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Studies on Scutellariae Radix. VII.[1] Anti-arthritic and Anti-inflammatory Actions of Methanolic Extract and Flavonoid Components from Scutellariae Radix

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A 70% methanol extract of Scutellariae Radix, the dried root of *Scutellaria baicalensis* GEORGI, and its main flavonoid components, baicalin, baicalein and wogonin, have been screened in comparison with three standard anti-inflammatory agents, phenylbutazone, indomethacin and dexamethasone, for activity in various experimental models of inflammation. All of the test substances were found to inhibit an increase in vascular permeability in mice induced by acetic acid and to reduce acute paw edema in rats induced by compound 48/80. They also suppressed the secondary lesion in developing adjuvant-induced arthritis in rats. Since these substances from Scutellariae Radix were found to be effective in both the acute and chronic phases of inflammation, the crude drug Scutellariae Radix can be considered as having anti-inflammatory activity.

Keywords——*Scutellaria baicalensis*; flavonoid; baicalein; baicalin; wogonin; inflammation; vascular permeability; compound 48/80; adjuvant-induced arthritis

Scutellariae Radix ("Ogon" in Japanese), the dried root of *Scutellaria baicalensis* GEORGI, has been used for treatment of some types of dermatitis, diarrhea and various inflammatory diseases as an antiphologistic and antipyretic in the traditional Chinese system of medicine. In our series of studies on Scutellariae Radix, we reported that the methanolic extract and its flavonoid components exhibited beneficial effects on lipid metabolism and lipid peroxidation of rat liver, ethanol-induced hyperlipemia in the rat and lipolysis in isolated fat cells of the rat.[2–6]

The present paper deals with a study on the anti-inflammatory action of 70% methanol extract, baicalein, baicalin and wogonin obtained from Scutellariae Radix.

Materials and Methods

Materials——The 70% methanol extract, baicalein, baicalin and wogonin used in this study were the same preparations as reported previously.[5]

Indomethacin, dexamethasone, phenylbutazone and compound 48/80 were purchased from Sigma Chemical Co. Carrageenin was obtained from Minsei Rikagaku Co. Dry heat-killed *Mycobacterium butyricum*, used as an adjuvant, was obtained from Difco Lab.

Animals——The adjuvant arthritis test was carried out using female Sprague-Dawley rats. Male Wistar-King rats were used in all other tests. Male ddY mice were used in the vascular permeability test.

The animals were housed for 7 d before the start of the experiment with free access to food and water.

Acetic Acid-Induced Vascular Permeability in Mice——The method is based on that of Whittle.[7] Groups of

fifteen mice (20—25 g) were dosed orally with the test substances 1 h before the intravenous injection of 4% pontamine sky blue (1 ml/kg). Fifteen min after the injection of the dye, 1% acetic acid (1 ml/kg) was injected intraperitoneally. After 20 min the mice were killed by dislocation of the neck and the viscera were exposed, after a 1 min period to allow blood to drain away from the abdominal wall. The animal was held by a flap of the abdominal wall and the viscera were irrigated with 10 ml of saline over a Petri dish. The washing was filtered through glass wool and transferred to a test tube. To each tube was added 0.1 ml of 0.1 N NaOH in order to clear any turbidity due to protein, and the absorbance was read at 590 nm with a Hitachi 200-10 spectrophotometer. Control animals were treated similarly except that they received an oral dose of the vehicle alone. The vascular permeability effects were expressed in terms of total dye amount which leaked into the intraperitoneal cavity.

Carrageenin-Induced Edema in Rats——The initial hind paw volume of the rats was determined volumetrically. A 1% suspension of carrageenin in saline (0.1 ml/rat) was injected subcutaneously into the right hind paw 1 h after the test substances had been administered orally. The control group ($n=10$) received the suspension vehicle. Paw volumes were measured up to 5 h at intervals of 1 h, and the volume of edema was determined. The results were expressed as percentage hind paw swelling, as compared with the initial hind paw volume.

Compound 48/80-Induced Edema in Rats——The initial hind paw volume of the rats was determined volumetrically. A 0.01% compound 48/80 solution (0.1 ml/rat) was injected subcutaneously into the right hind paw 1 h after the test substances had been administered orally. At 30 min after injection of compound 48/80 solution the paw volumes were mesured. Controls ($n=7$) received the suspension vehicle. The results were expressed as percentage hind paw swelling, as compared with the initial hind paw volume.

Cotton Pellet-Induced Granuloma in Rats——The method was based on that of Hicks.[8] Two cotton pellets (50 ± 3 mg, mean $\pm$ s.e.) were implanted subcutaneously. The test substances were administered in seven daily oral doses starting on the day of implantation. Controls ($n=10$) received the suspension vehicle and positive controls were treated with dexamethasone (0.5 mg/kg, *i.p.*). The rats were killed 7 d after implantation and the pellets were freed from extraneous tissue.

Adjuvant-Induced Arthritis in Rats——Arthritis was induced in rats in groups of seven by intradermal injection into the tail and right hind paw of a suspension of 0.05 mg of dry heat-killed *Mycobacterium butyricum* in 1 ml of Bayol F.

The hind paw volume of each injected rat was measured initially and then every 2 or 3 d thereafter, and the progress of the inflammation was plotted graphically. The test substances were administered in 27 daily oral doses starting on the day of injection of the adjuvant. The results were expressed as percentage hind paw swelling, as compared with that of non-injected rats.

The arthritic symptoms were also scored in the following four grades on the basis of the degree of degeneration or destruction of bone: score 3, severe; score 2, moderate; score 1, slight; score 0, not detectable. The observation of bone was performed with a SOFTEX CMB-2 microscope.

Results

Acetic Acid-Induced Vascular Permeability in Mice

Total dye amount which leaked into the peritoneal cavity was 123 ± 10 μg (mean $\pm$ s.e.)/mouse (20 g) in the vehicle control group. When 70% methanol extract (500 mg/kg), baicalein, baicalin (50 or 100 mg/kg, respectively) and wogonin (100 mg/kg) were administered orally to mice, the dye leakages were reduced significantly in a dose-dependent manner, as shown in Table I.

Carrageenin-Induced Edema in Rats

Indomethacin (10 mg/kg) caused strong inhibition of edema when given orally during 1 to 5 h after injection of carrageenin, but the test substances (except wogonin, which was ineffective) inhibited the increase of edema only at 1 h (Fig. 1).

Compound 48/80-Induced Edema in Rats

The increase of edema was inhibited significantly in rats orally given 100 mg/kg of baicalein, baicalin or wogonin. The inhibitory activity of indomethacin was greater than those of baicalein, baicalin and wogonin (Table II).

Cotton Pellet-Induced Granuloma in Rats

Dexamethasone (0.5 mg/kg, *i.p.*) was effective in this test, but the test substances were ineffective at the oral doses specified in Table III.

Table I. Effects of Components of Scutellariae Radix (70% Methanol Extract, Baicalein, Baicalin and Wogonin) on Vascular Permeability Induced by Acetic Acid in Mice

Treatment	Dose (mg/kg, *p.o.*)	No. of mice	Amount of P.S.B.[a] (μg/20 g, body weight)
Control		15	123±10
70% methanol extract	200	15	97±12
70% methanol extract	500	15	58±10[c]
Baicalein	50	15	90±10[b]
Baicalein	100	15	59±8[c]
Baicalin	50	15	87±7[b]
Baicalin	100	15	59±8[c]
Wogonin	50	15	103±12
Wogonin	100	15	76±9[c]
Indomethacin	10	15	88±11[b]

a) 4% pontamine sky blue. Each value represents the mean±S.E.
b) Significantly different from control, $p<0.05$.
c) Significantly different from control, $p<0.01$.

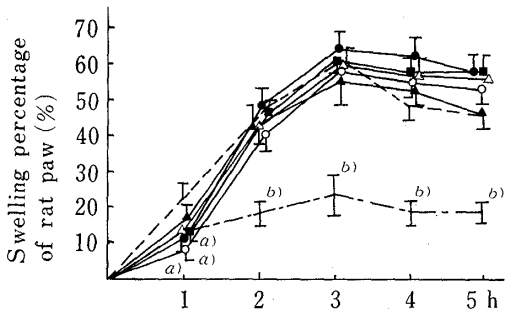

Fig. 1. Effects of Components of Scutellariae Radix (70% Methanol Extract, Baicalin, Baicalin and Wogonin) on Carrageenin Edema in Rats

----, control; △, 70% methanol extract 200 mg/kg; ○, 70% methanol extract 500 mg/kg; ■, baicalein 100 mg/kg; ●, baicalin 100 mg/kg; ▲, wogonin 100 mg/kg; —·—, indomethacin 10 mg/kg. Each value represents the mean±S.E. of 10 rats. *a*) Significantly different from control, $p<0.05$. *b*) Significantly different from control, $p<0.01$.

Table II. Effects of Components of Baicalein, Baicalin and Wogonin on Compound 48/80 Edema in Rats

Treatment	Dose (mg/kg)	Route	No. of rats	Swelling percentage (%)[a]
Control			7	48.0±3.3
Baicalein	50	*p.o.*	7	47.0±3.6
Baicalein	100	*p.o.*	7	41.1±2.5[b]
Baicalin	50	*p.o.*	7	43.0±4.0
Baicalin	100	*p.o.*	7	40.6±1.8[b]
Wogonin	50	*p.o.*	7	47.0±3.4
Wogonin	100	*p.o.*	7	38.1±5.0[b]
Indomethacin	10	*p.o.*	7	34.9±3.2[c]

a) Each value represents the mean±S.E.
b) Significantly different from control, $p<0.05$.
c) Significantly different from control, $p<0.01$.

Adjuvant-Induced Arthritis in Rats

The 70% methanol extract given orally (100 or 200 mg/kg) had no effect on adjuvant-induced arthritis during 1 to 9 d after injection of the adjuvant, but during 11 to 27 d it inhibited the development of edema. Baicalein, baicalin and wogonin given orally (each 100 mg/kg) also showed inhibitory effects on the edema during 11 to 27 d after injection of

TABLE III. Effects of Components of Scutellariae Radix (70% Methanol Extract, Baicalein, Baicalin and Wogonin) on Cotton Pellet-Induced Granuloma in Rats

Treatment	Dose (mg/kg)	Route	No. of rats	Wet granuloma (mg/100 g, b.w.)	Dry granuloma[a] (mg/100 g, b.w.)
Control			10	830±45	162±5
70% methanol extract	500	*p.o.*	10	825±42	158±3
Baicalein	100	*p.o.*	10	854±23	162±7
Baicalin	100	*p.o.*	10	783±43	154±6
Wogonin	100	*p.o.*	10	857±37	163±4
Dexamethasone	0.5	*i.p.*	10	600±32[b]	135±3[b]

a) Each value represents the mean±S.E.
b) Significantly different from control, $p<0.01$.

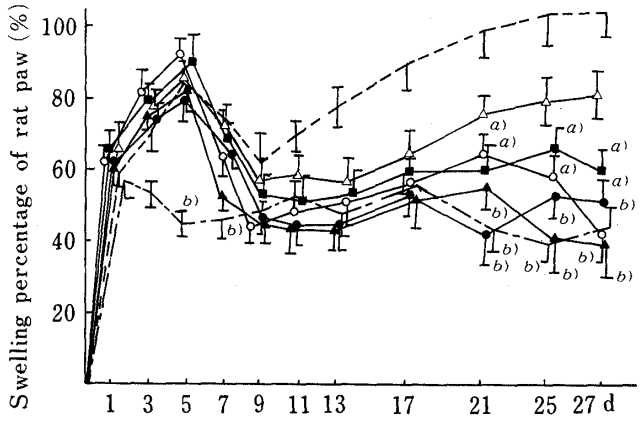

Fig. 2. Effects of Components of Scutellariae Radix (70% Methanol Extract, Baicalein, Baicalin and Wogonin) on Adjuvant-Induced Arthritis in Rats

----, control; △, 70% methanol extract 200 mg/kg; ○, 70% methanol extract 500 mg/kg; ■, baicalein 100 mg/kg; ●, baicalin 100 mg/kg; ▲, wogonin 100 mg/kg; —·—, phenylbutazone 50 mg/kg, Each value represents the mean±S.E. of 10 rats *a*) Significantly different from control, $p<0.05$. *b*) Significantly different from control, $p<0.01$.

TABLE IV. Effects of Components of Scutellariae Radix (70% Methanol Extract, Baicalein, Baicalin and Wogonin) on the Degeneration or Destruction of Bone in Adjuvant-Induced Arthritic Rats

Treatment	Dose (mg/kg)	Route	No. of rats	Arthritic score[a] and mean±S.E.									
				Right hind paw					Left hind paw				
				3	2	1	0		3	2	1	0	
Control			10	5	2	2	1[b]	2.1±0.3	1	4	3	2	1.4±0.3
70% methanol extract	200	*p.o.*	10	3	2	5	2	1.7±0.4	1	2	3	4	1.0±0.3
70% methanol extract	500	*p.o.*	10	1	2	4	2	1.2±0.3[c]	0	2	2	6	0.6±0.3[c]
Baicalein	100	*p.o.*	10	1	1	6	3	1.1±0.2[c]	0	1	3	6	0.5±0.4[c]
Baicalin	100	*p.o.*	10	1	3	4	2	1.0±0.3[c]	0	0	4	6	0.4±0.2[c]
Wogonin	100	*p.o.*	10	0	2	1	3	1.0±0.3[c]	0	0	3	5	0.4±0.2[c]
Phenylbutazone	50	*p.o.*	10	0	1	2	7	0.5±0.3[d]	0	0	2	8	0.2±0.1[d]

a) Arthritic score: 3, severe degeneration and destruction of bone; 2, moderate; 1, slight; 0, not detectable.
b) Number of rats.
c) Significantly different from control, $p<0.05$.
d) Significantly different from control, $p<0.01$.

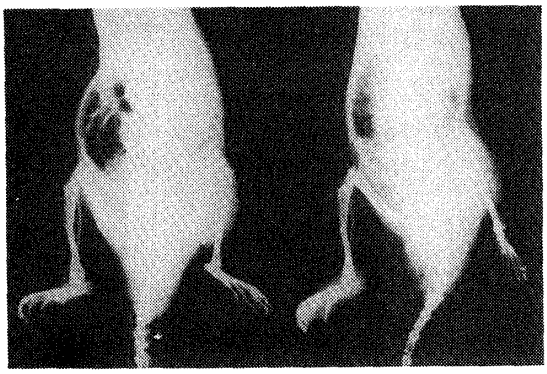

Fig. 3. Adjuvant-Induced Arthritic Rats

Adjuvant-induced degeneration and destruction of bone in rat hind paw.

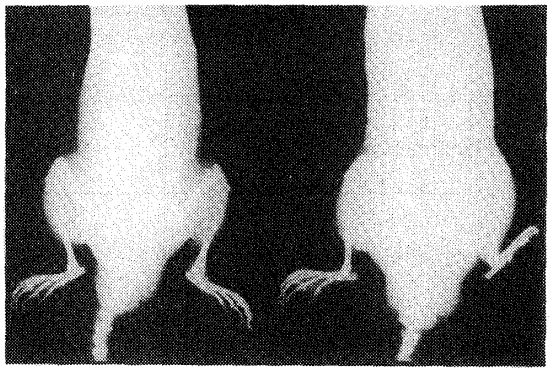

Fig. 4. 70% Methanol Extract-Treated Rats

70% methanol extract inhibited the degeneration and destruction of bone in rat hind paw as compared with control rats.

adjuvant. Phenylbutazone was more effective than the test substances (Fig. 2). X-Ray pictures (Figs. 3, 4) show the state of degeneration or destruction of bone in the control and 70% methanol extract-treated rats at 27 d after injection of adjuvant. From the arthritic scores given in Table IV, it can be seen that the test substances exhibited beneficial effects, but were less effective than phenylbutazone in suppressing the development of degeneration or destruction of bone caused by the injection of adjuvant.

Discussion

The present investigation has demonstrated anti-arthritic and anti-inflammatory properties of constituents of Scutellariae Radix, namely the 70% methanol extract, baicalein, baicalin and wogonin (components with multiple pharmacological actions).

These three flavonoids showed an inhibitory effect on the increased vascular permeability induced by acetic acid in mice and inhibited the increase of compound 48/80-induced edema in rats, though at rather high doses compared to the effective dose of indomethacin. Baicalein and baicalin inhibited the increase of carrageenin edema only when given 1 h after injection of carrageenin, but wogonin did not inhibit the edema at any time tested (Fig. 1).

Koda[9] reported that baicalein and baicalin inhibit the release of chemical mediator from mast cells. It seems likely that the inhibitory effects of these flavonoids on the increased vascular permeability and the compound 48/80-induced edema, namely the first phase of inflammation, are attributable to the inhibition of release of chemical mediators such as histamine or serotonin.[10]

On the other hand, these flavonoids had no effect on cotton pellet-induced granuloma (relevant to the third phase of inflammation), though they suppressed the secondary lesion in developing adjuvant-induced arthritis (the arthritis reappeared during 11 to 27 d after injection of the adjuvant).

In the adjuvant-induced arthritis, the secondary lesion is related to the formation of antibody or activation of complement and may involve type III or IV allergic reaction.[11,12] The anti-inflammatory effect of these flavonoids against the adjuvant-induced arthritis is considered to be attributable to inhibiton of delayed-type allergic reaction or activation of complement. It appears that the anti-arthritic and anti-inflammatory actions of 70% methanol extract are largely due to the actions of the main components, baicalein, baicalin and wogonin.

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