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Anti-Inflammatory Activities of Dehydrocorydaline Isolated from Corydalis Tuber

HIDEAKI MATSUDA,* KIYOSHI TOKUOKA, JIANXIN WU, HIDEMI SHIOMOTO and MICHINORI KUBO

Faculty of Pharmaceutical Sciences, Kinki University, 3-4-1, Kowakae, Higashiosaka, Osaka 577, Japan

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The inhibitory effects of dehydrocorydaline (DHC), a major component from Corydalis Tuber (tuber of *Corydalis turtschaninovii* forma *yanhusuo*, Papaveraceae), on acute inflammatory models were investigated. Oral administration of DHC at 0.125 mmol/kg inhibited the increases in the acetic acid-induced vascular permeability and the increase in the carrageenin-induced paw edema in mice. It exhibited an inhibitory effect on the edema derived by serotonin or bradykinin but no effect on the edema derived by histamine. It also showed inhibitory effects on the increase of ear edema induced by arachidonic acid (AA) and its metabolites, mixture of prostaglandin E_2 (PGE_2) and leukotriene C_4 (LTC_4). These results suggested that DHC might be considered to be a potent anti-inflammatory component in Corydalis Tuber, although its mechanisms have not yet been fully understood.

Keywords——*Corydalis turtschaninovii* forma *yanhusuo*; Papaveraceae; dehydrocorydaline; anti-inflammatory effect; alkaloid

Corydalis Tuber has been used as an analgesic mainly for treatment of stomachache in the traditional Chinese system of medicine. According to the ancient Chinese medicinal and herbal literature, it is also mentioned as being effective for treatment of inflammatory, hemorheological and allergic diseases. In a series of pharmacological studies of Corydalis Tuber, we reported that its methanol extract exhibited inhibitory effects on blood platelet aggregation and on allergic and inflammatory model animals.[1-6]

In order to identify the active component of Corydalis Tuber, the present paper deals with the anti-inflammatory effect of its major component, dehydrocorydaline isolated from Corydalis Tuber.

MATERIALS AND METHODS

Materials The Corydalis Tuber used in this study was originated from *Corydalis turtschaninovii* BESSER forma *yanhusuo* Y. H. CHOU et C. C. HSU produced in China. A commercial Corydalis Tuber was obtained from Nippon Funmatsu Yakuhin Co., Ltd. (Japan). Dehydrocorydaline (DHC) was isolated from a methanol extract of tubers by the method of Kaneko and Naruto.[7] The DHC content in the extract was determined by high performance liquid chromatography (HPLC) [conditions: column, YMC-ODS (AA-312) (6.0 mmϕ×150 mm); detection, UV absorption at 280 nm; mobile phase, 1,000 ml mixture of sodium citrate buffer and acetonitrile (3 : 2) containing sodium 1-octanesulfonate 4.0 g; flow rate, 1.0 ml/min; column temperature, 40℃; injection volume, 10 μl; the calibration curve was prepared by using an authentic DHC sample presented by Dr. Naruto of Dainippon Seiyaku Co. Ltd. (Japan)]. The DHC content in this extract was 4.98%. The following drugs were also used as reference in this study: λ-carrageenin, serotonin, bradykinin, arachidonic acid (AA), leukotriene C_4 (LTC_4), prostaglandin E_2 (PGE_2), carbobenzoxyl-L-phenylalanyl-L-arginine 4-methylcoumarinyl-7-amido (Z-Phe-Arg-MCA) (Sigma Chemicals Ltd.), indomethacin, histamine · 2HCl, cyproheptadine, soybean trypsin inhibitor (SBTI), kaolin (Nacalai Tesque Co. Ltd.), diphenhydramine, phenidone, pontamine sky blue (Tokyo Kasei Co. Ltd.), antipyrine (Hoei Yakko Co. Ltd.) and gabexate mesilate (FOY) (Onoyakuhin Co. Ltd.). For oral administration, DHC and indomethacin were suspended in a 0.2% carboxymethyl cellulose sodium salt (CMC · Na) solution. For intraperitoneal (i.p.) or intravenous (i.v.) injections, these drugs were dissolved in physiological saline.

Animals Male Slc : ddY strain mice (18-20 g, 30-32 g) and male Slc : Wistar strain rats (220-250 g) were used. They were maintained in a temperature- and humidity-controlled room (about 23℃, 60%) with lighting from 7 : 00 a.m. to 7 : 00 p.m. Animals were housed for at least one week in the laboratory animal room before experiment. A laboratory pellet chow (Labo MR Stock, Nihon Nosan Kogyo) and water were given freely.

Carrageenin-induced paw edema This test was

performed according to the method of Nakamura *et al.*[8] The initial hind paw thickness of the ddY strain mice was determined by a dial thickness gauge (Mitsutoyo). An 1.5% solution of carrageenin in saline (25 μl/animal) was injected subcutaneously into the right hind paw 1 h after the oral administration of test substances. The control group received the vehicle. The hind paw thickness was measured at intervals of 1 h for 5 h. The results were expressed as the percentage of hind paw swelling as compared with the initial hind paw thickness. Indomethacin was used as a standard drug.

Acetic acid-induced vascular permeability The test was performed according to the method of Whittle.[9] ddY strain mice were orally treated with the test substances 1 h before i.v. injection of 4% pontamine sky blue (10 ml/kg). Fifteen minutes after the injection of the dye, 0.7% 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 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 the amount of pontamine sky blue per mouse, which leaked into the intraperitoneal cavity. Indomethacin was used as a standard drug.

Chemical mediators-induced paw edema Following the determination of the initial hind paw thickness of the ddY strain mice, 1 h after oral administration of the test substances, 5 μl of 1.2% histamine, 0.02% serotonin or 0.6% bradykinin in saline was injected subcutaneously into the right hind paw. The control group received the vehicle. The paw thickness was measured for 30 min at intervals of 10 min, and the thickness of the edema was recorded. The results were expressed as the percentages of hind paw thickness as compared with the initial hind paw thickness. Diphenhydramine or cyproheptadine was used as a standard drug.

Assay of prekallikrein enzyme activity Whole blood samples were collected from the heart of pentobarbital-anesthetized Wistar strain rats. Nine milliliters of the blood was mixed with 1 ml of sodium citrate (3.8%) and centrifuged at 3,000 rpm for 10 min to obtain plasma. The assay of prekallikrein enzyme activity was performed by the method of Ohishi and Katori.[10] Citrated rat plasma (100 μl was mixed with 800 μl of test solution [2% dimethyl sulfoxide (DMSO)/acetone-Tris·saline buffer (buffer I; 0.02 M Tris · HCl, 0.15 M NaCl, pH 8.0)] and allowed to stand for 10 min at room temperature (about 25℃). Then, 100 μl of kaolin suspension (10 mg/ml buffer I) was added and mixed vigorously for 5 s. Five minutes after the addition of kaolin, 50 μl aliquots of the reaction mixture were incubated for 10 min at 37℃ with 1 ml of 50 mM Z-Phe-Arg-MCA in buffer II (0.05 M Tris·HCl, 0.1 M NaCl, 0.01 M $CaCl_2$, pH 8.0) in the presence (tube A) or the absence (tube B) of 40 μg SBTI. The reaction was terminated by the addition of 2 ml of 17% acetic acid and the fluorescence was read at 460 (emission) and 380 (excitation) nm in a Hitachi F-4010 fluorescence spectrophotometer. The difference between the values in tube A and tube B was taken as the prekallikrein activity and the inhibitory percentage of the test substance was determined. FOY was used as a standard drug.

Arachidonic acid (AA)-induced ear edema AA-induced ear swelling in mice was performed by the method of Young *et al.*[11] with modification. The initial thickness of right ear of ddY strain mice (30–32 g) was measured with a dial thickness gauge. One hour after the oral administration of test substance, each mouse was given 2 mg/ear of AA solution (100 mg AA/ml acetone) in the right ear and the thickness of the ear was measured 1 h after that. The control group received the vehicle. Phenidone dissolved in saline was administered intravenously as a standard drug.

Leukotriene C_4 (LTC_4) and/or prostaglandin E_2 (PGE_2)-induced ear edema Test substances were orally administered to mice, then after 1 h, 10 μl of LTC_4 (200 ng/ear) and/or PGE_2 (750 ng/ear) were intradermally injected into the right ear of male ddY strain mice. One hour after the injection, the mice were sacrificed. A circular tissue sample was cut off from each ear with a leather punch (5.5 mm in diameter) and weighted. Swelling was calculated from the difference of the ear weight.

Acetic acid-induced writhing Acetic acid-induced writhing test was performed by the method of Koster *et al.*[12] Samples were orally given 1 h prior to i.p. injection of 1.0% acetic acid (10 ml/kg). The events of writhing and stretching were counted for 10 min from 5 min after the injection.

Statistical analysis The experimental data were tested for the statistical differences by means of the Bonferroni/Dunn's Multiple Range Test.

RESULTS

Carrageenin-induced paw edema

Anti-inflammatory activity of DHC from Corydalis Tuber was screened in carrageenin-induced paw edema in mice. As shown in Fig. 1, DHC (0.125, 0.5 mmol/kg, *p.o.*) had a significant inhibitory effect on edema formation. Similar inhibition was shown by the standard drug indomethacin (10 mg/kg, *p.o.*).

Acetic acid-induced vascular permeability

As shown in Fig. 2, DHC at 0.125 mmol/kg (*p.o.*) inhibited the increase of dye leakage induced by acetic

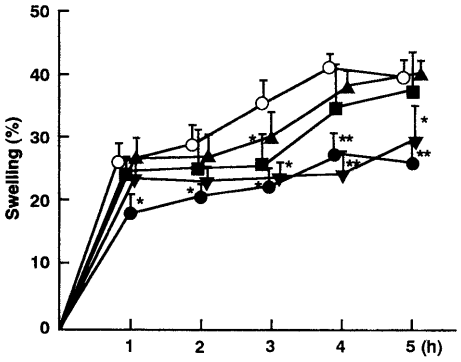

Fig. 1. Effects of Dehydrocorydaline (DHC) and Indomethacin on Carrageenin-Induced Paw Edema in Mice during 5 h period after Carrageenin Injection
Each test substance was administered orally 1 h before subcutaneous injection of 1.5% carrageenin (25 μl/animal). Control animals were orally administered with the vehicle. Each point represents the mean±S.E. of 9-10 mice. Significantly different from control, $^{*}p<0.05$, $^{**}p<0.01$. ○: control, ▲: DHC 0.025 mmol/kg, ■: DHC 0.125 mmol/kg, ▼: DHC 0.5 mmol/kg, ●: Indomethacin 10 mg/kg.

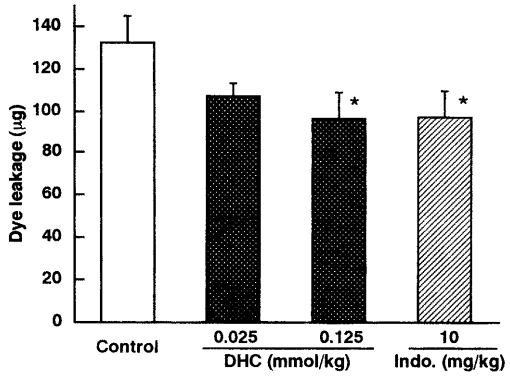

Fig. 2. Effects of Dehydrocorydaline (DHC) and Indomethacin (Indo.) on Vascular Permeability Induced by Acetic Acid in Mice
DHC and indomethacin were administered orally 1 h before the i.v. injection of 4% pontamine sky blue (10 ml/kg). Fifteen minutes after the injection of pontamine sky blue, 0.7% acetic acid was injected intraperitoneally (10 ml/kg). After 20 min, the mice were killed and the vascular permeability was measured and expressed in terms of the amount of leaked dye. Each column represents the mean±S.E. of 9-11 mice. Significantly different from the control group, $^{*}p<0.05$.

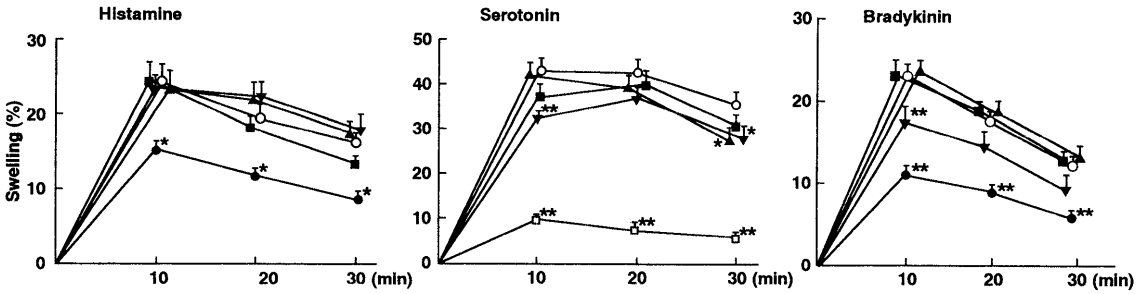

Fig. 3. Effects of Dehydrocorydaline (DHC), Diphenhydramine or Cyproheptadine on Histamine, Serotonin or Bradykinin-Induced Paw Edema in Mice for 30 min after Injection of Edema Inducers
One hour after oral administration of test substance, 1.2% histamine, 0.02% serotonin or 0.6% bradykinin solution (5 μl/animal) was subcutaneously injected. The control animals were administered with the vehicle. Each point represents the mean±S.E. of 9-10 mice. Significantly different from control, $^{*}p<0.05$, $^{**}p<0.01$. ○: control, ▲: DHC 0.025 mmol/kg, ■: DHC 0.125 mmol/kg, ▼: DHC 0.5 mmol/kg, ●: diphenhydramine 50 mg/kg, □: cyproheptadine 2 mg/kg.

acid in a dose response manner. The positive control agent, indomethacin, also reduced the leakage at 10 mg/kg, *p.o.*

Chemical mediators-induced paw edema

As shown in Fig. 3, the maximal edemas were observed 10 min after injections of the chemical mediators, histamine, serotonin or bradykinin in mice. DHC (0.5 mmol/kg, *p.o.*) inhibited the serotonin- or bradykinin-induced edema but not the histamine-induced edema. Positive control agent, diphenhydramine (50 mg/kg, *p.o.*) exhibited the inhibitory effects on the histamine-, and bradykinin-induced edema and cyproheptadine (2 mg/kg, *p.o.*) inhibited the serotonin-induced edema.

Assay of prekallikrein enzyme activity

As shown in TABLE I, DHC weakly inhibited the enzyme activity at concentrations 1.0-2.5 mM in a dose dependent manner. The positive reference drug FOY (50, 100 μg/ml) showed a strong inhibitory effect.

TABLE I. Effects of Dehydrocorydaline (DHC) and FOY on Prekallikrein Activity of Rat Plasma

Treatment	Concentration	Fluorescence	Inhibition(%)
Control	—	37.6±2.0	—
DHC	0.25(mM)	36.9±1.5	2.0
	1.0	32.9±1.7*	12.5
	1.25	31.1±1.6**	17.5
	2.5	24.3±1.0**	35.4
FOY	50(μg/ml)	10.2±0.1**	72.9
	100	4.2±0.4**	88.8

Each value represents the mean±S.E. of 3 experiments. Significantly different from control, *$p<0.05$, **$p<0.01$.

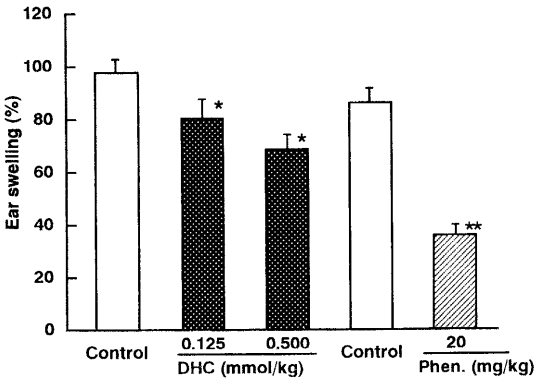

Fig. 4. Effects of Dehydrocorydaline (DHC) and Phenidone (Phen.) on Arachidonic Acid (AA)-Induced Ear Edema in Mice
One hour after oral administration of test substance, 2 mg AA dissolved in acetone (20 μl/animal) was applied to the ear of mice. One hour after the AA application the thickness of ear was measured and compared with that before AA application. The ear swelling is expressed as % swelling. Control was administered with the vehicle orally or intravenously. Each column represents the mean ±S.E. of 9-10 mice. Significantly different from control, *$p<0.05$, **$p<0.01$.

AA-induced ear swelling

As shown in Fig. 4, DHC at doses of 0.125, 0.5 mmol/kg (*p.o.*) significantly inhibited the AA-induced ear edema. The positive control agent phenidone significantly inhibited the edema at a dose of 20 mg/kg (i.v.).

Leukotriene C_4 (LTC_4)- and/or prostaglandin E_2 (PGE_2)-induced ear edema

One hour after the injection of LTC_4, the ear swelling was 116.8±6.1% (TABLE II). Injection of PGE_2 alone did not cause the ear edema, but it apparently enhanced the edema formation induced by LTC_4. DHC at 0.5 mmol/kg did not inhibit the LTC_4-induced edema formation whereas it significantly inhibited the edema induction by a mixture of LTC_4 and PGE_2.

Acetic acid-induced writhing

At oral doses of 0.125 and 0.5 mmol/kg, DHC did not have any antinociceptive effect on the writhing responses induced by 1% acetic acid solution in mice (data not shown). The positive control agent antipyrine (100 mg/kg, *p.o.*) showed the inhibitory effect.

TABLE II. Effects of Dehydrocorydaline (DHC) on Ear Swelling Induced by Leukotriene C_4 (LTC_4) or LTC_4 plus Prostaglandin E_2 (PGE_2) in Mice

Treatment	Dose (mmol/kg)	Swelling(%) (LTC_4)	Swelling(%) (LTC_4+PGE_2)
Control	—	117±6.1	141±6.0
DHC	0.025	109±5.0 (6.8%)	131±8.7 (7.1%)
	0.125	112±6.2 (4.3%)	123±4.9 (12.8%)
	0.5	109±4.0 (6.8%)	120±7.7*(14.9%)

Each value represents the mean± S.E. of 10-11 mice. Significantly different from control, *$p<0.05$. ( ) : Inhibition.

DISCUSSION

The anti-inflammatory effects of dehydrocorydaline (DHC) isolated from Corydalis Tuber were investigated by using various experimental models. DHC exhibited an inhibitory effect dose-dependently on the acetic acid-induced increased vascular permeability in mice, which is a typical model of the first stage of inflammatory reaction.

In the studies on the inhibitory effect on the carrageenin-induced edema, which was a model of the first and second stages of inflammatory reaction, mice were used in place of rats to reduce the quantity of test samples. The edema formed in mice is known to be slightly different from that in rats in the overall aspect and the formative process of swelling.[13] However, since indomethacin, a typical anti-inflammatory agent, inhibited the edema induced by carrageenin in mice as in rats, we considered that the effect of DHC on the acute inflammation induced by carrageenin can also be estimated even in mice.

DHC showed an inhibitory effect on carrageenin-induced edema in mice when given orally at doses of 0.125 to 0.5 mmol/kg. This effect was stronger on the increased edema 3 h after injection, and weaker on the edema 1 h after the injection. The biochemical mechanism for inflammatory reaction induced by carrageenin in animals is not yet clear. However, chemical mediators such as histamine, serotonin, prostaglandin (PGs) and kinin are presumed to be involved in the occurrence and development of inflammation. Edema induced by carrageenin is divided into the first phase in which the histamine and serotonin are involved and the second phase in which PGs and bradykinin participate through the advance and retreat of swelling.[14] It can therefore be presumed that DHC inhibit the production of chemical mediators and/or antagonizes the actions of the chemical mediators.

DHC significantly inhibited the serotonin-induced edema but was inactive on the histamine-induced

edema. We reported that DHC inhibits the histamine release from mast cells induced by compound 48/80.[5] Although the inhibitory effect of DHC on the production of serotonin is not known, it is believed that DHC has inhibitory effects on the production of histamine and on the action of serotonin itself involved in the first phase of carrageenin-induced edema. Since DHC inhibited the second phase of carrageenin-induced edema formation, there is no gainsaying the possibility that it possesses an effect on the formation or elimination of kinins or PGs in the edema.

The effects of DHC on the activation of prekallikrein (key enzyme in kinin formation) and on AA cascade were then studied. DHC showed an inhibitory effect on the activation of kallikrein and on bradykinin-induced edema. It also significantly inhibited the AA-induced ear edema. The inhibitory mechanism of DHC on the carrageenin-induced edema is thought to be related to its inhibitory effects on the kinin action, kinin formation and AA cascade.

It is known that PGE_2 and $PGF_{2\alpha}$ are produced on topic application of AA.[15] Accordingly, DHC may be considered to possess inhibitory actions on the conversion of AA into PGE_2 and on inflammation induced by PGE_2.

It is reported that the inflammation is not induced by topic application of PGE_2 itself, but that the edema formation induced by LTs, carrageenin and chemical mediators is increased by their combined use with PGE_2.[15-18] Since DHC significantly inhibited the ear edema induced by AA and LTC_4 plus PGE_2, it is suggested that DHC has some inhibitory effect on AA cascade. Its mechanisms have not yet been elucidated.

The major virtue of Corydalis Tuber, according to the herbal literature and books on the traditional Chinese system of medicine, is its possible anti-inflammatory effect. From the results of present studies, it has become clear that DHC is the main active component responsible for the expected anti-acute inflammation effect of Corydalis Tuber. Accordingly, Corydalis Tuber containing higher amount of DHC may be desired for therapeutic use.

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