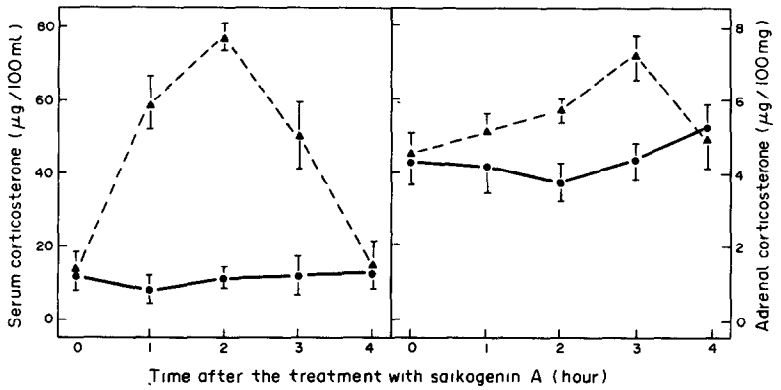

Fig. 5. Effect of saikogenin A on the level of corticosterone in serum and adrenal gland of rats. Saikogenin A (50 mg/kg, i.p.)-treated animal (broken line) was compared with the vehicle-treated animals (solid line). Each point indicates the mean ± S.E. of six studies.

increased significantly, when the adrenal slice was incubated with synthetic ACTH (10 ng/ml). Saikogenin A potentiated the ACTH-induced release of corticosterone in a dose-related fashion although it did not affect the spontaneous release of corticosterone (Table 1).

Effect of saikogenin A on ACTH and cyclic AMP

As shown in Table 2, concentration of ACTH in plasma was markedly increased by a 1 hr treatment of saikogenin A in a dose-dependent manner. Similarly, the amounts of cyclic AMP in isolated pituitary and adrenal glands were also increased although no changes can be detected in hypothalamus (Table 2). The results suggest that saikogenin A activated the cyclic AMP formation system resulting in the endogenous release of ACTH.

DISCUSSION

In this study, we found that saikogenin A possesses a similar anti-inflammatory property as its parent plant [4]. However, saikogenin A produced different effects on carrageenin-induced edema in the adrenalectomized rats and normal rats (Fig. 2). Because corticosterone has had an effective anti-inflammatory action in the rats [12], stimulation of the hypothalamopituitary–adrenal system may be responsible for the anti-inflammatory effect of saikogenin A. This view is further supported by the finding that plasma ACTH concentration was markedly increased with the treatment of saikogenin A (Table 2). It is generally recognized that endogenous ACTH is released from pituitary gland [13] and that circulating corticosterone plays a major control on the level of ACTH secretion [14]. Although the factors influencing the release of ACTH are still unknown [13], the role of intracellular cyclic AMP as second messenger has been suggested [15]. In the present study, we found that amounts of cyclic AMP in pituitary gland, but not in hypothalamus, were increased with the treatment of saikogenin A (Table 2). Thus, saikogenin A may induce the formation of cyclic AMP resulting in the secretion of ACTH. Interference in the feedback inhibition of corticosterone resulting in the release of ACTH by Tsai-Fu, the parent plant of saikogenin has been mentioned [5]. Regulation of the secretion of ACTH can be modulated by endogenous peptides such as vasopressin and oxytocin, as well as neurotransmitters like catecholamines and serotonin [16]. However, mechanism of the effect of saikogenin A on cyclic AMP required a further study.

Corticosterone released from adrenal gland by the perfusion with synthetic ACTH was markedly facilitated by saikogenin A whereas the spontaneous release was not affected (Table 1). The intracellular cyclic AMP concentration of adrenal gland was also increased in the saikogenin A-treated samples (Table 2). These results suggest that saikogenin A increased the intra-cellular cyclic AMP contents to induce the secretion of ACTH and the release of corticosterone.

Table 2. Effect of saikogenin A on the content of ACTH and cyclic AMP

	Control	Saikogenin A-treated	
		50 mg/kg	80 mg/kg
Plasma ACTH (pg/ml)	76.4 ± 19.8	336.9 ± 24.7*	527.4 ± 21.6*
Cyclic AMP content (nmol/g wet tissue)			
Hypothalamus	6.57 ± 0.35	6.74 ± 0.69	6.81 ± 0.56
Pituitary	1.22 ± 0.16	3.96 ± 0.58*	5.78 ± 0.43*
Adrenal gland	2.46 ± 0.52	9.25 ± 1.73*	14.33 ± 1.95*

Animals were decaptitated following an 1 hr treatment of saikogenin A. Each value represents the mean ± S.E. of six studies and * means a significant difference ($P < 0.01$) from the value of control which was taken from the vehicle-treated samples.

The anti-inflammatory effect of saikogenin A can thus be considered to be dependent on the secretion of endogenous corticosterone [12].

In addition, the effect of saikogenin A was not completely lost in the adrenalectomized rats and the release of histamine from mast cells *in vitro* was markedly reduced by saikogenin A (Fig. 4). The direct effect of saikogenin A on the inflammatory process may be one factor responsible for the anti-inflammatory action.

From these results, it is suggested that the main mechanism of the anti-inflammatory effect of saikogenin A could be due to a release of ACTH in the secretion of endogenous corticosterone. Other mechanisms, such as reduction of histamine release, might act simultaneously.

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