Anti-inflammatory Activities of Methanolic Extract and Alkaloidal Components from *Corydalis Tuber*

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A methanolic extract (CM-ext) from *Corydalis tuber* (*Corydalis turtschaninovii* BESSER forma *yanhusuo* Y. H. CHOU et C. C. HSU) has been screened for activity in experimental models of inflammation. CM-ext (200 or 500 mg/kg, *p.o.*) inhibited an increase in vascular permeability in mice induced by acetic acid, and reduced acute paw edema in rats induced by compound 48/80 or carrageenin. CM-ext suppressed the development of adjuvant-induced edema in arthritic rats. CM-ext and its alkaloidal components, dehydrocorydaline, *d*-glaucine and *l*-tetrahydrocorydaline inhibited compound 48/80-induced histamine release from peritoneal mast cells of rats. Since these substances from *C. tuber* were found to be effective in both the acute and chronic phases of inflammation, the crude drug *C. tuber* can be considered to exert anti-inflammatory activity.

Keywords *Corydalis turtschaninovii* forma *yanhusuo*; alkaloid; inflammation; dehydrocorydaline; *d*-glaucine; *l*-tetrahydrocorydaline

In a series of pharmacological studies on *Corydalis tuber* (Yan-hu-suo in Chinese, *Corydalis turtschaninovii* BESSER forma *yanhusuo* Y. H. CHOU et C. C. HSU),¹⁻⁴⁾ we reported that the methanolic extract (CM-ext) or its alkaloidal component, protopine, exhibited an inhibitory effect on *in vitro* and *in vivo* platelet aggregation. In China, *C. tuber* has been used for the treatment of various inflammatory diseases in the traditional Chinese system of medicine. The present paper deals with a study of the anti-inflammatory action of CM-ext and the alkaloidal components obtained from *C. tuber*.

MATERIALS AND METHODS

Materials CM-ext (yield: 2.95%) and seven alkaloidal components were prepared from *C. tuber* by the method of Kaneko and Naruto.⁵⁾ The structures of these alkaloidal

components are shown in Fig. 1. The following drugs were also used in this study: λ -carrageenin (Minsei Rikagaku Co.), compound 48/80, indomethacin, prednisolone (Sigma Chemical Co.), dry heat-killed *Mycobacterium butyricum* (Difco Lab.), *o*-phthaldialdehyde (Nakalai Tesque), disodium cromoglycate (DSCG, Funakoshi), diphenhydramine·HCl, cortisone, pontamine sky blue (Tokyo Kasei), histamine·HCl (Kishida Chemical).

Animals Male Slc, Wistar strain rats (180–200 g); female Jcl, Sprague-Dawley strain rats (180–200 g); male Kwl, ddY strain mice (18–22 g) and male Slc, Hartley strain guinea pigs (250–300 g) were used. They were maintained in an air-conditioned room with lighting from 7 a.m. to 7 p.m. The room temperature (about 23 °C) and humidity (about 60%) were controlled automatically. A laboratory pellet chow (CLEA Japan Inc.) and water were given freely.

Acetic Acid-Induced Vascular Permeability in Mice The method was based on that of Whittle.⁶⁾ The ddY strain mice were dosed orally with the test substances suspended in a 0.5% sodium carboxymethylcellulose (CMC·Na) solution for 1 h before the intravenous injection of 4% pontamine sky blue (10 ml/kg). Fifteen min after the injection of the dye, 1% acetic acid (10 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. Each 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 washed matter was filtered through glass wool and transferred to a test tube. To each tube was added 0.1 ml of 1 N NaOH in order to clear any turbidity due to protein, and the absorbance was read at 590 nm with a Shimadzu model UV-160 spectrophotometer. Control animals were treated similarly, except that they received an oral dose of the vehicle alone. Vascular permeability was expressed in terms of absorbance value per 20 g weight of mouse which leaked into the intraperitoneal cavity. Indomethacin was used as a standard

Fig. 1. Structures of Alkaloidal Components Obtained from a CM-ext of *C. Tuber*

drug.

Carrageenin-Induced Edema in Rats This method was based on that of Nakamura *et al.*⁷⁾ The initial hind paw volume of the Wistar strain rats was determined volumetrically. A 1% solution 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 received the vehicle. Paw volumes were measured up to 3 h at intervals of 15 or 60 min, and the volume of edema was determined. The results were expressed as the percentage of hind paw swelling, as compared with the initial hind paw volume. Indomethacin was used as a standard drug.

Compound 48/80-Induced Edema in Rats The initial hind paw volume of Wistar strain rats was determined volumetrically. A 0.01% solution of compound 48/80 in saline (0.1 ml/rat) was injected subcutaneously into the right hind paw 1 h after the test substances had been administered orally. Paw volumes were measured up to 30 min at intervals of 15 min, and the volume of edema was determined. Controls received the vehicle. The results are expressed as the percentage hind paw swelling, as compared with the initial hind paw volume. Indomethacin was used as a standard drug.

Measurement of Histamine Release from Mast Cells Induced by Compound 48/80 Mast cells were prepared from the peritoneal cavity fluid of Wistar strain rats by a slight modification of the method described by Uvnäs and Thon.⁸⁾ The cells were suspended in Hanks' solution containing heparin (10 U/ml), then layered on 30 and 40% Ficoll in a test tube for 30 min. After centrifugation at $150 \times g$ and 4°C for 10 min, the layer containing mast cells was pipetted out. The cells were washed three times with 5 ml of phosphate-buffered saline (pH 7.0) and suspended in the same medium at 2.9×10^6 cells/ml. The cell suspensions contained 85–90% or more viable mast cells, as determined by the toluidine blue (0.1% in 50% ethanol) staining test of Bray and Van Arsdel.⁹⁾

The test substances solved with 5% dimethyl sulfoxide were added to the mast cell suspension, then the mixture was incubated at 37°C . After 10 min, 0.1 ml of compound 48/80 solution (0.2 mg/ml) was added, and the mixture was incubated at 37°C for 10 min in a final volume of 2 ml. The reaction was terminated by cooling the mixture on ice. The mixture was centrifuged at $150 \times g$ and 5°C for 5 min, then histamine in the supernatant fluid was assayed fluorometrically according to the method of Shore *et al.*¹⁰⁾ The activity of the test substance on histamine release from mast cells induced by compound 48/80 was expressed as the inhibitory percentage. DSCG was used as a standard drug.

Measurement of Histamine-Induced Contraction in the Isolated Guinea Pig Ileum Each guinea pig was sacrificed by means of a blow on the head and the ileum was isolated. A length of 1.5 to 2.0 cm was suspended in Tyrode's solution bubbled through with air in an organ bath maintained at 37°C . Ileum contractions were isotonicity recorded by means of a lever loaded with 0.5 g on a smoke drum. The ileum was preincubated with CM-ext (10^{-4} g/ml, solved 5% dimethyl sulfoxide/Tyrode's solution) or diphenhydramine·HCl (10^{-6} mmol/ml, solved Tyrode's

solution) for 3 min, and then histamine (10^{-9} – 10^{-4} mmol/ml) was added and incubation was carried out for 3 min at 37°C . The extent of contraction was estimated from the maximal contraction of the isolated ileum after the addition of histamine (10^{-4} mmol/ml) alone.

Adjuvant-Induced Arthritis in Rats The method was based on that of Nakamura and Shimizu.¹¹⁾ Arthritis was induced by intradermal injection of a 0.05 ml suspension of dry heat-killed *Mycobacterium butyricum* (10 mg) in Bayol F (1 ml) as an adjuvant agent into the tail and right hind paw of SD strain rats. The right hind paw volume of injected adjuvant agent was measured initially, and then every 1–3 d thereafter for 21 d, and the volume of edema was determined. The results were expressed as the percentage hind paw swelling, as compared with the initial hind paw volume.

Statistical Analysis The experimental data were tested for statistically significant differences by means of the Williams' Multiple Range test.

RESULTS

Acetic Acid-Induced Vascular Permeability The total dye amount which leaked into the peritoneal cavity was 0.78 ± 0.06 OD_{590nm}/20 g body weight in the vehicle control group. When CM-ext (500 mg/kg) was administered to mice, the dye leakage was reduced, as shown in Fig. 2. A standard drug, indomethacin 10 mg/kg, also reduced the leakage.

Carrageenin-Induced Edema CM-ext (500 mg/kg) had a significant inhibitory effect on the edema 2 or 3 h after the injection of carrageenin, as shown in Fig. 3. Standard drug, indomethacin (10 mg/kg), showed more potent inhibition than that of CM-ext.

Compound 48/80-Induced Edema The percentage of swelling was significantly decreased by the administration of CM-ext (200 or 500 mg/kg), as shown in Fig. 4. The standard drug, indomethacin (10 mg/kg), significantly inhibited the swelling.

Histamine Release from Mast Cells Induced by Compound 48/80 As shown in Fig. 5, the control value for compound 48/80-induced histamine release was

Fig. 2. Effects of CM-ext from *C. Tuber* and Indomethacin on Vascular Permeability Induced by Acetic Acid in Mice

CM-ext or indomethacin suspended with 0.5% CMC·Na was orally administered 1 h before the intravenous injection of 4% pontamine sky blue. Fifteen min after the injection of the dye, 1% acetic acid was injected intraperitoneally. After 20 min the mice were killed, and vascular permeability was expressed in terms of OD_{590nm} as the amount of dye which leaked into the intraperitoneal cavity. Each value represents the mean $\pm$ S.E. Significantly different from control group, a) $p < 0.05$.

TABLE I. Effects of Alkaloidal Components of a CM-ext from *C. Tuber* and DSCG on Compound 48/80-Induced Histamine Release from Peritoneal Mast Cells of Rat

Treatment	Concentration (mM)	Histamine release (%)	Inhibitory (%)	Treatment	Concentration (mM)	Histamine release (%)	Inhibitory (%)
Control	—	78.3 ± 0.3		Control	—	78.3 ± 0.3	
<i>d</i> -Corydaline	0.1	71.9 ± 0.5 ^b	8.2	<i>dl</i> -Tetrahydropalmatine	0.1	80.1 ± 0.3	−2.3
	0.25	73.7 ± 0.8 ^b	5.9		0.25	80.3 ± 0.2	−2.6
	0.5	73.8 ± 0.6 ^b	5.7		0.5	75.7 ± 0.7 ^b	3.3
Control	—	80.3 ± 0.1		Control	—	78.2 ± 0.5	
<i>d</i> -Glaucine	0.1	77.2 ± 0.4 ^b	3.9	<i>l</i> -Tetrahydrocolumbamine	0.1	78.8 ± 0.8	−0.8
	0.25	72.0 ± 0.4 ^b	10.3		0.25	77.4 ± 0.2	1.0
	0.5	68.1 ± 0.5 ^b	15.2		0.5	77.3 ± 0.1	1.2
Control	—	79.8 ± 0.4		Control	—	78.2 ± 0.5	
Protopine	0.1	78.5 ± 0.4 ^b	1.6	Dehydrocorydaline	0.1	63.8 ± 0.3 ^b	18.4
	0.25	78.5 ± 0.2 ^b	1.6		0.25	57.2 ± 0.4 ^b	26.9
	0.5	78.8 ± 1.0	1.3		0.5	37.7 ± 0.5 ^b	51.8
Control	—	80.3 ± 0.1		Control	—	83.3 ± 0.3	
<i>l</i> -Tetrahydrocoptisine	0.1	77.7 ± 0.4 ^b	3.2	DSCG	1.0	52.9 ± 2.2 ^b	37.2
	0.25	75.7 ± 0.7 ^b	5.7				
	0.5	71.8 ± 0.4 ^b	10.6				

Mast cells prepared from the peritoneal cavity fluid of rats were suspended in phosphate-buffered saline at 2.9×10^6 cells/ml. The mast cell suspension, treated with alkaloidal components isolated from CM-ext for 5 min, were stimulated by compound 48/80 (10 μ g/ml) for 10 min at 37°C. Each value represents the mean $\pm$ S.E. of 5 experiments. Significantly different from the control group, a) $p < 0.05$, b) $p < 0.01$.

Fig. 3. Effects of CM-ext from *C. Tuber* and Indomethacin on Carageenin-Induced Acute Edema in Rats

○, control; ■, CM-ext 50 mg/kg; ▲, CM-ext 200 mg/kg; ●, CM-ext 500 mg/kg; □, indomethacin 10 mg/kg. CM-ext or indomethacin suspended with 0.5% CMC·Na was orally administered 1 h before the subcutaneous injection of 1% λ -carageenin. The foot paw swelling percentage was measured. Each value represents the mean $\pm$ S.E. of 5–7 rats. Significantly different from the control group, a) $p < 0.05$, b) $p < 0.01$.

Fig. 4. Effects of CM-ext from *C. Tuber* and Indomethacin on Compound 48/80-Induced Acute Edema in Rats

○, control; ■, CM-ext 50 mg/kg; ▲, CM-ext 200 mg/kg; ●, CM-ext 500 mg/kg; □, indomethacin 10 mg/kg. CM-ext or indomethacin suspended with 0.5% CMC·Na was orally administered 1 h before the subcutaneous injection of a 0.01% compound 48/80. The foot paw swelling percentage was measured. Each value represents the mean $\pm$ S.E. of 5–7 rats. Significantly different from the control group, a) $p < 0.05$, b) $p < 0.01$.

83.3 $\pm$ 0.3%. Treatment with CM-ext (50, 100 or 250 μ g/ml) significantly suppressed the histamine release. Among the alkaloidal components obtained from CM-ext, dehydrocorydaline showed a stronger inhibitory effect than that of DSCG, but that of *d*-glaucine and *l*-tetrahydrocoptisine were weak (Table I).

Histamine-Induced Contraction in the Isolated Guinea Pig Ileum In the experiment shown in Fig. 6, histamine by itself caused the contraction of isolated guinea pig ileum with an S-shaped log (dose)–response curve at concentrations of 10^{-9} – 10^{-4} mmol/ml. In the presence of CM-ext (10^{-4} g/ml) or diphenhydramine (10^{-6} mmol/ml) the dose–response curve was unaltered in shape but was shifted to the right.

Adjuvant-Induced Arthritis As shown in Fig. 7, CM-ext (500 mg/kg) had an inhibitory effect on the development of edema in adjuvant-induced arthritis in rats. The

Fig. 5. Effects of CM-ext from *C. Tuber* and DSCG on Compound 48/80-Induced Histamine Release from Peritoneal Mast Cells of Rat

Mast cells prepared from the peritoneal cavity fluid of rats were suspended in phosphate-buffered saline at 2.9×10^6 cells/ml. The mast cell-suspension treated with CM-ext for 5 min was stimulated by compound 48/80 (10 μ g/ml) for 10 min at 37°C. Each value represents the mean $\pm$ S.E. of 5 experiments. Significantly different from the control group, a) $p < 0.01$.

Fig. 6. Effects of CM-ext from *C. Tuber* and Diphenhydramine on the Concentration-Action Curves of Phasic Contraction by Histamine with the Isolated Ileum of Guinea Pig

○, histamine (His); ●, CM-ext 10^{-4} g/ml + His; □, diphenhydramine 10^{-6} mmol/ml + His. Each value represents the mean of 3 experiments.

Fig. 7. Effects of CM-ext from *C. Tuber* and Prednisolone on Right Hind Paw Edema of Adjuvant-Induced Arthritis in Rats

○, control; ■, CM-ext 50 mg/kg; ▲, CM-ext 200 mg/kg; ●, CM-ext 500 mg/kg; □, prednisolone 10 mg/kg. Arthritis induced by the intradermal injection of a suspension (0.05 ml) of dry heat-killed *Mycobacterium butyricum* 10 mg in Bayol F 1 ml as an adjuvant agent was measured. CM-ext or prednisolone suspended with 0.5% CMC-Na was orally administered once a day for 21 d immediately after the injection of the adjuvant agent. Each value represents the mean $\pm$ S.E. of 15–17 rats. Significantly different from the control group, a) $p < 0.05$, b) $p < 0.01$.

standard drug, prednisolone (10 mg/kg), showed a strong inhibition of the edema.

DISCUSSION

Corydalis tuber has been used for the treatment of various inflammatory diseases in the traditional Chinese system of medicine. At this time, the effect of CM-ext from *Corydalis tuber* on various inflammatory experimental models were investigated. CM-ext significantly inhibited compound 48/80- and carrageenin-induced edema. It is generally known that compound 48/80 induces histamine release from mast cells without the participation of an immunological mechanism as caused by an antigen-antibody reaction.^{12,13} CM-ext showed an inhibitory effect against histamine release from mast cells and on histamine-induced contractions in the isolated guinea pig ileum. Accordingly, it was suggested that CM-ext not only inhibited histamine release from mast cells, but also had

an inhibitory effect on the released histamine. It is said that carrageenin-induced edema is suppressed by the inhibition of histamine release and an anti-histamine effect, and by the inhibition of prostaglandins (PGs) production in the second step of edema. The inhibitory effect of CM-ext on PGs production of CM-ext was not examined, but the inhibitory effect may be expected, since an inhibitory effect of CM-ext in platelets was observed in our previous investigations.^{2,3} Indomethacin has a strong inhibitory effect against the increase of vascular permeability induced by acetic acid, so it is anticipated that CM-ext has also an inhibitory effect on PGs production.

It has been considered that adjuvant-induced arthritis is closely related to either the formation of antibodies or the activation of a complement, and may involve type III or IV allergic reactions.¹⁴ In this study, CM-ext suppressed the development of adjuvant-induced edema in arthritic rats. Thus, it seems that CM-ext interacts with type III or IV allergic reactions. A study is now in progress on the effects of CM-ext against various types of allergic reactions. In order to clarify the active component of CM-ext, the alkaloidal components obtained from CM-ext following histamine release induced by compound 48/80 from mast cells were estimated. Among the tested alkaloidal components, dehydrocorydaline showed a stronger inhibitory effect than that of the standard drug, DSCG, but the effects of *d*-glucine and *l*-tetrahydrocoptisine were weak. It is anticipated that the inhibitory effects of CM-ext on acute inflammatory models are due to these three alkaloids with dehydrocorydaline.

The present study clearly demonstrates that CM-ext has an inhibitory effect on either acute or chronic inflammation. This is interesting in light of the fact that *C. tuber* has been employed for the treatment of various inflammatory diseases in the traditional Chinese system of medicine. It suggests that these findings have potentially important implications for the therapeutic use of *C. tuber* in inflammatory diseases.

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