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Immunopharmacological studies of Unsei-in (Wen-Qing-Yin), a Chinese blended medicine. Examination of most effective one of Chinese herbs composed of Unsei-in on type IV hypersensitivity reaction

Yutaka ONO[a], Takeshi NISHIYORI[a], Akito MASE[a], Takanori SENGOKU[a], Qiang XU[a], Hiroshi MORI[a], Akihide KODA*[a] and Itsuo NISHIOKA[b]

[a] *Department of Pharmacology, Gifu Pharmaceutical University*
[b] *Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Kyushu University*

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Abstract

In the present paper, the Chinese herbs composed of Unsei-in were studied for their individual inhibitory activity on the induction phase of the type IV hypersensitivity reaction and GvH-R. The following results were obtained : 1) Since Unsei-in contains both Simotu-tô (Si-Wu-Tang) and Ôren-gedoku-tô (Huang-Lian-Jie-Du-Tang), the effect of each component on the contact dermatitis as induced in mice by picryl chloride (PC-CD) was studied. When compared to Simotsu-tô, Ôren-gedoku-tô was found to markedly inhibit the primary and secondary immune responses. 2) On the GvH-R Simotu-tô showed only a tendency to inhibit, while Ôren-gedoku-tô inhibited significantly. 3) Phellodendri C. of 4 kinds of Chinese herbs composed of Ôren-gedoku-tô was found to exhibit the most potent inhibitory activity on the primary and secondary immune responses in PC-CD. It was also found to inhibit GvH-R. From these results, Phellodendri C. was shown to be the most potent Chinese herb in Unsei-in in the inhibition of cellular immune response.

Key words graft versus host reaction, Ôren-gedoku-tô, picryl chloride-induced contact dermatitis, Simotsu-tô, type IV hypersensitivity reaction, Unsei-in

Abbreviations GvH-R, graft versus host reaction ; Hank's BSS, Hank's balanced salt solution ; PC, picryl chloride ; PC-CD, picryl chloride-induced contact dermatitis ; PLN, popliteal lymph nodes ; Ôren-gedoku-tô (Huang-Lian-Jie-Du-Tang), 黄連解毒湯 ; Simotu-tô (Si-Wu-Tang), 四物湯 ; Unsei-in (Wen-Qing-Ying), 温清飲

Introduction

Behçet's syndrome is an intractable disease characterized by a protracted course of acute inflammatory lesions of the oral mucosa, skin, eyes and external genitalia. Cellular immunity plays an important role in the onset of Behçet syndrome,[1-3] and immunosuppressive drugs have mainly been used for the therapy.[4-6] Unsei-in (Wen-Qing-Yin), a Chinese blended medicine, has been reported to be effective for the oral mucosal lesions and genital ulcers of Behçet's syndrome.[7-9] Previously we[10] studied the effects of Unsei-in on the Type IV hypersensitivity reaction and the reaction caused by cytotoxic T cells, and found that the medicine inhibited the induction phase of the Type IV hypersensitivity reaction as well as the reaction caused by cytotoxic T cells. On the other hand, Unsei-in showed only a tendency to inhibit the production of humoral antibodies. These findings suggest that Unsei-in has a rather specific inhibitory activity on cellular immune response. The examination of the most potent one

*〒502 岐阜市三田洞東5-6-1
岐阜薬科大学薬理学教室　江田昭英
5-6-1, Mitahora-higashi, Gifu 502, Japan

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of Chinese herbs composed of Unsei-in and its active component may be contributed to development of the specific inhibitor of cellular immune response.

With this thought in mind, the present study was designed to find the most potent of the Chinese herbs composed of Unsei-in. Since Unsei-in contains both Chinese blended medicines of Simotu-tô (Si-Wu-Tang) and Ôren-gedoku-tô (Huang-Lian-Jie-Du-Tang) which are each composed of four Chinese herbs, initially the effects of both preparations on the Type IV hypersensitivity reaction were studied. According to results, the most potent inhibition was found in Ôren-gedoku-tô. Secondarily, the effects of four Chinese herbs composed of Ôren-gedoku-tô were individually studied on the Type IV reaction.

Materials and Methods

Drugs : Aqueous extracts of Unsei-in, Simotu-tô and Ôren-gedoku-tô were prepared with the respective Chinese herbs, and were extracted by water using the usual method. The aqueous extracts of Chinese herbs composed of Ôren-gedoku-tô including Coptidis Rh., Phellodendri C., Scutellariae R. and Gardeniae F. were similarly made. Each of the extracts was dissolved into distilled water before use and given p.o. throughout the experiments. Prednisolone acetate (Takeda Chemical Industries, Ltd.) was used as a comparative.

Experimental animals : ddY strain male mice, 8－9 weeks old, BALB/c strain male mice, 5 weeks old, and CBF_1 strain male mice, 5 weeks old, were used. All of these animals were purchased from Shizuoka Laboratory Animal Center and were fed solid feed and water *ad libitum* in an air-conditioned room at 22±1℃ and 60% humidity.

Picryl chloride-induced contact dermatitis (PC-CD) : Using the modified method of Asherson and Ptak,[11] ddy mice were sensitized with an application of 0.1 ml of 1% picryl chloride (PC) in ethanol solution on the skin of the abdomen which had been shaved one day before (primary sensitization).

After 6 days, the primary challenge was performed by the application of 15 μl each of 1% PC solution in olive oil on both sides of the ear lobes bilaterally. The thickness of the lobe after 24 hr was measured with a dial thickness gauge (Ozaki), and the thickness before challenge was subtracted from this thickness to obtain the swelling rate due to PC-CD. In the experiment on secondary sensitization, animals were sensitized again 2 days after the measurement of lobe thickness in the same way and the secondary challenge was performed 6 days later. The mean value of both lobes thickness obtained from individual mice was used to express the results. Each test drug was given for 5 consecutive days commencing with the primary sensitization to evaluate its individual effect on the induction phase.

Local graft versus host reaction (GvH-R) : The experimental method as described by Ford et al.[12] was employed. The spleens of BALB/c mice and CBF_1 mice were surgically removed using aseptic technique, and passed through a stainless wire mesh (200 mesh) in Hank's balanced salt solution (Hank's BSS) containing 5 U/ml of heparin. Red blood cells were effectively hemolyzed by adding an adequate amount of 0.75% ammonium chloride-Tris solution. The suspension was then washed three times with Hank's BSS and the number of cells was adjusted to 1×10^8 cells/ml. Spleen cell suspensions were injected s.c. to the posterior footpads of CBF_1 mice. The right rear footpad of CBF_1 mouse was injected s.c. with 0.05 ml of BALB/c spleen cells and the left rear footpad received the same dose of CBF_1 spleen cells. Eight days later, the popliteal lymph nodes (PLN) were removed bilaterally and immediately weighed. The intensity of GvH-R was determined by subtracting the left PLN weight from that of the right. Each test drug was given for 8 days commencing with the cell-transfer.

Statistical analysis : The difference between the control group and the experimental groups were statistically evaluated using the Student's *t*-test.

Results

1. Effect of Simotu-tô and Ôren-gedoku-tô on PC-CD

For 5 consecutive days following the primary sensitization, 50−200 mg/kg of Simotu-tô and Ôren-gedoku-tô were administered daily to evaluate their effects on the induction phase of PC-CD. As illustrated by Fig. 1, even at high doses Simotu-tô showed no effect or a tendency to augment on the primary immune response, yet a significant inhibition was seen on the secondary immune response in doses of 50 and 200 mg/kg. On the other hand, Ôren-gedoku-tô showed a tendency to inhibit the primary immune response. The secondary immune response was significantly inhibited in each dose level employed. Used as a comparative, Unsei-in in a dose of 100 mg/kg significantly inhibited both the primary and secondary immune responses, while 10 mg/kg of prednisolone demonstrated an inhibitory effect on the secondary response.

2. Effects of Simotu-tô and Ôren-gedoku-tô on GvH-R

To study their effects on GvH-R, Simotu-tô and Ôren-gedoku-tô in doses of 50−200 mg/kg respectively were given for 8 days commencing with the cell-transfer. As shown in Fig. 2, Simotu-tô showed only a mild inhibition in each dose level, while Ôren-gedoku-tô showed a tendency to inhibit in 50 mg/kg and a significant inhibition in doses of 100 and 200 mg/kg. Likewise, 100 and 200 mg/kg of Unsei-in and 10 mg/kg of prednisolone which were used as comparatives showed a significant inhibitory activity.

3. Effects of Chinese herbs composed of Ôren-gedoku-tô on PC-CD

Since as shown in the results of 1 and 2, Ôren-gedoku-tô has a more potent inhibitory activity on the induction phase of PC-CD and GvH-R than that of Simotu-tô, individual Chinese herbs composed of Ôren-gedoku-tô were evaluated for their effect on PC-CD.

Ôren-gedoku-tô is composed of 4 kinds of Chinese herbs including Coptidis Rh., Phellodendri C., Scutellariae R. and Gardeniae F. The aqueous extract of each herb in doses of 100−500 mg/kg was given for 5 consecutive days following the primary sensitization. Regardless of the dos-

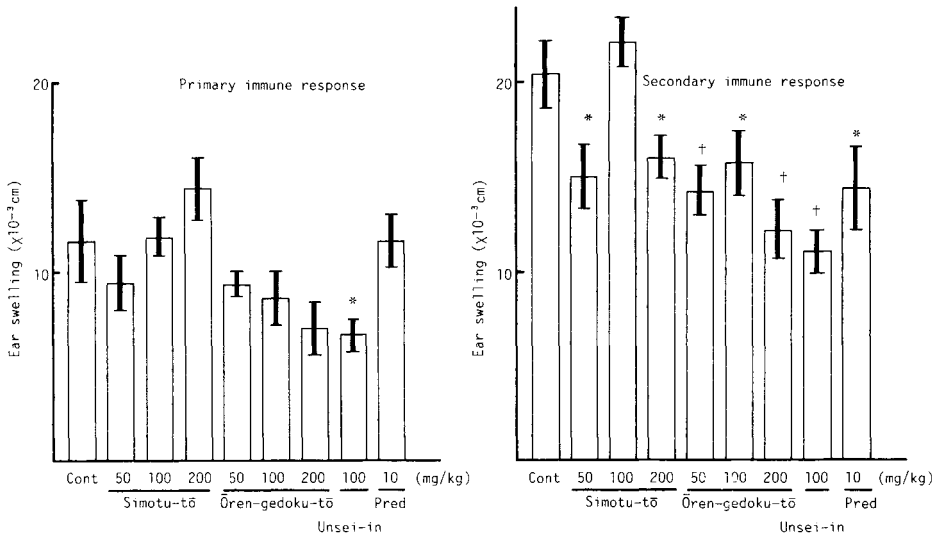

Fig. 1 Effect of Simotu-tô, Ôren-gedoku-tô, Unsei-in and prednisolone (Pred) on PC-induced cellular immune response in mice.

Drugs were given p.o. for 5 days following the first sensitization. Each group included 8 to 10 animals. *, †: Statistical significance from the control at $p<0.05$ and $p<0.01$, respectively.

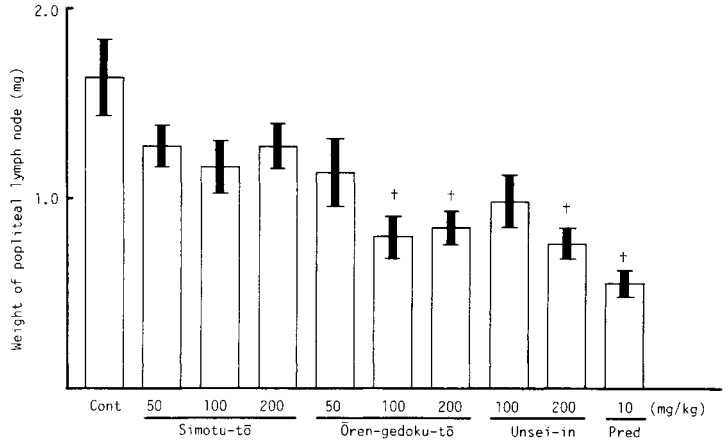

Fig. 2 Effect of Simotu-tô, Ôren-gedoku-tô, Unsei-in and prednisolone (Pred) on local graft versus host reaction (GvH-R) in mice.

Drugs were given p.o. for 8 days following the cell transfer. An increase in the weight of popliteal lymph node resulted in GVH-R was measured 9 days after the cell transfer. Each column indicates the mean±S.E. of 15 to 17 animals. *, † : Statistical significance from the control at $p<0.05$ and $p<0.01$, respectively.

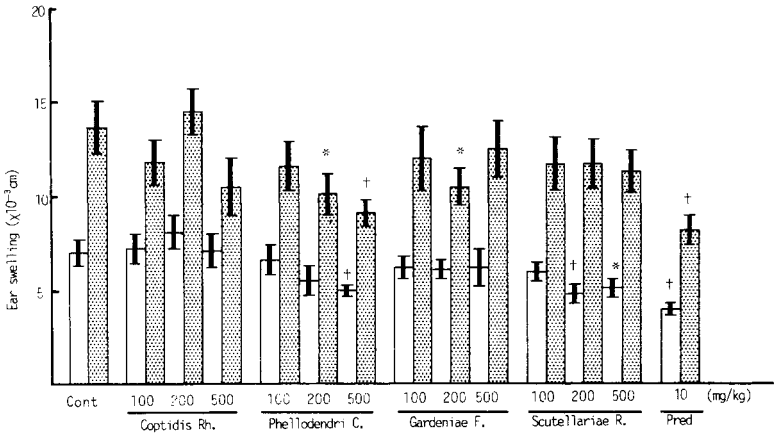

Fig. 3 Effect of Coptidis Rh., Phellodendri C., Gardeniae F., Scutellariae R. and prednisolone (Pred) on PC-induced cellular immune response in mice.

Drugs were given p.o. for 5 days following the first sensitization. Each group included 15 to 19 animals. *, † : Statistical significance from the control at $p<0.05$ and $p<0.01$, respectively. ☐ : primary immune response, ▨ : secondary immune response.

age, neither Coptidis Rh. nor Gardeniae F. had a significant influence on the primary immune response as shown in Fig. 3. On the other hand, Phellodendri C. exerted a dose-dependent inhibitory activity which was significant in 500 mg/kg. Scutellariae R. was also found to have a significant inhibitory activity in doses of 200 and 500 mg/kg. When the secondary immune response was evaluated, no effect was seen in any dose with the administration of either Coptidis Rh. or

Scutellariae R. On the other hand, Phellodendri C. inhibited the secondary immune response in a dose-dependent fashion which was significant in doses of more than 200 mg/kg. Gardenia F. also exerted a significant inhibition in a dose of 200 mg/kg, though a dose-dependent inhibition was not observed. A comparative, 10 mg/kg of prednisolone, displayed a significant inhibitory activity on both the primary and secondary immune responses.

4. Effect of Phellodendri C. on GvH-R

Of all Chinese herbs composed of Ôren-gedoku-tô, Phellodendri C. had the most dramatic effect on the induction phase of PC-CD through the primary and secondary immune responses as shown in Result 3. Therefore 100—500 mg/kg of Phellodendri C. was administered for 8 days commencing with the day of the cell transfer to observe its effect on GvH-R. Fig. 4 shows what a dose-dependent inhibitory activity with the activity being significant in doses more than 200 mg/kg. The inhibitory activity by Phellodendri C. in a dose of 200 mg/kg was approximately equal to that of prednisolone in 10 mg/kg.

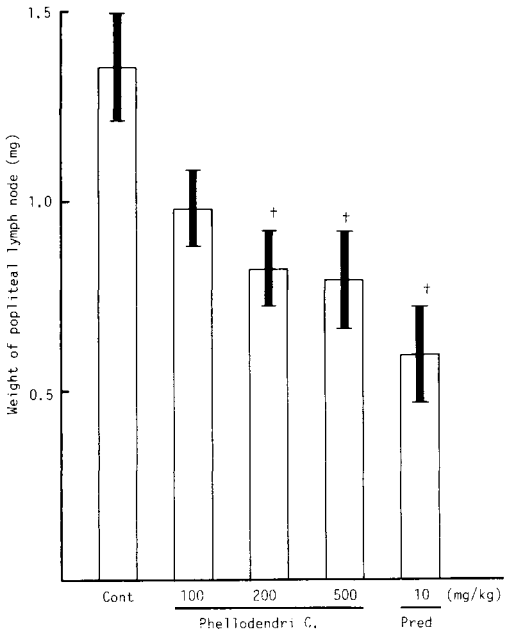

Fig. 4 Effect of Phellodendri C. and prednisolone (Pred) on local graft versus host reaction (GvH-R) in mice.

Drugs were given p.o. for 8 days following the cell transfer. An increase in the weight of popliteal lymph node resulted in GvH-R was measured 9 days after the cell transfer. Each column indicates the mean±S.E. of 9 to 11 animals. †: Statistical significance from the control at $p<0.01$.

Discussion

Long term immunosuppressive therapy is the accepted treatment for Behçet's syndrome. Unfortunately the chronic use of immunosuppressive drugs including glucocorticoids, cyclophosphamide, colchicine and others is associated with serious side effects.[4-6] The combination use of these drugs with Unsei-in, a Chinese blended medicine, has negligible side effects and has been reported to improve the oral, genital ulcers and digestive symptoms of Lipschutz disease, which is described as an incomplete type of Behçet's syndrome.[7-9] In our previous immunopharmacological study on Unsei-in, we obtained the following results.

1) Unsei-in had little effect on the effector phase of the type IV hypersensitivity reaction but clearly inhibited the induction phase.

2) It exhibited a dose-dependent inhibition of GvH-R.

3) It showed only a tendency to inhibit the humoral antibody production. From these findings a study on the search for the most potent of the Chinese herbs composed of Unsei-in and the isolation of its principal substances may lead to the development of a drug with specific inhibitory activity on cellular immune response.

The present study was carried out to identify the most potent of the Chinese herbs composed of Unsei-in regarding 1) and 2) reactions. Because Unsei-in is a combination of Ôren-gedoku-tô and Simotu-tô, the individual effect of each was studied on PC-CD and GvH-R. The reason is not clear why a dose-related inhibition of Ôren-gedoku-tô has not been observed on the secondary immune response of the former reaction. On the whole, the activity of Ôren-gedoku-tô showed a tendency to be potent compared to that of Simotsu-tô. Although Unsei-in inhibited significantly

not only the primary response but also the secondary one, the components, Simotsu-tô and Ôren-gedoku-tô, did not show a significant inhibition of the primary response. There may be certain synergisms in the inhibition mechanism of Unsei-in. On the latter reaction, even a high dose of Simotu-tô such as 200 mg/kg showed an only tendency to inhibit the reaction, while Ôren-gedoku-tô in doses more than 100 mg/kg significantly inhibited. Of the two, Ôren-gedoku-tô was discovered to have more potent inhibitory activity on these two reactions. The four subcomponents of Ôren-gedoku-tô were then examined for their effects on PC-CD. Among them, Phellodendri C. was shown to have the most potent inhibitory activity on the PC-CD, although a significant inhibitory dose was more than 200 mg/kg. There may also be a synergism in the inhibition of PC-CD by Ôren-gedoku-tô or Unsei-in, because Oren-gedoku-tô and Unsei-in inhibited the reaction significantly in doses of less than 200 mg/kg and 100 mg/kg, respectively. Phellodendri C. showed the inhibition of GvH-R dose-dependently. Attempts are, therefore, now under investigation to find the principal substances of Phellodendri C.

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和文抄録

本研究では，IV型アレルギー反応の誘導期およびGvH・Rに対する個々の温清飲構成生薬の抑制作用を検討し，以下の成績を得た。1）温清飲は四物湯（Si-Wu-Tang）および黄連解毒湯（Huang-Lian-Jie-Du-Tang）の合剤であるので，それぞれの方剤のPCによるマウスの接触性皮膚炎に及ぼす影響を検討した。四物湯に比して黄連解毒湯は一次および二次免疫応答を強く抑制することを見出した。2）GvH・Rに対して四物湯は抑制傾向を示すにすぎなかったが，黄連解毒湯は有意な抑制作用を示した。3）4種の黄連解毒湯構成生薬のうちで，黄柏はPCによる接触性皮膚炎の一次および二次免疫応答を最も強く抑制した。また，黄柏はGvH・Rをも抑制した。以上の成績から，湯清飲構成生薬中で黄柏は細胞性免疫反応抑制作用の最も強い生薬であることが明らかである。

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