The effect of methanolic extract from Corydalis Tuber on cytokine production and allergic reactions in experimental animals

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Abstract

The effects of methanolic extract of Corydalis Tuber (MECT) on the production of cytokines and some allergic reactions in experimental animals were investigated.

1. MECT inhibited bacterial lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF)-α production and showed a tendency to inhibit the production of interleukin (IL)-1β and IL-6 in mice that had been pretreated with Propionibacterium acnes.

2. MECT inhibited the LPS-induced TNF-α production by J774.1 cells, whereas it failed to affect the TNF-α-induced cytotoxicity to L929 cells.

3. MECT inhibited IgE-mediated biphasic cutaneous reaction in mice. When MECT was administered for 6 days, the magnitude of the inhibition was potentiated.

4. MECT inhibited dinitrofluorobenzene-induced contact dermatitis in mice, although little inhibition was observed in Arthus reaction in rats and Forssman cutaneous vasculitis in guinea pigs.

5. The inhibitory action of TNF-α production is one of the possible mechanisms of anti-allergic action of MECT.

Key words TNF-α, Corydalis Tuber, Allergic reaction, Cytokine.

Abbreviations DNFB, dinitrofluorobenzene ; FCV, Forssman cutaneous vasculitis ; IL, Interleukin ; MECT, Methanolic extract of Corydalis Tuber ; P. acnes, Propionibacterium acnes ; TNF, Tumor necrosis factor.

Introduction

Many mediators play a role in the induction and effector phases of immunity-mediated inflammations including allergy and autoimmune diseases. Proinflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 are especially important for the onset and development of a certain kind of allergy and autoimmune diseases.[1-3] In our laboratory, we have widely investigated the effects of Chinese herbal medicines on experimental allergic and autoimmune diseases, and already reported an efficacy of the crude extracts from Scuterallia baicalensis, Nandina domestica and Phellodendron amurense on experimental allergic reactions.[4-8] On the other hand, the anti-allergic and anti-inflammatory actions of methanol extract from Corydalis Tuber (MECT) have extensively been investigated.[9-12] These reports indicated that MECT inhibited some allergic reactions mediated by IgE antibody, IgG antibody and effector T cells, beside chemical mediator-induced inflammatory reactions in animal models. However, as anti-allergic and anti-inflammatory actions of MECT are wide, the mechanisms are still obscure. In the present study, therefore, we have

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examined the effects of MECT on proinflammatory cytokine production and on experimental allergic cutaneous reactions.

Materials and Methods

Materials : MECT was kindly donated from Daiichi Pharmaceutical Co., Ltd. (Tokyo). The powdered Corydalis Tuber was purchased from Nippon Funmatsu Yakuhin Co., Ltd. (Osaka).

Lipopolysaccharide (LPS)-induced cytokine production in mice : The effects of MECT on the production of proinflammatory cytokines (TNF-α, IL-1β and IL-6) *in vivo* was examined by Propionibacterium acnes (P. acnes) and LPS system. In brief, P. acnes at a dose of 0.3 mg (in 0.2 ml saline) was injected intravenously into male C57BL/6 mice. Seven days later LPS (Difco Laboratories, Detroit, MI, USA) at a dose of 5 mg/kg was injected intravenously into the mice. Blood samples were obtained 2 hr after the LPS injection. Each level of TNF-α, IL-1β and IL-6 was measured by ELISA (Endogen, Woburn, MA, USA).

LPS-induced TNF-α production by J774.1 cells in vitro : Murine macrophage-like cell, J774.1 was cultured in RPMI 1640 medium supplemented with 10 % fetal calf serum, 50 μM mercaptoethanol, 50 U/ml penicillin and 50 μM streptomycin (FCS-RPMI). The cells were adjusted to a concentration of 2×10^5 cells/ml using FCS-RPMI and plated into 24-well plates (Corning Coster Japan, Tokyo) at 1 ml/well. After incubation at 37°C for 30 min in 5 % CO_2, LPS was added at a final concentration of 1 μg/ml. After incubation for 18 hr, the supernatant was harvested. The supernatant was centrifuged to remove cell debris and then TNF-α was assayed by ELISA (Endogen).

TNF-α-induced cytotoxicity in L929 cells : The effect of MECT on the TNF-α activity was evaluated by using TNF-α-induced L929 cell cytotoxicity. L929 cells in Eagle MEM supplemented with 10 % fetal calf serum and 1 μg/ml of actinomycin D (5×10^4 cells/50 μl/well) were plated in flat-bottomed wells. Twenty five microliters of murine recombinant TNF-α (Genzyme, Cambridge, MA, USA) at a concentration of 160 U/ml and the same volume of MECT were added. Cells were incubated at 37°C in 5 % CO_2 for 24 hr. After the incubation, 10 μl of Alamar blue solution was added to each well and further incubated for 4 hr. Fluorescence intensity of each well was monitored by using a Millipore Cytofluor plate reader at an excitation wavelength of 560 nm and an emission wavelength of 590 nm.

IgE-mediated biphasic cutaneous reaction : The cntaneous reaction was elicited by the method previously described.[13,14] Briefly, BALB/c mice were passively sensitized by an intravenous injection of anti-dinitrophenyl monoclonal IgE 24 hr before the test. Cutaeous reaction was elicited by painting 25 μl of 0.15 % dinitrofluorobenzene (DNFB) acetone-olive oil solution (3 : 1) to each side of each ear. The ear thickness was measured by a micrometer, Upright Dial Gauge (Peacock, Ozaki, Tokyo) before and at appropriate times after challenge.

Arthus cutaneous reaction in rats : Rabbit anti-ovalbumin serum was diluted 2-fold with physiologic saline, and injected at a volume of 100 μl into the plantar pad of the hind paw of a rat. Immediately afterwards, 25 mg/kg of ovalbumin saline solution was injected intravenously. The volume of the paw was measured with a mercury plethysmometer (KN-357, Natsume, Tokyo) at 0 and 4 hr. At time 0, the average rat paw volume amounted to 1.63 ml.

Forssman cutaneous vasculitis (FCV) : FCV was carried out according to the method described previously.[15] Guinea pigs were injected with 0.1 ml of rabbit anti-sheep red blood cell serum diluted 8-fold with physiologic saline intradermally into their shaved backs, followed by an intravenous injection of 1.0 ml of 1 % Evans blue. After 1 hr the animals were sacrificed by exsanguination, and the skin was removed. The bluing spot caused by the FCV was evaluated by the amount of extravasated dye.

DNFB-induced contact dermatitis : Ears of BALB/c mice were painted with DNFB acetone-olive oil (3 : 1) solution or vehicle once each week for 3 weeks. A total of 25 μl of 0.15 % DNFB in vehicle was applied to each side of the ear. Ear thickness was measured using the same method as described above.

Statistics : Data are presented as mean$\pm$S.E.M. Differences between two groups were analyzed by Student's t-test. Differences among three groups or more were analyzed by Dunnett's test. $P<0.05$ was

considered to be significant.

Results

Effect of MECT on cytokine production

The injection of LPS into P. acnes-pretreated BALB/c mice resulted in the production of a large amount of TNF-α, IL-1β and IL-6 in the serum (Fig. 1). MECT at doses of 100 and 300 mg/kg significantly inhibited the production of TNF-α, and showed a tendency to inhibit the production of IL-1β and IL-6. Prednisolone at a dose of 3 mg/kg, used as a reference drug, clearly inhibited the production of all three kinds of proinflammatory cytokines.

Effect of MECT on the production of TNF-α by J774.1 cells was examined. As shown in Fig. 2, MECT inhibited the LPS-induced production of TNF-α ([A]) without affecting the cell viability ([B]). MECT at doses between 10^{-8} and 10^{-5} g/ml showed an inhibition of TNF-α production in a dose-dependent

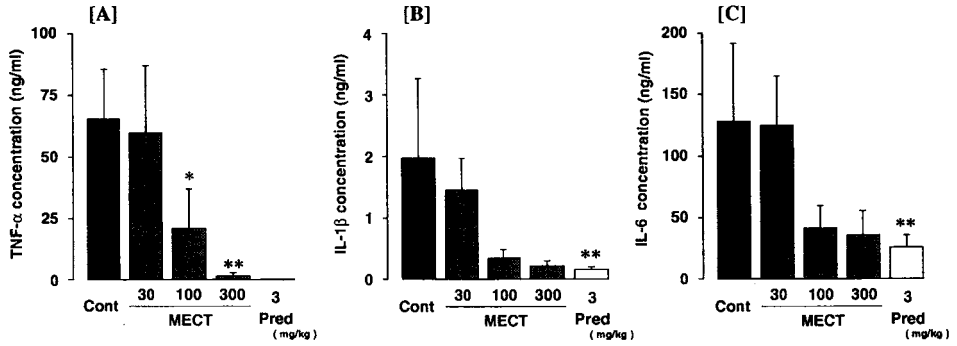

Fig. 1 Effects of methanolic extract from Corydalis Tuber (MECT) and prednisolone (Pred) on lipopolysaccharide (LPS)-induced cytokine production in P. acnes-pretreated mice. MECT and Pred were administered orally 1 hr before LPS injection. Each column and vertical bar represents the mean±S.E.M. for 6 mice. [A] tumor necrosis factor (TNF)-α, [B] interleukin (IL)-1β, [C] IL-6, $^{*}p<0.05$, $^{**}p<0.01$

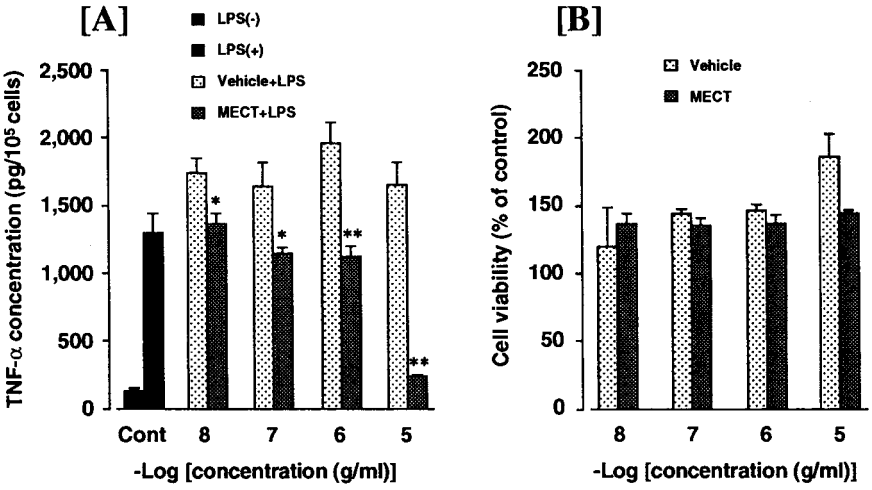

Fig. 2 Effects of methanolic extract from Corydalis Tuber (MECT) on lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF)-α production by J774.1 cells. [A] TNF-α production by J774.1 cells, [B] viability of J774.1 cells estimated with Alamar blue assay. Each column and vertical bar represents the mean±S.E.M. for 5 experiments. $^{*}p<0.05$, $^{**}p<0.01$

fashion.

Effect of MECT on TNF-α-induced cytotoxicity to L929 cells was examined. MECT at doses between 10^{-8} and 10^{-5} g/ml showed no effect on the TNF-α-induced cytotoxicity to L929 cells (Fig. 3).

Effect on IgE-mediated biphasic cutaneous reaction in mice

Since our previous studies indicate that TNF-α plays an important role for the onset and development of IgE-mediated biphasic cutaneous reaction,[13,14] we have examined the effects of MECT on the IgE-mediated biphasic cutaneous reaction. As shown in Fig. 4 [A], MECT at a dose of 300 mg/kg clearly inhibited the immediate and late phase reactions when administered 1 hr before challenge. Prednisolone at a dose of 10 mg/kg also inhibited the biphasic cutaneous reaction significantly. When MECT was administered for 6 days before the skin reaction, both immediate and late phase reactions were inhibited in a dose-

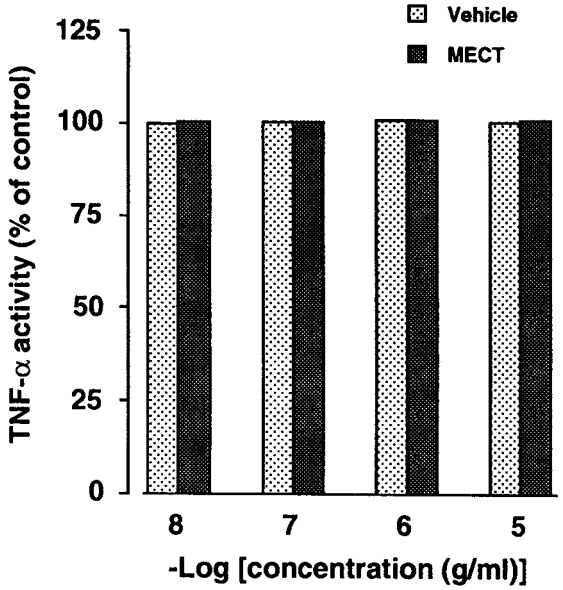

Fig. 3 Effects of methanolic extract from Corydalis Tuber (MECT) on murine tumor necrosis factor (TNF)-α-induced cytotoxicity to L929 cells. Cytotoxicity was evaluated by Alamar blue assay. Each column represents the mean value for 5 experiments.

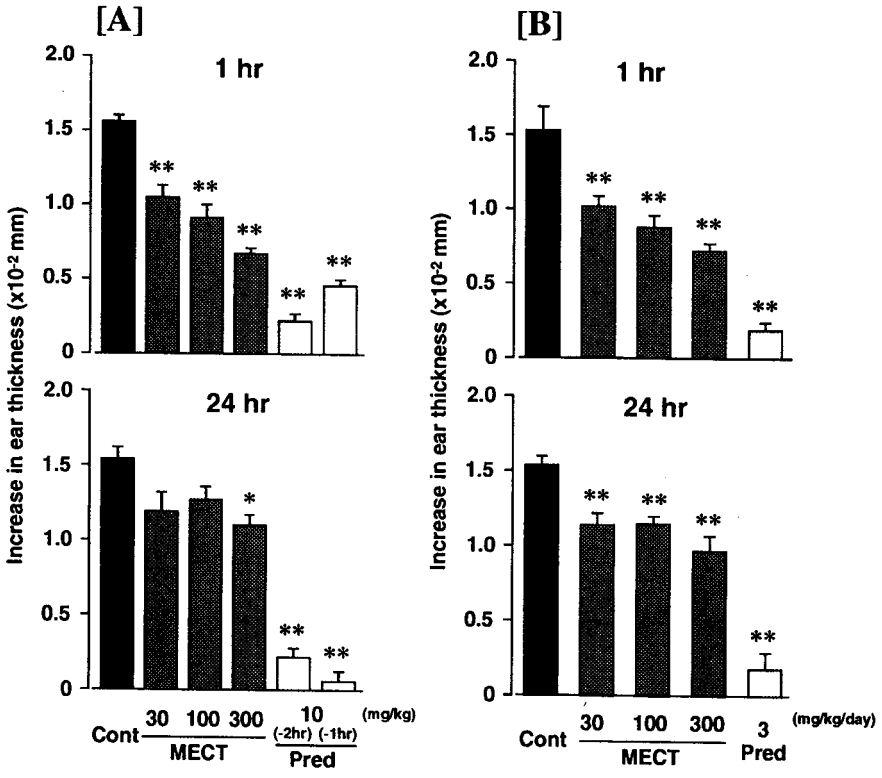

Fig. 4 Effects of methanolic extract from Corydalis Tuber (MECT) and prednisolone (Pred) on IgE-mediated biphasic cutaneous reaction in mice. [A] MECT was administered orally 1 hr before challenge, and Pred was given 1 or 2 hr before challenge. [B] MECT and Pred were administered orally once a day for 6 days before challenge. Each column and vertical bar represents the mean±S.E.M. for 6 mice. $*p<0.05$, $**p<0.01$

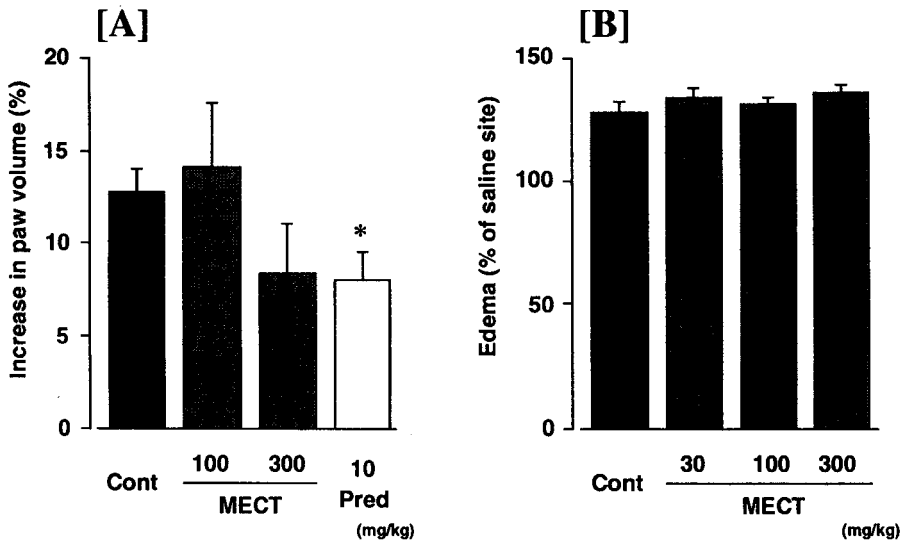

Fig. 5 Effects of methanolic extract from Corydalis Tuber (MECT) and prednisolone (Pred) on Arthus reaction in rats and Forssman cutaneous vasculitis (FCV) in guinea pigs. MECT and Pred were administered orally 1 hr before challenge. Each column and vertical bar represents the mean±S.E.M. of 5 animals. [A] Arthus reaction, [B] FCV, $*p<0.05$

related fashion (Fig. 4 [B]). Prednisolone at a dose of 3 mg/kg administered for 6 days also significantly inhibited the IgE-mediated biphasic cutaneous reaction.

Effect on Arthus reaction and FCV

The Arthus reaction in rats is a typical animal model for an immune complex-induced reaction, and FCV in guinea pigs is a typical model for a cytolytic reaction. The effects of MECT on these two reactions were examined. MECT at doses of 100 and 300 mg/kg did not affect both reactions, whereas prednisolone inhibited the Arthus reaction significantly (Fig. 5).

Effect on DNFB-induced contact dermatitis

The effect of MECT on DNFB-induced contact dermatitis in BALB/c mice was investigated. As shown in Fig. 6, MECT at doses between 30 and 300 mg/kg inhibited the increase in ear thickness caused by contact dermatitis by repeated application of DNFB on the mice ears. Prednisolone at a dose of 3 mg/kg also clearly inhibited the increase in ear thickness caused by DNFB.

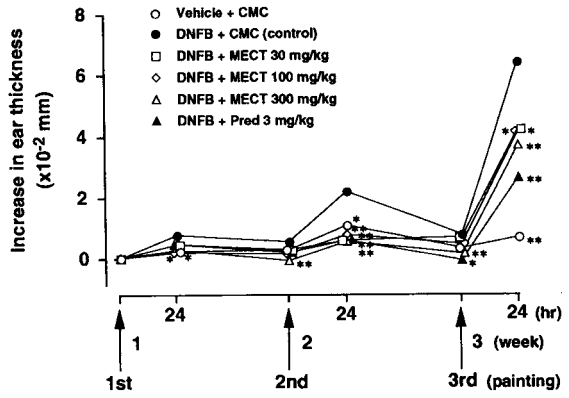

Fig. 6 Effects of methanolic extract from Corydalis Tuber (MECT) and prednisolone (Pred) on dinitrofluorobenzene-induced contact dermatitis in mice. MECT and Pred were administered orally once a day throughout the experiment. Each point represents the mean value for 7 mice. S.E.M. was not indicated for clarity. Each S.E.M. was within 11.2 % of the corresponding mean value. CMC : carboxymethyl cellulose, $*p<0.05$, $**p<0.01$

Discussion

Present results indicate that MECT exhibits a potent inhibitory action of TNF-α production both *in vivo* and *in vitro*. Simultaneously, MECT inhibits allergic reactions that have been reported to involve TNF-α for their onset.[13,14] MECT has been reported to have many pharmacological actions including analgesic, smooth muscle relaxing and anti-peptic ulcer activities.[16,17] In addition, there are some reports to show an anti-inflammatory activity of MECT.[9,12]

Regarding the anti-peptic ulcer action, dehydrocorydaline shows a potent action as well as MECT. In our previous study, we have confirmed anti-allergic actions of tertial alkaloids, such as phellodendrine and tetrandrine.[6] In our recent preliminary experiments, we have also demonstrated an inhibitory action of dehydrocorydaline on TNF-α production in murine system.

Concerning the role of TNF-α in allergic inflammation, there are many studies showing a central role of TNF-α in chronic allergic diseases.[18-21] We have already reported the importance of TNF-α in IgE-mediated biphasic cutaneous reaction in mice.[13,14] In addition, there are some reports indicating a role of TNF-α in delayed type hypersensitivity.[22,23] On the contrary, there is little information about the role of TNF-α in Arthus reaction and Forssman reaction. The present results indicate that the anti-allergic effects of MECT are, at least in part, due to the inhibition of TNF-α production.

Recent extensive investigations revealed that TNF-α plays an important role for the onset and development of arthritis. Matsuda *et al.*[12] have reported that MECT inhibited the development of collagen-induced arthritis in mice. The action of MECT may also be related to the inhibitory action for TNF-α production.

In the present study, we have not examined the active principles in MECT. Our preliminary experiments, however, have suggested that dehydrocorydaline is one of the active principles. We are now extensively investigating the anti-allergic actions of dehydrocorydaline, especially the effects on the production of cytokines and their actions. Further detailed investigations will be necessary to identify the active principles in MECT.

In conclusion, we have demonstrated the anti-allergic actions of MECT and its possible mechanisms. The inhibitory action of TNF-α production is one of the possible mechanisms of anti-allergic action of MECT.

和文抄録

延胡索（Corydalis Tuber）のメタノール抽出エキス（MECT）のサイトカイン産生および実験的アレルギー性炎症に及ぼす影響を検討した。MECTはマウスの腫瘍壊死因子（TNF-α）産生をin vivoおよびin vitroのいずれの系においても抑制した。さらに，MECTはTNF-αが発症に関与すると思われるIgEによる遅発型アレルギー反応および接触性皮膚炎を強く抑制した。しかし，TNF-αの関与のないArthus反応およびForssman反応には影響を与えなかった。これらのことから，TNF-α産生抑制作用はMECTの抗アレルギー作用機序の一つであると考えられる。

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