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Effects of Kampô-hôzai (traditional Chinese medicine) on immune responses
In vivo studies of Syô-saiko-tô and Dai-saiko-tô on antibody responses to sheep red blood cell and lipopolysaccharide

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Abstract

The prednisolone-induced suppression of antibody responses to sheep red blood cell (SRBC) in mice was restored by Syô-saiko-tô and Dai-saiko-tô. On the other hand, the suppression of antibody responses to lipopolysaccharide (LPS) induced by cyclophosphamide was restored by Dai-saiko-tô, although the carrageenan-induced reduction of antibody responses to LPS was restored by Syô-saiko-tô. In addition, Syô-saiko-tô showed a phagocytosis enhancing effect as determined by the carbon clearance test. These results suggest that Syô-saiko-tô and Dai-saiko-tô show different effect on immune responses. Generally, Syô-saiko-tô is conceivable to augment the immune response by affecting macrophage function while Dai-saiko-tô enhance immune response by different manner.

Key words carbon clearance test, Dai-saiko-tô, hemolytic plaque forming cell, immune response, lipopolysaccharide, sheep red blood cell, Syô-saiko-tô

Abbreviations HPFC, hemolytic plaque forming cell ; HPLC, high performance liquid chromatography ; LPS, lipopolysaccharide ; SRBC, sheep red blood cell ; Dai-saiko-tô (Da-Chai-Hu-Tang), 大柴胡湯 ; Syô-saiko-tô (Xiao-Chai-Hu-Tang), 小柴胡湯

Introduction

Syô-saiko-tô (Xiao-Chai-Hu-Tang) and Dai-saiko-tô (Da-Chai-Hu-Tang) are well known Kampô-hôzai (traditional Chinese medicines) which contains various components extracted from several plants and used frequently for the treatment of chronic diseases in Japan and China. Especially, these medicines have been used for the treatment of chronic hepatitis and nephritis, although their pharmacological basis is poorly understood. In our previous paper, we reported that Syô-saiko-tô increased the anti-inflammatory action of prednisolone[1] and reversed the adrenal cortex atrophy induced by prednisolone.[1,2] It is thought that the stimulatory action of Syô-saiko-tô on the pituitary-adrenocortical axis plays an important role in the combination therapy of prednisolone and Syô-saiko-tô.

In current experiments, the effects of Syô-saiko-tô and Dai-saiko-tô on the immune response of mice against T cell-dependent antigen, sheep red blood cell (SRBC), and a T cell-independent antigen, lipopolysaccharide (LPS), were investigated. Furthermore, the effects of Syô-saiko-tô and Dai-saiko-tô on phagocytosis were also studied.

Materials and Methods

Animals : Female Balb/c mice, five weeks old, and male ICR mice, eight weeks old, were purchased from the Charles River (Tokyo, Japan) and given standard laboratory chow and tap water *ad libitum*.

Preparation of Kampô-hôzai : The herbs which were given from Daiko-Shoyaku Co., Ltd.

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Table I Components of herbs in Kampô-hôzai.

	Syô-saiko-tô	Dai-saiko-tô
Bupleuri Radix (*Bupleurum chinense* DC., China)	7	6
Pinelliae Tuber (*Pinellia ternata* BREITENBACH, China)	5	4
Zingiberis Rhizoma (*Zingiber officinale* ROSCOE, Japan)	4	4
Scutellariae Radix (*Scutellaria baicalensis* GEORGI, China)	3	3
Zizyphi Fructus (*Zizyphus vulgaris* LAM. var *inermis* BUNGE, China)	3	3
Ginseng Radix (*Panax ginseng* C.A. MEYER, Korea)	3	
Glycyrrhizae Radix (*Glycyrrhiza glabra* L. var. *grandulifera* REGEL et HERDER, China)	2	
Paeoniae Radix (*Paeonia lactiflora* PALLAS, Japan)		3
Auranti Fructus Immaturus (*Citrus aurantium* L. var. *daidai* MAKINO, Japan)		2
Rhei Rhizoma (*Rheum tanguticum* MAXIM. ex BALF., China)		2

Each number indicated in the Table shows the gram of composed crude drug per daily dose of human.

(Nagoya, Japan) were mixed as indicated in Table I, suspended in 700 ml of water, and extracted at 100℃ for 1 hr. The extracts were then concentrated to 300 ml followed by lyophilization to produce powdered extracts. Yields were 7.2 g in Syô-saiko-tô and 8.0 g in Dai-saiko-tô, respectively. These are the standard human daily doses.

Quantitative analysis of main components in Kampô-hôzai : Powdered extracts of Syô-saiko-tô, 0.12 g, and those of Dai-saiko-tô, 0.13 g, were dissolved in 5 ml of distilled water.

After centrifugation at $1200\times g$ for 10 min, 2 ml of supernatant solution (for the analysis of saikosaponins and glycyrrhizin) was injected into a C_{18}-cartridge column (TSK-gel ODS, Toyo Soda Mfg. Co., Ltd., Tokyo, Japan), washed with 4 ml of water and eluted with 4 ml of methanol. The eluted methanolic solution was then passed through a membrane filter (0.45 μm, Toyokagaku Sangyo Co., Ltd., Tokyo, Japan). The filtrate was injected into a high performance liquid chromatography (HPLC) column (stainless steel column, 250×4 mm i.d.). A Shimadzu Model 4A chromatograph equipped with a Shimadzu Model SPD 2A ultraviolet detector was employed. Peak area was measured using a Shimadzu Model 4A computing integrator. For the analysis of paeoniflorin, the supernatant solution obtained by the centrifugation of aqueous extracts of Kampô-hôzai was passed through a membrane filter and the filtrate was injected into a same HPLC column. Other analytical conditions were as follows :

a) Analysis of saikosaponins[3] Packing : Hypersil ODS (5 μm, Erma Optical Works Ltd.), Mobile phase : acetonitrile-water (46 : 54), Column temperature : 50℃, Flow rate : 1.0 ml/min, Chart speed : 2.5 ml/min, Detection wavelength : 210 and 254 nm, Sensitivity : 0.005 absorbance unit full scale (a.u.f.s.).

b) Analysis of glycyrrhizin[4,5] Packing : Develosil ODS (5 μm, Nomura Chem. Co., Ltd., Aichi, Japan), Mobile phase : acetonitrile-0.03% sulfuric acid solution (37 : 63), Column temperature : 45℃, Flow rate : 1.0 ml/min, Chart speed : 2.5 ml/min, Detection wavelength : 254 nm, Sensitivity : 0.005 a.u.f.s.

c) Analysis of paeoniflorin[6] Packing : Hypersil ODS (5 μm, Erma Optical Works Ltd., Tokyo, Japan), Mobile phase : acetonitrile-0.03% sulfuric acid solution (15 : 85), Column temperature : 50℃,

Flow rate : 1.0 ml/min, Chart speed : 2.5 ml/min, Detection wavelength : 230 nm, Sensitivity : 0.005 a.u.f.s.

Administration of medicines : Syô-saiko-tô and Dai-saiko-tô were dissolved in distilled water and administered orally using a stomach tube. The doses of Syô-saiko-tô were 0.24 g/kg body weight or 1.2 g/kg body weight and those of Dai-saiko-tô were 0.26 g/kg body weight and 1.3 g/kg body weight. As a positive control, levamisole (Aldrich Chemical Co., Inc., Milwaukee, USA) was given orally at doses of 0.001, 0.01 and 0.03 g/kg body weight in a same manner as mentioned above. The doses of Kanpô-hôzai are roughly equal to two and ten times the human dose per day. Cyclophosphamide (Nakarai Chem. Co., Kyoto, Japan) was suspended in 5%-tween 80 and injected subcutaneously at a dose of 0.01 g/kg body weight for three days from 1 day before to 1 day after antigenic stimulation. Carrageenan (Sigma Chem. Co., St. Louis, USA) was dissolved in physiological saline at the concentration of 0.25 mg/ml, sterilized by autoclaving and an aliquot, 0.2 ml (0.0025 g/kg), was given into each mouse intraperitoneally at 3 day and 1 day before antigenic stimulation.[7]

Antigens and immunization : SRBC was purchased from Nippon Bio. Supp. Center Co., Ltd. (Tokyo, Japan). SRBC stored in Alsever's solution at 3−6℃ was washed with saline by centrifugation at $180\times g$ for 10 min. This procedure was repeated three times and SRBC suspension (5×10^8/ml) was prepared. SRBC suspension, 0.2 ml (1×10^8 cells) was injected into mice intravenously. Lipopolysaccharide (LPS) of *E. coli* 055 : B5 was obtained from Difco Laboratories (Michigan, USA). The alkaline-treated LPS was prepared according to the method of Neter.[8] Ten micrograms of LPS was injected intravenously for immunization.

Preparation of spleen cell suspension : Mice were sacrificed by exsanguination from the carotid arteries. Their spleens were aseptically removed, shredded with scissors and strained through a 60-gauge nylon sieve in Eagle's minimal essential medium (Eagle's MEM) (Research Foundation for Microbiol. Diseases of Osaka University, Osaka, Japan) adjusted pH to 7.2 with sodium bicarbonate. Further disruption was achieved by gentle aspiration with a pasteur pipette. The single cells thus obtained were washed twice with Eagle's MEM by centrifugation for 10 min at $180\times g$, resuspended and counted under a microscope.

Assay of the antibody forming cells : Hemolytic plaque forming cell (HPFC) was determined at 4 or 5 day after antigenic stimulation by the method of Cunningham and Szenberg[9] with a slight modification.[10] For the assay of anti-LPS HPFC, SRBC was coated with LPS according to the method of Andersson and Blomgren.[11] Three small compartments each with a capacity of 20 μl, covered with cover glass, were made on a slide glass. An aliquot of a mixture of 0.2 ml of 1×10^7/ml of spleen cells, and 25 μl of SRBC, 1×10^{10}/ml, or LPS-SRBC, 1×10^{10}/ml, was packed into the three compartments. After the addition of a mixed solution without spleen cells to the remaining space of the compartment and closing its hole with paraffin, the slide glass was incubated at 37℃ for 1 hr. After that, the number of HPFC was determined under a microscope.

Assay of HA titer : HA titer was assayed by the methods described by Ceglowski and Friedman.[12] Blood was collected from the mouse carotid artery and centrifuged at $6000\times g$ for 10 min. The serum obtained was inactivated at 56℃ for 60 min. After a two-fold serial dilution of serum with 0.15 M phosphate buffered saline (PBS, pH 7.2), 0.025 ml of SRBC (2×10^8/ml) was added to 0.05 ml of each diluted solution and mixed. After standing at 10℃ for 18 hr, HA titer was assayed. 7S HA titer was assayed by adding 0.2 M 2-mercaptethanol to the inactivated serum, 0.025 ml, and allowing to stand at 37℃ for 1 hr.[13]

Phagocytic activity assay : The phagocytic function was measured by the method of Biozzi *et al*.[14] Colloidal carbon (Pelikan Drawing Ink., Gunther Wagner, Germany) was injected into tail vein of mice and 25 μl of blood samples were taken from the orbital veins 3, 6, 9, 12, and 15 min after the carbon injection.

The blood was discharged into 5 ml of 0.1%

(w/v) Na_2CO_3 solution and the absorbance at 675 nm was determined. The phagocytic activity is expressed as the phagocytic index, K, which was calculated by means of the following equation:

$$K=(\ln OD_1-\ln OD_2)/(t_2-t_1),$$

where OD_1 and OD_2 are the optical densities at time t_1 and t_2, respectively.

Results

Quantitative analysis of main components in Kampô-hôzai

To evaluate Kampô-hôzai used in this experiment, the main components, glycyrrhizin, saikosaponin a, saikosaponin b_2 and paeoniflorin, were determined by HPLC. As shown in Table II, the contents of glycyrrhizin, saikosaponin a and saikosaponin b_2 in the extracts of Syô-saiko-tô compared to each dried herb (Glycyrrhizae Radix, 2 g, and Bupleuri Radix, 7 g) were 1.5%, 0.033% and 0.034%, respectively. The contents of paeoniflorin, saikosaponin a and saikosaponin b_2 in the extracts of Dai-saiko-tô compared to each dried herb (Paeoniae Radix, 3 g, Bupleuri Radix, 6 g) were 2.5%, 0.013% and 0.031%, respectively.

Judging from the average content of glycyrrhizin in dried Glycyrrhizae Radix is 1.0 − 5.0 %, that of saikosaponin a and b_2 in dried Bupleuri Radix was 0.01 − 0.05% and that of paeoniflorin in dried Paeoniae Radix is 2 − 5%, the two Kampô-hôzai used in this experiment possess average quality.

Effects on antibody responses to SRBC

The effects of Kampô-hôzai on antibody response to SRBC were investigated by evaluating HPFC and HA titer. Kampô-hôzai and levamisole were administered to ICR mice orally for 7 days from 2 day before to 4 day after antigenic stimulation. All doses of Syô-saiko-tô and Dai-saiko-tô tested did not cause any change

Table II Contents of glycyrrhizin, saikosaponin a, saikosaponin b_2 and paeoniflorin in Kampô-hôzai.

	Components (mg/human dose/day and % against dry weight)							
	Glycyrrhizin		Saikosaponin a (%)		Saikosaponin b_2 (%)		Paeoniflorin (%)	
Syô-saiko-tô	30.42±0.27	1.5	2.30±0.15	0.033	2.38±0.08	0.024		
Dai-saiko-tô			0.80±0.11	0.013	1.86±0.07	0.031	76.35±0.30	2.5

Each datum indicates the mean±S.E.M. of three samples.

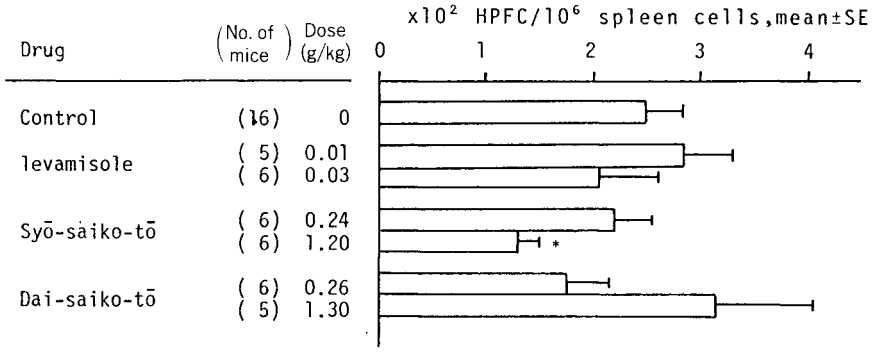

Fig. 1 Effects of Kampô-hôzai on the development of HPFC to SRBC in ICR mice.
Each group of ICR mice was immunized intravenously with 10^8 SRBC and given Kampô-hôzai and levamisole orally for 7 days from 2 day before to 4 day after antigenic stimulation. The HPFC in the spleen cells was assessed on day 5.
* : $p<0.05$ vs. control

in both spleen weight and the number of spleen nucleated cell. When 1.2 g/kg body weight of Syô-saiko-tô was given, decrease in the number of HPFC to SRBC was observed as shown in Fig. 1, but 0.24 g/kg body weight of Syô-saiko-tô and 0.26 or 1.3 g/kg body weight of Dai-saiko-tô did not affect on the number of HPFC to SRBC. Such change in antibody responses to SRBC was not detectable when effects of Syô-saiko-tô and Dai-saiko-tô were estimated by HA titer as shown in Fig. 2. These results suggested that Syô-saiko-tô and Dai-saiko-tô might give little effect on immune responses of normal individuals. We therfore investigated the effects of those Kampô - hôzai on the decreased antibody responses induced by glucocorticoid. Prednisolone was given subcutaneously for 3 days from 1 day before to 1 day after antigenic stimulation.

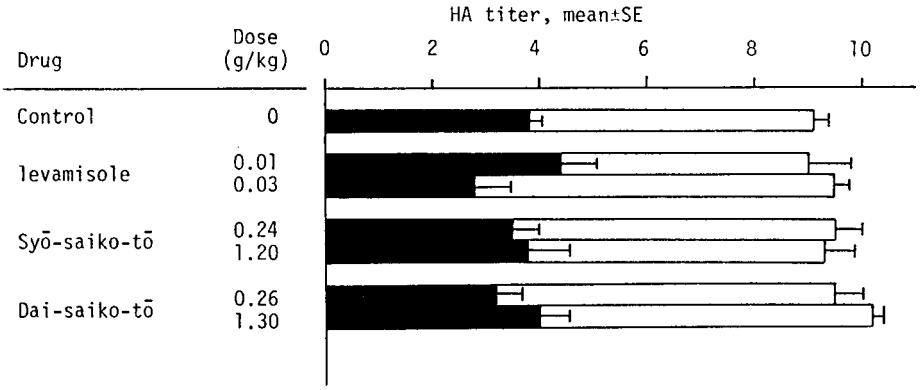

Fig. 2 Effects of Kampô-hôzai on HA titer to SRBC in ICR mice.
Each group of ICR mice was immunized intravenously with 10^8 SRBC and given Kampô-hôzai and levamisole orally for 7 days from 2 day before to 4 day after antigenic stimulation. HA titer in the serum was assessed on 5 day.
■ : 7S HA titer, □ : 19S HA titer

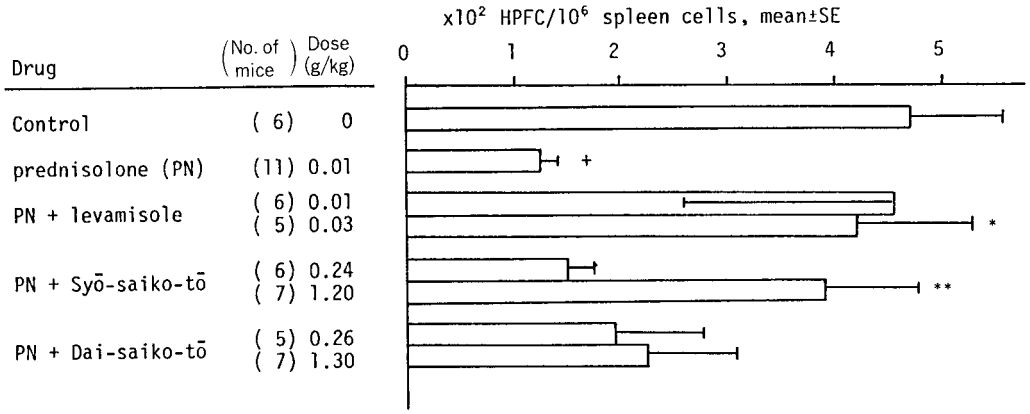

Fig. 3 Effects of Kampô-hôzai on the development of HPFC to SRBC in ICR mice treated with prednisolone, 0.01 g/kg.
Each group of ICR mice was immunized intravenously with 10^8 SRBC and given Kampô-hôzai and levamisole orally for 7 days from 2 day before to 4 day after antigenic stimulation. Prednisolone was given subcutaneously for 3 days from 1 day before to 1 day after antigenic stimulation. The HPFC in the spleen cells was assessed on day 5.
+ : $p<0.01$ vs. control
* : $p<0.05$, ** : $p<0.01$ vs. prednisolone-treated group

All doses of Syô-saiko-tô and Dai-saiko-tô did not restore the decrease in spleen weight and the number of spleen nucleated cell induced by prednisolone. When 0.01 g/kg body weight of prednisolone was injected to mice, the number of HPFC was decreased remarkably as shown in Fig. 3. By the combination of 0.01 or 0.03 g/kg body weight of levamisole and 0.01 g/kg body weight of prednisolone, the suppression of the number of HPFC induced by prednisolone was restored. Furthermore, 1.2 g/kg body weight of Syô-saiko-tô restored the suppression of the number of HPFC induced by 0.01 g/kg body weight of prednisolone. Furthermore, 1.2 g/kg body

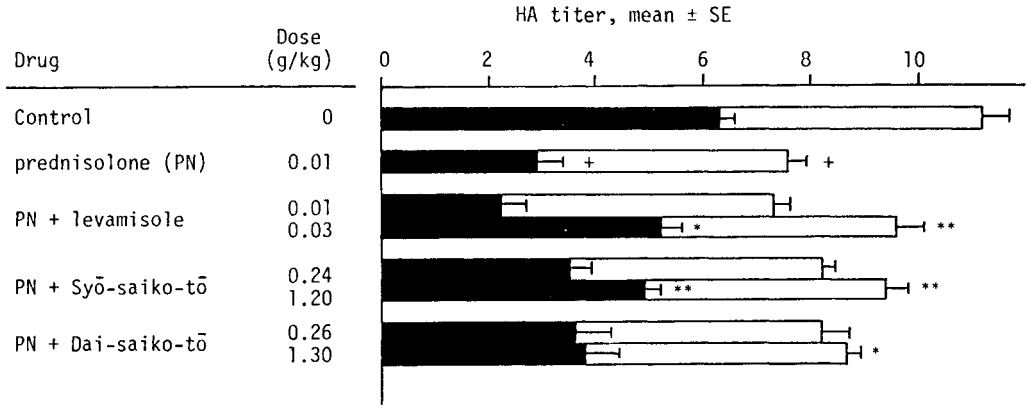

Fig. 4 Effects of Kampô-hôzai on HA titer to SRBC in ICR mice treated with prednisolone, 0.01 g/kg.

Each group of ICR mice was immunized intravenously with 10^8 SRBC and given Kampô-hôzai and levamisole orally for 7 days from 2 day before to 4 day after antigenic stimulation. Prednisolone was given subcutaneously for 3 days from 1 day before to 1 day after antigenic stimulation. HA titer in the serum was assessed on 5 day.

■ : 7S HA titer, □ : 19S HA titer

+ : $p<0.01$ vs. control, * : $p<0.05$, * * : $p<0.01$ vs. prednisolone-treated control

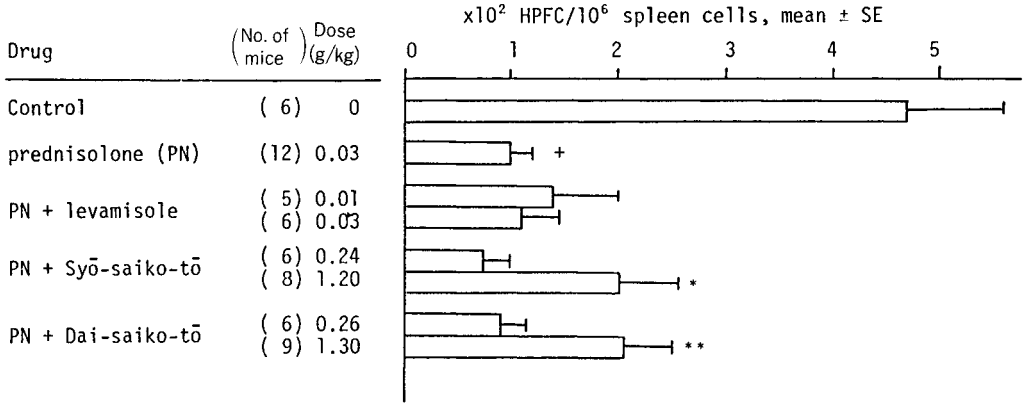

Fig. 5 Effects of Kampô-hôzai on the development of HPFC to SRBC in ICR mice treated with prednisolone, 0.03 g/kg.

Each group of ICR mice was immunized intravenously with 10^8 SRBC and given Kampô-hôzai and levamisole orally for 7 days from 2 day before to 4 day after antigenic stimulation. Prednisolone was given subcutaneously for 3 days from 1 day before to 1 day after antigenic stimulation. The HPFC in the spleen was assessed on 5 day.

+ : $p<0.01$ vs. control

* : $p<0.05$, * * : $p<0.01$ vs. prednisolone-treated group

weight of Syô-saiko-tô restored the suppression of 7S HA titer induced by 0.01 g/kg body weight of prednisolone as shown in Fig. 4. Dai-saiko-tô also restored the suppression of total HA titer at a dose of 1.3 g/kg body weight, which was expected to be due to the restoration of 7S HA titer. By the combination of 1.2 g/kg body weight of Syô-saiko-tô or 1.3 g/kg body weight of Dai-saiko-tô and 0.03 g/kg body weight of prednisolone, the suppression of the number of HPFC induced by prednisolone was restored as shown in Fig. 5. However, the decreased 7S HA titer by 0.03 g/kg body weight of prednisolone was not restored by both Kampô-hôzai as shown in Fig. 6.

Effects on antibody responses to LPS

The effects of Kampô-hôzai on antibody responses to LPS were studied by evaluating the number of HPFC. Levamisole and Kampô-hôzai were administered to Balb/c mice orally for 6 days 2 day before to 3 day after antigenic stimulation. Levamisole and Kampô-hôzai did not

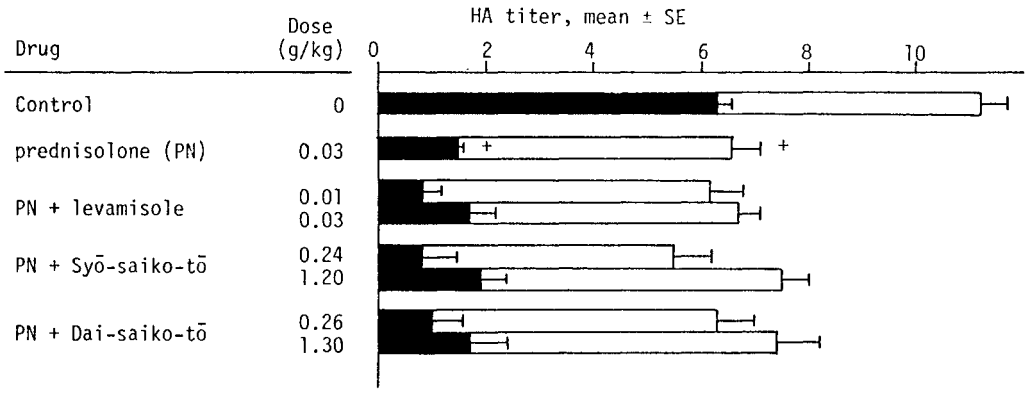

Fig. 6 Effects of Kampô-hôzai on HA titer to SRBC in ICR mice treated with prednisolone, 0.03 g/kg.
Each group of ICR mice was immunized intravenously with 10^8 SRBC and given Kampô-hôzai and levamisole orally for 7 days from 2 day before to 4 day after antigenic stimulation. Prednisolone was given subcutaneously for 3 days from 1 day before to 1 day after antigenic stimulation. HA titer in the serum was assessed on 5 day.
■ : 7S HA titer, □ : 19S HA titer
+ : $p<0.01$ vs. control

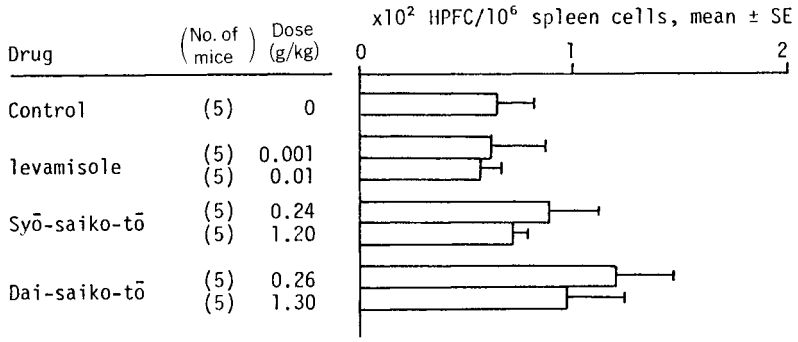

Fig. 7 Effects of Kampô-hôzai on the development of HPFC to LPS in Balb/c mice.
Each group of mice was immunized intravenously with LPS at a dose of 10 μg/mouse and treated orally with Kampô-hôzai and levamisole for 6 consecutive days from 2 day before to 3 day after antigenic stimulation. The HPFC in the spleen cells was assessed on 4 day.

cause any change in both spleen weight and the number of spleen nucleated cell. Although levamisole showed no increasing effect on the number of HPFC, Syô-saiko-tô and Dai-saiko-tô showed a tendency to increase the number of HPFC as shown in Fig. 7. Syô-saiko-tô elevated the number of HPFC by 36% at a dose of 0.24 g/kg body weight and Dai-saiko-tô increased it by 80 and 47% at doses of 0.26 and 1.3 g/kg body weight, respectively. However, there was no significant difference between control and the Kampô-hôzai treated group.

Therefore the effects of Kampô-hôzai on the suppression of antibody responses to LPS induced by cyclophosphamide were examined. Levamisole and Kampô-hôzai were administered for 6 days from 2 day before to 3 day after antigenic stimulation to mice treated with 0.01 g/kg body weight of cyclophosphamide for 3 days from 1 day before to 1 day after antigenic stimulation. The number of HPFC was determined 4 day after antigenic stimulation. Although, spleen weight and the number of spleen nucleated cell were decreased by the treatment with cyclophosphamide, the suppressed spleen weight and the number of spleen nucleated cell were not restored by the treatment with levamisole and Kampô-hôzai. However, the suppressed number of HPFC by cyclophosphamide was restored by 1.3 g/kg body weight of Dai-saiko-tô. On the other hand, Syô-saiko-tô and levamisole did not affect the suppressed number of HPFC to LPS induced by cyclophosphamide.

Next, the effects of Kampô-hôzai on the suppression of antibody responses to LPS induced by carrageenan which was cytotoxic for macrophages were investigated. Levamisole and Kampô-hôzai were administered for 6 days from 2 day before to 3 day after antigenic stimulation to mice treated with 0.0025 g/kg body weight of carrageenan intraperitoneally 3 day and 1 day before antigenic stimulation. Carrageenan did not cause any change in both spleen weight and the number of spleen nucleated cell. All doses of levamisole and Kampô-hôzai tested also did not affect on the spleen weight and the number of spleen nucleated cell. However, carrageenan suppressed the number of HPFC to LPS remarkably as shown in Fig. 9. When 0.01 g/kg body weight of levamisole or 0.24 and 1.2 g/kg body weight of Syô-saiko-tô were given, restoration of the suppressed number of HPFC by carrageenan was observed. But, Dai-saiko-tô did not affect the number of HPFC that was suppressed by

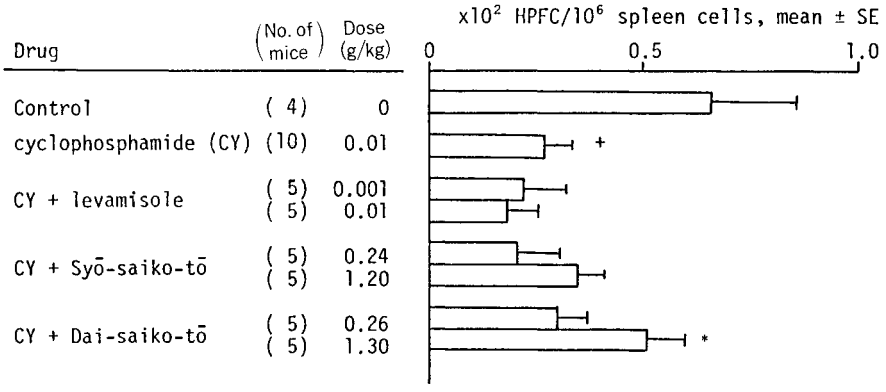

Fig. 8 Effects of Kampô-hôzai on the development of HPFC to LPS in Balb/c mice treated with cyclophosphamide.

Each group of mice was immunized intravenously with LPS at a dose of 10 μg/mouse and treated orally with Kampô-hôzai and levamisole for 6 consecutive days from 2 day before to 3 day after antigenic stimulation. Cyclophosphamide was given subcutaneously for 3 consecutive days from 1 day before to 1 day after antigenic stimulation. The HPFC in the spleen cells was assessed on 4 day.

+ : $p<0.05$ vs. control, * : $p<0.05$ vs. cyclophosphamide-treated group.

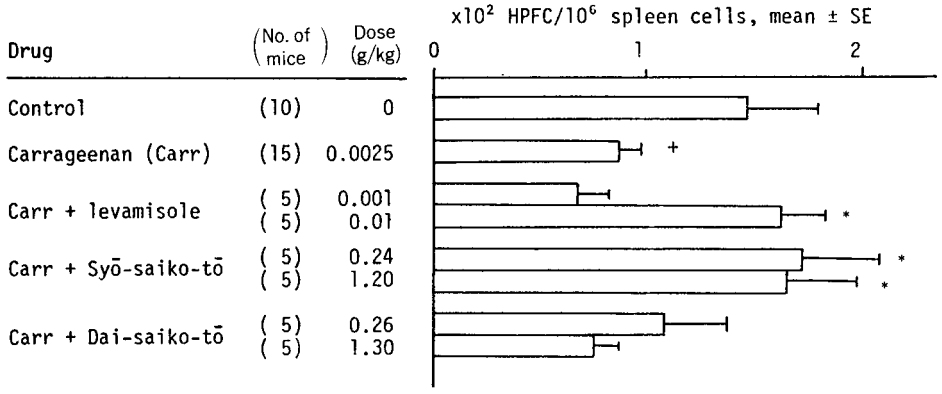

Fig. 9 Effects of Kampô-hôzai on the development of HPFC to LPS in Balb/c mice treated with carrageenan.
Each group of mice was immunized intravenously with LPS at a dose of 10 μg/mouse and treated orally with Kampô-hôzai and levamisole for 6 consecutive days from 2 day before to 3 day after antigenic stimulation. Carrageenan was given intraperitoneally on 3 and 1 day before antigenic stimulation. The HPFC in the spleen cells was assessed on 4 day.
+ : $p<0.05$ vs. control, * : $p<0.01$ vs. carrageenan-treated group

Table III Effects of Kampô-hôzai on carbon clearance activity.

Drug	Dose (g/kg)	Route	No. of mice	K Index
Control	0	p.o.	7	0.020±0.001
Syô-saiko-tô	0.24	p.o.	9	0.028±0.003[a)]
	1.20	p.o.	7	0.019±0.002
Dai-saiko-tô	0.26	p.o.	8	0.021±0.002
	1.30	p.o.	5	0.025±0.004
Control (saline)	0	i.p.	8	0.016±0.001
Zymosan	0.05	i.p.	6	0.041±0.004[b)]

Kampô-hôzai was administered orally for eight successive days. Zymosan was injected intraperitoneally for eight successive days. The carbon clearance activities were determined one hour after the last treatment.
a) : $p<0.05$, b) : $p<0.01$ vs. control.

carrageenan.

Effects on carbon clearance activity

To examine the effects of Kampô-hôzai on carbon clearance activity, mice were administered Kampô-hôzai orally and zymosan intraperitoneally for 8 successive days. The carbon clearance activities were determined 1 hour after administration. As shown in Table III, 0.05 g/kg body weight of zymosan enhanced the carbon clearance activity remarkably. Furthermore, 0.24 g/kg body weight of Syô-saikô-tô showed a weak enhancing activity compared with zymosan. On the other hand, the high dose of Syô-saiko-tô did not cause any effect. Both doses of Dai-saiko-tô also gave little effect on carbon clearance activity.

Discussion

Kampô-hôzai (traditional Chinese medicines), aqueous extracts of a mixture of natural crude drugs, have been used as drug therapy for some thousand years in China and is administered orally as decoction. Numerous components in Kampô-hôzai with various interactions make it quite difficult to elucidate its mechanism of

action. But, the pharmacological actions should be elucidated since hundreds of Kampô-hôzai are widely applied in Japan and China. Therefore, we have investigated the pharmacological action of Kampô-hôzai using *in vivo* methods.

In the present study, Syô-saiko-tô and Dai-saiko-tô restored the immunosuppression induced by prednisolone in the experiment using SRBC, a T cell dependent antigen. In a HA titer study, both Kampô-hôzai restored the decrease in 7S HA titer by prednisolone. These data show the possibility that both Kampô-hôzai stimulate T cell function. On the other hand, Syô-saiko-tô and Dai-saiko-tô showed a tendency to increase the number of HPFC to LPS by 36−80%. Since LPS has been reported to be a T cell-independent antigen and a polyclonal B cell mitogen,[11] the potentiation of the number of HPFC to LPS by these Kampô-hôzai may be due to the stimulation of B cell function. Goto *et al.*[15] reported that the LPS-SRBC PFC contained the LPS non-specific SRBC-PFC by 10% due to the polyclonal B cell activation by LPS. However, in our experimental conditions, the LPS-nonspecific SRBC-PFC was about 5% and both Kampô-hôzai showed no increasing effect on the LPS-nonspecific SRBC-PFC production. This data show that Syô-saiko-tô and Dai-saiko-tô do not stimulate B cell activity polyclonally in an *in vivo* experiment. However, Syô-saiko-tô and Dai-saiko-tô are reported to stimulate B cell activity polyclonally[16] in an *in vitro* method. Although, this report suggests that both Kampô-hôzai stimulate B cell activity polyclonally, more detailed investigations are needed to clarify these differences by both *in vivo* and *in vitro* methods.

Ishizaka *et al.*,[7] Rumjanek *et al.*[17] and Wong *et al.*[18] reported that most of the thymus-independent antigens were likely to be macrophage-independent. Among the thymus-independent antigens, LPS is the only exception. Ishizaka *et al.*[7] reported that the antibody response to TNP-LPS, a T cell-independent antigen, was inhibited by carrageenan which is cytotoxic for macrophages. The reason why this immune response is sensitive to carrageenan needs further investigation. This suggests that the response to TNP-LPS is also macrophage-dependent. In addition, Janezic *et al.*[19] reported that the antibody response to LPS in carrageenan-treated rats was reduced. Their conclusion was that LPS, a T cell-independent antigen, was macrophage-dependent. Cruchaud *et al.*[20] and Merluzzi *et al.*[21] reported that levamisole which stimulated macrophages and did not affect B cell elevated the number of HPFC to LPS. Our results obtained from the experiment using levamisole and carrageenan also suggest that the antibody response to LPS is macrophage-dependent. These results suggest that Syô-saiko-tô and Dai-saiko-tô stimulate B cell or macrophage function. On the other hand, cyclophosphamide, an inhibitor of B cell function, suppressed the number of HPFC to LPS. Levamisole and Syô-saiko-tô showed no effect on the suppressed antibody response induced by the pretreatment with cyclophosphamide. In contrast, Dai-saiko-tô restored the number of HPFC which was suppressed by cyclophosphamide. These results indicate the possibility that Dai-saiko-tô affects B cell function, but Syô-saiko-tô as well as levamisole dose not. Furthermore, the suppressed number of HPFC to LPS induced by the pretreatment with carrageenan was found to be restored by Syô-saiko-tô as well as levamisole. However, Dai-saiko-tô did not affect the suppression of antibody response by carrageenan. Although it is clear that macrophages are required for antibody synthesis *in vitro*,[22-25] in the generation of cytotoxic cells,[26] in the mixed lymphocyte reaction, in antigen or mitogen-induced lymphocyte proliferation[27-30] and in the cooperation of T and B cells,[31] their exact role in these processes is still not understood. The immunosuppressive effect of carrageenan may be derived from its action on macrophages because other reports[32] have shown that carrageenan is cytotoxic to macrophages but not to lymphocytes *in vitro*. Carrageenan is readily taken up by macrophages and stored in lysosomes, which subsequently swell and rupture, apparently resulting in cell death. Consequently, our data have suggested that Syô-saiko-tô augments the immune response by affecting macrophage functions *in vivo*. The results obtained from the carbon

clearance test also explain the stimulative action of Syô-saiko-tô on macrophage function. On the other hand, the results that both Kampô-hôzai restore the suppression of antibody responses to SRBC, a T cell-dependent antigen, by prednisolone shows that further studies regarding T cell function are needed. Furthermore, it is necessary to clarify whether these Kampô-hôzai repair the cell injury induced by immunosuppressors or that they potentiate the survival cell function which are not injured by immunosuppressors.

Syô-saiko-tô and Dai-saiko-tô contain five common crude drugs, Bupleuri Radix (Saiko), Pinelliae Tuber (Hange), Zizyphi Fructus (Taiso), Scutellariae Radix (Ogon) and Zingeberis Rhizoma (Shokyo). But, their immune actions have not been reported. On the other hand, Mizutani *et al.*[33] reported that the water soluble polysaccharide from Sanchi-Ginseng (Panax Notoginseng Radix) activated macrophage function. Takada and Kumagai[34] reported the immunosuppressive action of the fraction from Glycyrrhizae Radix (Kanzo), but there has been no report on the activation of macrophage function by the fraction of Glycyrrhizae Radix. Therefore, the characteristic action of Syô-saiko-tô on the immune response may be due to the components in Ginseng Radix. Furthermore, the plural ingredients involved in Syô-saiko-tô may have an affect on immune responses synergistically.

It is of great interest that Kampô-hôzai possess different mechanisms of immunostimulatory activity on macrophage, B lymphocyte and T lymphocyte by different combinations of crude drugs. However, the mechanisms of action of prednisolone, cyclophosphamide and carrageenan as immunosuppressors are not completely apparent and further studies of Kampô-hôzai on the effector cells are required.

和文抄録

羊赤血球（SRBC）に対する抗体産生のプレドニゾロンによる抑制は，小柴胡湯及び大柴胡湯により改善された。一方，T細胞非依存性抗原リポポリサッカライド（LPS）に対する抗体産生のシクロホスファミドによる抑制は，大柴胡湯により改善されたものの，小柴胡湯では改善されなかった。しかし，小柴胡湯はマクロファージ障害作用を有するカラゲニンによるLPSに対するHPFC産性の抑制を改善させた。さらに，小柴胡湯は，カーボンクリアランス試験においても促進効果を示した。これらの結果は，小柴胡湯，大柴胡湯が，免疫応答において異なる作用を有することを示している。すなわち，小柴胡湯はマクロファージに作用して抗体産生抑制を改善させるのに対し，大柴胡湯はマクロファージを介さずに，抗体産生抑制を改善している可能性が考えられる。

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