Inhibitory Effects of Methanolic Extract from *Corydalis Tuber* against Types I–IV Allergic Models

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Methanolic extract (CM-ext) from tubers of *Corydalis turtschaninovii* forma *yanhusuo* has been screened for activity in experimental models of types I–IV allergy. In type I allergic models, CM-ext at doses of 200, 500 mg/kg, *p.o.* inhibited 48-h homologous passive cutaneous anaphylaxis (PCA) in rats which is related to IgE, and 4-h heterologous PCA in guinea pigs which is related to IgG. The inhibition of CM-ext on 48-h PCA was also recognized in adrenalectomized rats. CM-ext exhibited the inhibitory effect on formation of IgE antibody in BALB/c mice. In type II allergic model, it was found that CM-ext inhibits reversed cutaneous anaphylaxis (RCA). In type III allergic model, CM-ext showed the inhibitory effect on direct passive arthus reaction (DPAR) in rats. Furthermore, in type IV allergic model, CM-ext had the inhibitory effects on induction phase and effector phase in picryl chloride-induced contact dermatitis (PC-CD). It also showed therapeutic action on PC-CD. These results indicated that CM-ext not only inhibits antibody-mediated allergic reactions but also influences cell-mediated allergic reactions and should be recognized as a potent material for allergic reactions, although the mechanisms and active principles of CM-ext have not yet been completely determined.

Key words *Corydalis turtschaninovii* forma *yanhusuo*; Chinese traditional crude drug; types I–IV allergic reaction; IgE antibody

Corydalis tuber has been used mainly for treatment of stomachache as an analgesic 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 the methanolic extract and its alkaloidal components exhibited inhibitory effects on blood platelet aggregation and inflammatory models.^{1–5)}

The present paper deals with a study on an anti-allergic effect of methanolic extract from *C. Tuber*.

MATERIALS AND METHODS

Materials The *C. Tuber* used in this study originated from *Corydalis turtschaninovii* BESSER forma *yanhusuo* Y.H. CHOU *et* C.C. HSU in China. A commercial *C. Tuber* was obtained from Nippon Funmatsu Yakuhin Co., Ltd. (Japan). The powdered *C. Tuber* was refluxed for 2 h (two times) in methanol of decuple of the powder. The filtrate was evaporated to dryness at 40 °C in vacuum to give a brownish extract (CM-ext, yield: 2.95%). The following drugs were also used in this study: aluminum hydroxide gel (Maruishi), egg albumin (EWA, Sigma), absorbed diphtheria-purified pertussis-tetanus combined vaccine (Kitasawa), disodium cromoglycate (DSCG, Funakoshi Yakuhin), fresh sheep red blood cells (SRBC), picryl chloride, cyclophosphamide, dexamethasone, prednisolone (Nacalai Tesque) and Freund's complete adjuvant (Difco).

Subjects Male Slc: Wistar strain rats (150–170, 180–200 g), male Slc: Sprague-Dawley (SD) strain rats (150–170 g), male Slc: ICR strain mice (30–32 g), female Slc: BALB/c strain mice (18–20 g), Slc: Hartley strain

male guinea pigs (250–300 g) and Slc: JW strain male rabbits (2.0–2.5 kg) 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 and water were given freely.

Forty-eight-hour Homologous Passive Cutaneous Anaphylaxis (PCA, Type I Allergic Model) Rat anti-EWA serum containing IgE was prepared by the method of Stotland and Share⁶⁾ in male Wistar strain rats weighing 180 to 200 g. The rats were immunized with 1.0 mg of EWA, 20 mg of aluminum hydroxide gel suspended in 0.5 ml of absorbed diphtheria-purified pertussis-tetanus combined vaccine subcutaneously. Seven days later, the rats were immunized again with *i.p.* injection of the same mixture. Fourteen days later, the rats were anesthetized with pentobarbital, blood was withdrawn from the carotid arteries and rat anti-EWA serum was obtained. Serum was stored at –80 °C until use.

The antiserum diluted 8-fold with physiologic saline was injected into 4 sites on the shaved dorsal skin of male Wistar strain rats weighing 180 to 200 g intradermally in a 0.05 ml dose. Forty-eight h after sensitization, the rats were challenged with 0.5 ml of saline containing 10 mg of EWA and 5 mg of Evans blue *via* the tail vein. Thirty min later, the rats were sacrificed, the dorsal skin was removed for measurement of blue area, the amount of leaked dye was then determined colorimetrically after extraction with 1.0 N KOH and a mixture of acetone and phosphoric acid based on the method of Katayama *et al.*⁷⁾ Methanolic-extract (CM-ext) was administered orally 1 h before the challenge of antigen. DSCG was administered intravenously 1 min before the challenge.

Adrenal glands of male Wistar strain rats weighing 180 to 200 g were inoculated, and the rats were fed physiologi-

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cal saline for 2 weeks thereafter. Forty-eight-h homologous PCA in adrenalectomized rats was carried out by the method described above.

Formation of IgE Antibody On the basis of the method of Levine and Vaz,⁸⁾ 1 mg EWA and 1.5 mg of aluminum hydroxide gel in 0.2 ml of saline was injected i.p. to BALB/c female mice weighing 18–20 g. Seven and fourteen days thereafter, the mice were bled from eye grounds and serum was obtained to determine serum IgE antibody. CM-ext was administered orally from the day of immunization. A standard drug, cyclophosphamide (dissolved in saline) was i.p. administered to the mice from the day of immunization. The anti-EWA IgE antibody was determined by PCA in Wistar rats. The PCA titer was expressed as the highest dilution causing a lesion more than 5 mm in diameter.

Four-hour Heterologous PCA in Guinea Pigs (Type I Allergic Model) Rabbit anti-EWA serum containing IgG was prepared according to the method of Koda *et al.*⁹⁾; briefly, 10 ml of Freund's complete adjuvant was mixed with 10 ml of physiological saline containing 20 mg of EWA. One ml of this mixture was intramuscularly injected into rabbits weighing 2.0–2.5 kg once weekly for 4 weeks and 1 week after the final injection, rabbit anti-EWA serum was obtained. The serum was stored at -80°C until use.

Rabbit anti-EWA serum and physiological saline were injected into 3 sites on the right and left shaved dorsal skin, respectively, in a 0.1 ml dose. Four h after immunization, the guinea pigs were challenged intravenously with 1 ml of physiological saline containing 2 mg of EWA and 10 mg of Evans blue. Thirty min after challenge, the guinea pigs were sacrificed, the area of bluing and the amount of leaked dye were measured by the method of Katayama *et al.*⁷⁾ described in the 48-h homologous PCA in rats. CM-ext was orally administered 1 h before the challenge of antigen, and DSCG was intravenously administered 1 min before that challenge.

Reversed Cutaneous Anaphylaxis (RCA, Type II Allergic Model) Rabbit anti-rat serum was obtained by the method of Ungar *et al.*¹⁰⁾; briefly, 1 ml of normal rat serum was i.v. injected into rabbits weighing 2.0–2.5 kg, and this was repeated 10 times at a 1-d interval. One day after the final immunization, antiserum obtained was stored at -80°C until use.

Based on the method of Ungar *et al.*,¹⁰⁾ RCA in rats was carried out as follows. Rabbit anti-rat serum (0.05 ml) containing 1% Evans blue or the same volume of physiological saline containing 1% Evans blue was injected into the shaved dorsal skin of Wistar strain rats weighing 150–170 g. Two h later, the rats were sacrificed, and the dorsal skin was removed. The inflamed areas were cut out with a leather punch (12 mm in diameter). Each piece of skin was weighed immediately after removal. The swelling percentage was expressed by the following equation: $\text{swelling}\% = (W_i - W_s)/W_s \times 100$, W_i is the weight of the inflamed site and W_s is the weight of the saline-injected site. CM-ext was orally administered 1 h before the injection of antiserum, and dexamethasone was orally administered 30 min before the injection.

Direct Passive Arthus Reaction (DPAR, Type III Allergic Model) The procedure was based on the method of

Terasawa *et al.*¹¹⁾ Rabbit anti-EWA serum was intravenously injected into male SD strain rats weighing from 150–170 g via the tail vein; 30 min later, 0.1 ml of physiological saline containing 0.025 mg of EWA was intracutaneously injected into the right hind paw of the rats to induce an arthus reaction. Paw swellings were measured volumetrically 2 h after the challenge of EWA, and the results were expressed as a percentage of the swelling compared with the initial hind paw volume. CM-ext was administered orally 1 h before the challenge. A standard drug, prednisolone was administered orally 30 min before the challenge.

Picryl Chloride-Induced Contact Dermatitis (PC-CD, Type IV Allergic Model) The procedure was in accordance with the method reported by Asherson and Ptak.¹²⁾ Male ICR strain mice weighing 30–32 g were sensitized by applying 0.1 ml of 7% picryl chloride solution in ethanol to the shaved abdomen. After a 6-d sensitization period, the mice were challenged by painting the inside of the right ear with 0.02 ml of 1% picryl chloride solution in olive oil to induce PC-CD. In research on the induction phase of PC-CD, the ear thickness was measured with a dial thickness gage (Mitsutoyo, Japan) before and 24 h after the challenge and the difference in thickness was calculated. CM-ext was administered orally from day -1 to day 6 after immunization. Prednisolone was also administered orally from day 0 to day 6 after immunization.

In the study of the effector phase of PC-CD, mice with a certain percentage of ear swelling after sensitization and challenge were chosen; 3 d thereafter they were subjected to sensitization and challenge again by the same procedure and the percentage of ear swelling was again determined. CM-ext was administered orally before and 16 h after challenge. Prednisolone was administered orally 16 h after challenge. Furthermore, in research on the therapeutic effects of CM-ext and prednisolone on PC-CD, CM-ext and prednisolone were administered orally 24 h after challenge. The ear swelling was measured from 2 to 10 h after administration at 2 h intervals.

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

RESULTS

Forty-eight-hour Homologous PCA As shown in Fig. 1, the dye leakage caused by PCA in intact rats was significantly decreased by CM-ext at doses of 200, 500 mg/kg, *p.o.* Control agent, DSCG at a dose of 5 mg/kg, *i.v.*, caused inhibition. Inhibitions by CM-ext and DSCG were also noted in adrenalectomized rats (Fig. 2).

Formation of IgE Antibody As shown in Fig. 3, CM-ext at a dose of 500 mg/kg, *p.o.* had an inhibitory effect on the formation of IgE antibody 1 or 2 weeks after the immunization. CM-ext at a dose of 200 mg/kg, *p.o.* inhibited the IgE formation 1 week after the immunization. Control agent, cyclophosphamide (10 mg/kg, *i.p.*) had the strong inhibition.

Four-hour Heterologous PCA The inhibitory effects of CM-ext and DSCG on 4-h heterologous PCA are shown

Fig. 1. Effects of Methanolic Extract (CM-ext) from *C. Tuber* and Disodium Cromoglycate (DSCG) on 48-h Homologous PCA in Rats

CM-ext suspended with 0.5% CMC·Na was orally administered to rats mediated by the rat anti-EWA serum 1 h before the challenge with antigen. DSCG dissolved with saline was intravenously administered 1 min before the challenge. Control was orally administered 0.5% CMC·Na or intravenously administered saline alone. Each column indicates mean $\pm$ S.E. of 7–8 rats. Significantly different from the control group, *, $p < 0.05$; **, $p < 0.01$.

Fig. 2. Effects of Methanolic Extract (CM-ext) from *C. Tuber* and Disodium Cromoglycate (DSCG) on 48-h Homologous PCA in Adrenalectomized Rats

CM-ext suspended with 0.5% CMC·Na was orally administered to adrenalectomized rats mediated by the rat anti-EWA serum 1 h before the challenge with antigen. DSCG dissolved with saline was intravenously administered 1 min before the challenge. Control was orally administered 0.5% CMC·Na alone or intravenously administered saline alone. Each column indicates mean $\pm$ S.E. of 7 rats. Significantly different from the control group, *, $p < 0.05$.

Fig. 3. Effects of Methanolic Extract (CM-ext) from *C. Tuber* and Cyclophosphamide (CY) on the Formation of IgE Antibody in BALB/c Mice

Mice were immunized with 1 mg of EWA and 1.5 mg of aluminum hydroxide gel. CM-ext suspended with 0.5% CMC·Na was orally administered and cyclophosphamide dissolved with saline was i.p. administered once a day for 14 d immediately after the implantation of immunization. The animals were bled 7 or 14 d after the immunization. Each column indicates mean $\pm$ S.E. of 6–7 mice. Significantly different from the control group, *, $p < 0.05$; **, $p < 0.01$.

in Fig. 4. CM-ext (500 mg/kg) and DSCG (5 mg/kg) inhibited the leakage of dye induced by 4-h heterologous PCA in guinea pigs.

RCA As shown in Fig. 5, CM-ext inhibited the skin swelling induced by RCA in rats. The mode of inhibition with CM-ext was dose-dependent. Dexamethasone at a dose of 5 mg/kg also inhibited this increase.

DPAR Figure 6 shows the effects of CM-ext and prednisolone on the DPAR reaction. CM-ext at doses of 200 and 500 mg/kg was effective in reducing the paw swelling in rats induced by DPAR. Prednisolone at a dose of 25 mg/kg also inhibited this reaction.

PC-CD The effects of CM-ext and prednisolone on the induction phase of PC-CD are shown in Fig. 7. CM-ext at doses of 50, 200, 500 mg/kg had dose-dependent and inhibitory effects on the induction phase. Prednisolone also showed the inhibition.

Figure 8 shows the effects of CM-ext and prednisolone on the effector phase of PC-CD. CM-ext at doses of 50, 200, 500 mg/kg had inhibitory effects on the ear swelling in mice induced by PC-CD. Prednisolone at a dose of 10 mg/kg also inhibited this ear swelling.

Finally, the therapeutic effects of CM-ext and prednisolone on PC-CD were determined. Twenty-four h after challenge, ear swelling reached the maximum. CM-ext and prednisolone were administered orally and the ear swelling at this point was 100%. The ear swellings at 2, 4, 6, 8 and 10 h after administration were measured. As shown in Fig. 9, CM-ext at a dose of 500 mg/kg significantly reduced the swellings at 6, 8, 10 h after the administration. A standard drug, prednisolone at a dose of 20 mg/kg exhibited the same effect on the ear swellings from 4 to 10 h after administration.

DISCUSSION

We reported here that methanolic extract (CM-ext) of *Corydalis Tuber* exhibited an inhibitory effect on acute inflammatory models and adjuvant arthritis in rats. Anti-allergic effects of CM-ext were investigated using experimental allergic models divided into four types by Coombs and Gell.¹³⁾

PCA, active systemic anaphylaxis and Schultz–Dale reactions which are classified as type I allergic reactions,

Fig. 4. Effects of Methanolic Extract (CM-ext) from *C. Tuber* and Disodium Cromoglycate (DSCG) on 4-h Heterologous PCA in Guinea Pigs

CM-ext suspended with 0.5% CMC·Na was orally administered to guinea pigs mediated by the rabbit anti-EWA serum 1 h before the challenge with antigen. DSCG dissolved with saline was intravenously administered 1 min before the challenge. Control was orally administered 0.5% CMC·Na alone or intravenously administered saline alone. Each column indicates mean $\pm$ S.E. of 7 guinea pigs. Significantly different from the control group, *, $p < 0.05$.

Fig. 5. Effects of Methanolic Extract (CM-ext) from *C. Tuber* and Dexamethasone on Reversed Cutaneous Anaphylaxis (RCA) in Rats

CM-ext suspended with 0.5% CMC·Na was orally administered to rats 1 h before the injection of rabbit anti-rat serum. Dexamethasone suspended with 0.5% CMC·Na was administered 30 min before the injection of antibody. Control was administered orally with 0.5% CMC·Na alone. Each column indicates mean $\pm$ S.E. of 7 rats. Significantly different from the control group, *, $p < 0.05$; **, $p < 0.01$.

Fig. 6. Effects of Methanolic Extract (CM-ext) from *C. Tuber* and Prednisolone on Direct Passive Arthus Reaction (DPAR) in Rats

CM-ext suspended with 0.5% CMC·Na was orally administered to rats 1 h before the injection of antigen. Prednisolone suspended with 0.5% CMC·Na was administered 30 min before the injection of antigen. Control was administered orally with 0.5% CMC·Na alone. Two h after the challenge, paw swellings were measured. Each column indicates mean $\pm$ S.E. of 9–10 rats. Significantly different from the control group, *, $p < 0.05$.

Fig. 7. Effects of Methanolic Extract (CM-ext) from *C. Tuber* and Prednisolone on the Induction Phase of Picryl Chloride-Induced Contact Dermatitis (PC-CD) in Mice

CM-ext suspended with 0.5% CMC·Na was orally administered from day -1 to day 6 after sensitization, prednisolone was orally administered from day 0 to 6 after sensitization, and percentage of ear swelling was measured 24 h after the challenge. Each column indicates mean $\pm$ S.E. of 15 mice. Significantly different from the control group, *, $p < 0.05$; **, $p < 0.01$.

Fig. 8. Effects of Methanolic Extract (CM-ext) from *C. Tuber* and Prednisolone on the Effector Phase of Picryl Chloride-Induced Contact Dermatitis (PC-CD) in Mice

CM-ext suspended with 0.5% CMC·Na was orally administered before and 16 h after challenge, prednisolone was orally administered 16 h after challenge. Percentage of ear swelling was measured 24 h after the challenge. Each column indicates mean $\pm$ S.E. of 15–19 mice. Significantly different from the control group, *, $p < 0.05$; **, $p < 0.01$.

Fig. 9. Effects of Methanolic Extract (CM-ext) from *C. Tuber* and Prednisolone on the Therapeutic Effects of Picryl Chloride-Induced Contact Dermatitis (PC-CD) in Mice

CM-ext and prednisolone suspended with 0.5% CMC·Na were orally administered 24 h after challenge. Percentage of ear swelling was measured after administration. Each point indicates mean of 13–15 mice. Significantly different from the control group, *, $p < 0.05$; **, $p < 0.01$. —○—, control; —●—, CM-ext 50 mg/kg; —▲—, CM-ext 200 mg/kg; —■—, CM-ext 500 mg/kg; —▼—, prednisolone 20 mg/kg.

are believed to be caused by chemical mediators such as histamine and leucotriens being released from mast cells and basophils by an IgE-related mechanism. CM-ext at oral doses of 200 or 500 mg/kg inhibited significantly 48 h PCA. CM-ext also inhibited IgE antibody production in EWA sensitized BALB/c mice. The inhibitory effect of CM-ext on histamine release from mast cells has been confirmed.⁵⁾ Accordingly, the inhibitory effect against PCA observed in the present study may be attributed to the inhibition of IgE antibody production and of histamine release from mast cells, and seems to be an action without participation of the adrenal function, since the effect did not disappear even in adrenal gland excised-rats. CM-ext exhibited the inhibitory effects on reversed cutaneous anaphylaxis, Arthus reaction and delayed type hypersensitivity, which are classified as types II, III and IV allergic reactions, respectively. Type II is an allergic reaction which produces the dissolution or destruction of cell or tissue and is elicited by the combination of the antibody to surface antigen on all membrane. The participation of complement is also required to cause this reaction.

Type III is the allergic reaction which antigen-antibody complex activates kinin system, forming anaphylatoxin with participation of complement, and further aggregating platelets to result in injury of cell or tissue. These allergic reactions have been shown to depend on complement activity.

Although the precise mechanism for inhibitory effects of CM-ext on types II and III allergic reactions is ambiguous, it might be partly attributable to its anti-complement activity.

On the other hand, type IV differs from types I to III in being a reaction of cellular mediated immunity related closely to immune cell. This delayed type reaction is introduced by the release of chemical mediators such as lymphokines derived from lymphocytes through contact

with the corresponding antigen of T-cell sensitized with antibody and complement.

In conclusion, it is clear that CM-ext was effective in all the experimental models of types I to IV allergic reactions. These results seem to indicate that a certain effectiveness of Yan-Hu-Suo (延胡索) which has been described in herbal literatures of China and Japan is supported experimentally. Accordingly, Yan-Hu-Suo is expected to be effective against allergic diseases. Further studies seem mandatory on the mechanism of the anti-allergic activity observed in this study and on elucidation of its active principles.

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