Effect of Kampo medicines on IgE-mediated biphasic cutaneous reaction in mice

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Abstract

The effect of 20 Kampo formulations on murine IgE-mediated biphasic cutaneous reaction was investigated in BALB/c mice. Mice were passively sensitized by an intravenous injection of monoclonal anti-dinitrophenol IgE antibody. Skin reactions were elicited by an epicutaneous challenge of dinitrofluorobenzene and occurred biphasically with immediate phase response (IPR) and late phase response (LPR) at 1 and 24 h, respectively. The inhibitory effect of 20 Kampo formulations on the biphasic cutaneous reaction was divided into three groups, expressed according to the inhibition rate of ear swelling. The first group included Sho-seiryu-to (小青竜湯), Toki-shakuyaku-san (当帰芍薬散), Byakko-ka-ninjin-to (白虎加人参湯) and Tokaku-joki-to (桃核承気湯), and significantly inhibited both IPR and LPR (IPR/LPR, +/+ group), similarly to the effect by prednisolone. The second group inhibited mainly LPR, but not IPR (-/+ group), and included Gorei-san (五苓散), Unsei-in (温清飲), Shimotsu-to (四物湯) and Ogi-kenchu-to (黄耆建中湯). The third group did not result in any inhibition of IPR and LPR (-/- group), and comprised Oren-gedoku-to (黄連解毒湯), Yoku-kan-san (抑肝散), Rokumi-gan (六味丸) and Inchinko-to (茵蔯蒿湯). These findings may be useful for the determination of treatment modality using Kampo medicines in some of the allergic diseases.

Key words Kampo formulations, IgE-mediated biphasic cutaneous reaction, anti-DNP IgE antibody, LPR.

Abbreviations DNP, dinitrophenol ; DNFB, dinitrofluorobenzene ; IPR, immediate phase response ; IL-1 β, interleukin-1β ; LPR, late phase response ; mAb, monoclonal antibody ; TNF-α,tumor necrosis factor-α.

Introduction

A recent increase of patients with chronic allergic diseases, such as bronchial asthma, allergic rhinitis and atopic dermatitis, has been reported.[1,2] Although glucocorticoids are effective for the treatment of chronic allergic disease, they cause several undesirable effects, such as decreased resistance to microbial infections, digestive ulcers, diabetes mellitus, osteoporosis, and dysfunction of the adrenal cortex.

To search for new antiallergic agents, we have investigated the effect of several plant materials and Kampo medicines on murine IgE-mediated cutaneous reaction. We recently reported that the administration of a nondialysable water extract of spikelets of *Miscanthus sinensis* significantly inhibited the IgE-mediated biphasic cutaneous reaction in mice.[3] In this model, passive sensitization with a murine monoclonal IgE antibody specific for the dinitrophenyl group (anti-DNP IgE mAb) followed by the challenge of dinitrofluorobenzene (DNFB) to mouse ears could

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induce biphasic cutaneous reactions with immediate phase reaction (IPR) and late phase reaction (LPR) at 1 and 24 h after the antigen challenge, respectively.[4] In addition, many Kampo medicines have been experimentally or clinically used for long-term treatment of chronic allergic disease, and some of their effectiveness has been reported.[5] But there has been no systematic report on the effects of Kampo formulations on allergic inflammation.

In the present study, to find effective Kampo formulations for LPR which is considered to be associated with severe or chronic allergic reactions, we examined the effect of 20 Kampo formulations on murine IgE-mediated biphasic cutaneous reaction.

Materials and Methods

Mice : Specific pathogen-free BALB/c mice (6 weeks old, female) were purchased from Japan SLC Inc., Hamamatsu, Japan, and maintained in the Laboratory for Animal Experiments, Research Institute for Wakan-yaku, Toyama Medical and Pharmaceutical University.

Antigens and chemicals : DNFB was purchased from Nacalai Tesque, Kyoto, Japan, and dissolved in 100 % ethanol. Prednisolone 21-acetate and diphenhydramine were purchased from Sigma Chemical Co., St. Louis, MO. Prednisolone 21-acetate were suspended in 0.5 % methylcellulose solution, and administered intraperitoneally 2 h prior to antigen challenge. Kampo medicines were kindly donated by Tsumura & Co. Ltd (Tokyo, Japan). Kampo formulations used in this study and their applications were listed in Table I : Sho-saiko-to (小柴胡湯 ; TJ-9, lot.no. 24009040), Saiko-keishi-to (柴胡桂枝湯 ; TJ-10, lot.no. 26010020), Saiko-keishi-kankyo-to (柴胡桂枝乾姜湯 ; TJ-11, lot.no. 25011010), Oren-gedoku-to (黄連解毒湯 ; TJ-15, lot.no. 250015010), Gorei-san (五苓散 ; TJ-17, lot.no. 260017010), Sho-seiryu-to (小青竜湯 ; TJ-19, lot.no. 260019030), Shofu-san (消風散 ; TJ-22, lot.no. 250022010), Toki-shakuyaku-san (当帰芍薬散 ; TJ-23, lot.no. 260023020), Byakko-ka-ninjin-to (白虎加人参湯 ; TJ-34, lot.no. 260034010), Hochu-ekki-to (補中益気湯 ; TJ-41, lot.no. 930041001PO), Juzen-taiho-to (十全大補湯 ; TJ-48, lot.no. 260048010), Keigai-rengyo-to (荊芥連翹湯 ; TJ-50, lot.no. 250050010), Yoku-kan-san (抑肝散 ; TJ-54, lot.no. 260054010), Unsei-in (温清飲 ; TJ-57, lot.no. 260057010), Ji-zuso-ippo (治頭瘡一方 ; TJ-57, lot.no. 260057010), Tokaku-joki-to (桃核承気湯 ; J-61, lot.no. 250061010), Shimotsu-to (四物湯 ; TJ-71, lot.no. 250071010), Rokumi-gan (六味丸 ; TJ-87, lot.no. 250087010), Ogi-kenchu-to (黄耆建中湯 ; TJ-

Table I List of Kampo formulations in this study[10]

Kampo formulations	in Japanese	TJ number	particular symptoms and accompanying condition
Sho-saiko-to	小柴胡湯	9	resistance tender on pressure of the right subcostal region, loss of appetite, esnsation in the mouth cavity
Saiko-keishi-to	柴胡桂枝湯	10	resistance tender on pressure of the right subcostal region, abdominal pain, nervousness
Saiko-keishi-kankyo to	柴胡桂枝乾姜湯	11	reduced abdominal tension, palpitation of the abdominal aorta, red face, dry mouth, red of the tongue
Oren-gedoku-to	黄連解毒湯	15	red face, emotional instability, feeling of uprising heat, depression, lower abdomen tender on pressure
Gorei-san	五苓散	17	thirst, oliguria, emesis, diarrhea, headache, sweating
Sho-seiryu to	小青竜湯	19	natural sweating, pulse of deficiency type, serious rhinitis, stasis of body fluids
Shofu-san	消風散	22	chronic skin diseases with exdation and pruritis persistently moist then scaling eczema, erythema, thirst
Toki-shakuyaku-san	当帰芍薬散	23	cold lumbar region, anemia, tension of cervical muscles, vertigo, dysmenorrhea, edema, stasis of body fiuids
Byakko-ka-ninjin to	白虎加人参湯	34	deficiency body fluids, thirst
Hochu-ekki to	補中益気湯	41	weakness of muscles, fatigue, loss of appetite, gastric ptosis, hemorroids, systemic KI deficiency
Juzen-taiho-to	十全大補湯	48	weakness, tiring easily, exhaustion, loss of appetite night sweat, cold hands and feet, anemia, marked KI deficiency
Keigai-rengyo-to	荊芥連翹湯	50	inflammation of the upper respiratory system, pale face, sweaty palms and foot soles
Yoku-kan-san	抑肝散	54	emotional instability, nervousness, fibrillation of muscles
Unsei-in	温清飲	57	red face, emotional instability, somatitis, angular somatitis, eczema, dry skin, local blood deficiency
Ji-zuso-ippo	治頭瘡一方	59	alteration in the demis especially in the region of the head, fedness, vesicles, papula, scaling skin, pruritus
Tokaku-joki-to	桃核承気湯	61	umbilical region tender on pressure, excoration of the skin in the sigmoid region
Shimotsu to	四物湯	71	physical weakness, marked reduction of abdominal tension, blood deficiency
Rokumi-gan	六味丸	87	vertigo, tinnitus, sore throat, thirst, weakness of leg muscles, hot hands and feet
Ogi-kenchu-to	黄耆建中湯	98	abdominal pain, hot hands and feet, night sweat, fatigue, eczema, KI and blood deficiency
Inchinko-to	茵蔯蒿湯	135	icterus, head sweat, oliguria, thirst, constipation

Table II The composition of crude drugs in the formulation used in this study

Scientific name	in Japanese	Kampo formulation (TJ number)																			
		9	10	11	15	17	19	22	23	34	41	48	50	54	57	59	61	71	87	98	135
Artemisia capillaris THUNBERG	inchinko																				○4
Astragalus membranaceus BUNGE	ogi										○1	○3								○4	
Scutellaria bacicalensis GEORGI	ogon	○3*	○2	○3	○3								○1.5		○1.5						
Phellodendron amurense RUPRECHT	obaku				○1.5								○1.5		○1.5						
Coptis japonica MAKINO	oren				○2								○1.5		○1.5						
Polygala tenuifolia WILLDENOW	onji																				
Trichosanthes kirilowii MAXIMOWICZ	karokon			○3																	
Zingiber officinale ROSCOE	kankyo			○2			○3														
Glycyrrhiza uralensis FISCHER	kanzo	○2	○2				○3	○1		○2	○1.5	○1.5	○1	○1.5		○1	○1.5			○2	
Platycodon grandiflorum A.DE CANDOLLE	kikyo												○1.5								
Citrus aurantium LINNÉ *var. daidai* MAKINO	kijitsu												○1.5								
Sophora flavescens AITON	kujin							○1													
Schizonepeta tenuifolia BRIQUET (*var. japonica* KITAGAWA)	keigai							○1					○1.5			○1					
Cinnamomum cassia BLUME	keihi		○2	○3		○1.5	○3					○3					○4			○4	
Carthamus tinctorius LINNÉ	koka															○1					
Oryza sativa LINNÉ	kobei									○8											
Evodia rutaecarpa BENTHAM	goshuyu																				
Arctium lappa LINNÉ	goboshi							○2													
Sesamum indicum LINNÉ	goma							○1.5													
Schisandra chinensis BAILLON	gomishi						○3														
Bupleurum falcatum LINNÉ	saiko	○7	○5	○6							○2		○1.5	○2							
Asiasarum sieboldi F.MAEKAWA	saishin						○3														
Gardenia jasminoides ELLIS	sanshishi				○2								○1.5		○1.5						○3
Cornus officinalis SIEBOLD *et* ZUCCARINI	sanshuyu																		○3		
Dioscorea japonica THUNBERG	sanyaku																		○3		
Rehmannia glutinosa LIBOSCHITZ *var. purpurea* MAKINO	jio							○3				○3	○1.5		○3			○3	○5		
Paeonia lactiflora PALLAS	shakuyaku		○2				○3		○1			○3	○1.5		○3			○3		○6	
Zingiber officinale ROSCOE	shokyo	○2	○1								○0.5									○1	
Cimicifuga simplex WORMSKIORD	shoma										○1										
Gypsum Fibrosum	sekko							○3		○15											
Cnidium officinale MAKINO	senkyo								○3			○3	○1.5	○3	○3	○3		○3			
Cryptotympana tustulata FABRICIUS	zentai							○1													
Atractylodes lancea DE CANDOLLE	sojutsu					○3		○2	○1		○1	○3		○4		○3					
Rheum palmatum LINNÉ	daio															○0.5	○3				○1
Zizyphus jujuba MILLER *var. inermis* REHDER	taiso	○3	○2								○2									○4	
Alisma orientale JUZEPCZUK	takusha					○1			○1										○3		
Anemarrhena asphodeloides BUNGE	chimo							○1.5		○5											
Uncaria rhynchophylla MIQUEL	chotoko													○3							
Polyporus umbellatus FRIES	chorei					○3															
Citrus unshiu MARKOVICH	chinpi										○2										
Asparagus cochinchinensis MERRILL	tenmondo																				
Angelica acutiloba KITAGAWA	toki							○3	○3		○3	○3	○1.5	○3	○3			○3			
Prunus persica BATSCH	tonin																○5				
Panax ginseng C.A.MEYER	ninjin	○3	○2							○1.5	○4	○3									
Lonicera japonica THUNBERG	nindo															○2					
Mentha arvensis LINNÉ *var. piperascens* MALINVAUD	hakka												○1.5								
Pinellia ternata BREITENBACH	hange	○5	○4				○6														
Angelica dahurica BENTHAM *et.* HOOKER	byakushi												○1.5								
Atractylodes japonica KOIDZUMI *ex* KITAMURA	byakujutsu																				
Poria cocos WOLF	bukuryo					○3						○3		○4					○3		
Natril Sulfas	bosho																○0.9				
Saposhnikovia divaricata SCHISCHKIN	bofu							○2					○1.5			○2					
Paeonia suffruticosa ANDREWS (*Paeonia moutan* SIMS)	botanpi																		○3		
Ostrea gigas THUNBERG	borei			○3																	
Ephedra sinica STAPF	mao						○3														
Akebia quinata DECAISNE	mokutsu							○2													
Forsythia suspensa VAHL	rengyo												○1.5								

; Numbers represents the ratio for preparing the formulations

98, lot.no. 260098010), Inchinko-to (茵蔯蒿湯 ; TJ-135, lot.no. 260135010). The composition of crude drugs in the formulations was summarized in Table II. Ogi-kenchu-to was prepared without Saccharum granorum. Each formulation was administered orally 2 h prior to antigen challenge.

Anti-DNP IgE preparation : An anti-DNP mAb-producing cell line (EC1) was cultured in 10 ml of an equal volume mixture of RPMI-1640 and Dulbecco's modified Eagle minimum essential medium with high glucose supplemented with 10 % heat-inactivated fetal bovine serum (GIBCO Laboratories, Life Technologies, Inc., Grand Island, NY) and 2 mM glutamine until reaching a confluent state. The supernatant was harvested, centrifuged at 400×g and stored at -80°C until use.[6] The IgE antibody titer was estimated to be 1 : 1024 by heterologous passive cutaneous anaphylaxis in rats injected intravenously with DNP-bovine serum albumin as an antigen.[7]

Induction of skin reaction in mouse ears : BALB/c mice were given an i.v. injection of a 1 ml aliquot of anti-DNP IgE mAb-containing fluid 24 h before DNFB challenge. Skin reaction was elicited by applying 10 μl of 0.1 % or 0.05 % DNFB in 100 % ethanol to each side of each ear of sensitized mice. The reaction to DNFB was evaluated by measuring ear thickness using a dial thickness gauge (G-1A type, Peacock, Ozaki MFG., Co., LTD., Osaka, Japan) immediately before the challenge and at appropriate times after. The results were expressed as average ear swelling (increase in ear thickness, μm) ±S.D. of three mice.

Statistical analysis : Statistical significance of difference between the groups was determined by using Mann-Whitney's U-test.

Results and Discussion

DNFB challenge caused biphasic cutaneous reactions with IPR and LPR at 1 and 24 h in BALB/c mice which were passively sensitized with anti-DNP IgE mAb (Fig. 1). The response was dose-dependent on the challenge by DNFB. Diphenhydramine, an H1 receptor antagonist, inhibited IPR but not LPR. Our recent study has shown that only IPR was suppressed by the histamine-release inhibitor amlexanox.[3] These results indicate that IPR is mainly caused by

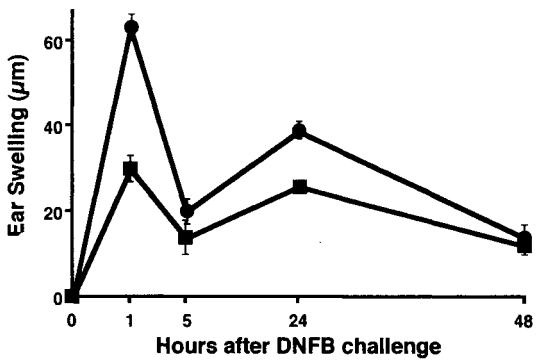

Fig. 1 Time course of IgE-mediated skin reaction in passively sensitized mice. Mice were given i.v. injection of 1.0 ml of anti-DNP IgE mAb preparation 24 h before skin testing with 0.1 % (●) and 0.05 % (■) DNFB in 100 % ethanol. Each value represents the mean±S.D. of 3 mice.

histamine released from mast cells.[8] In contrast, the intraperitoneally administration of prednisolone 2 h before the challenge resulted in significant inhibition of the ear swelling in both IPR and LPR in a dose-dependent manner. Although the mechanism for the development of LPR has not been clearly understood, inflammatory cells and their derived cytokines such as IL-1 β and TNF-α are considered to be involved. Nagai *et al.*[6,7,9] have reported that prednisolone inhibited the edematous reaction caused by IL-1 β and TNF-α. Therefore, predonisolone may partly inhibit LPR by suppressing the action of cytokines.

To search for anti-allergic Kampo formulations, we have investigated the effect of many Kampo formulations on murine IgE-mediated biphasic cutaneous reaction. Table I summarizes 20 Kampo formulations, their particular symptoms and accompanying conditions for clinical use.[10] The inhibitory effect of the 20 formulations on the biphasic cutaneous reaction was divided into three groups in terms of their inhibition rate of ear swelling (Table IIIa, b and c). The first group (Table. IIIa) consisting of Sho-saiko-to, Sho-seiryu-to, Shofu-san, Toki-shakuyaku-san, Byakko-ka-ninjin-to, Hochu-ekki-to and Tokaku-joki-to significantly inhibited both IPR (IPR/LPR, +/+ group), similar to the effect of prednisolone. Clinically, Byakko-ka-ninjin-to is often used to treat patients of atopic dermatitis and is actually effective in some of the cases. Although Sho-seiryu-to is mainly used for respiratory diseases including bronchial asthma or

Table IIIa Effect of Kampo formulations on IgE-mediated biphasic skin reactions in passively sensitized mice

	dose (g/kg)	IPR				LPR			
		Ear swelling (μm)	SD	% inhibition	P value	Ear swelling (μm)	SD	% inhibition	P value
control		51	3			53	2		
	0.5	45	2	12 %	*	46	3	13 %	**
Sho-saiko-to	1	44	2	14 %	**	44	3	17 %	***
TJ-9	2	43	1	16 %	**	42	2	21 %	***
Prednisolone	0.01	42	2	18 %	**	40	3	25 %	***
control		56	3			48	2		
	0.5	45	1	20 %	***	42	1	13 %	***
Sho-seiryu-to	1	42	3	25 %	***	40	3	17 %	***
TJ-19	2	37	2	34 %	***	37	2	23 %	***
Prednisolone	0.01	42	3	25 %	***	37	3	23 %	***
control		51	4			43	2		
	0.5	43	3	16 %	**	40	2	7 %	**
Shofu-san	1	40	3	22 %	***	39	3	9 %	
TJ-22	2	46	4	10 %		42	1	2 %	
Prednisolone	0.01	39	3	24 %	***	37	2	14 %	***
control		56	2			48	2		
	0.5	48	2	14 %	***	41	1	15 %	***
Toki-shakuyaku-san	1	48	2	14 %	***	42	2	13 %	***
TJ-23	2	53	3	5 %		43	2	10 %	**
Prednisolone	0.01	42	3	25 %	***	37	3	23 %	***
control		52	3			47	2		
	0.5	43	5	17 %	**	39	4	17 %	***
Byakko-ka-ninjin-to	1	42	3	19 %	***	38	3	19 %	***
TJ-34	2	44	4	15 %	**	39	2	17 %	***
Prednisolone	0.01	44	3	15 %	***	41	2	13 %	***
control		51	3			53	2		
	0.5	49	5	4 %		54	3	-2 %	
Hochu-ekki-to	1	43	1	16 %	***	44	2	17 %	***
TJ-41	2	43	2	16 %	**	45	3	15 %	**
Prednisolone	0.01	42	2	18 %	**	40	3	25 %	***
control		52	3			47	2		
	0.5	49	3	6 %		46	1	2 %	
Tokaku-joki-to	1	45	1	13 %	***	41	2	13 %	***
TJ-61	2	41	3	21 %	***	39	3	17 %	***
Prednisolone	0.01	44	3	15 %	***	41	2	13 %	***

Mice were given i.v. injection of 1.0 ml of anti-DNP IgE mAb preparation 24 h before skin testing with 0.1 % DNFB in 100 % ethanol. Kampo formulations at doses of 0.5, 1.0 and 2.0 g/kg were administered orally 2 h before the challenge.
Prednisolone was administered i.p. 2 h before DNFB challenge. Ear swelling was assessed 1 h (IPR) and 24 h (LPR) after the challenge.
Each value represents the mean±S.D. of 3 mice. *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$ by Mann-Whitney's U-test.

rhinitis,[10] Table. IIIa suggests that Sho-seiryu-to may also be effective for patients with atopic dermatitis. Toki-shakuyaku-san and Tokaku-joki-to are "OKETSU" -improving drugs in Kampo medicine. Terasawa *et al.*[11] have reported four cases of atopic dermatitis successfully treated with Tokaku-joki-to. Therefore, it may be of particular interest to investigate the possible effects of other "OKETSU"-improving medicines on IgE-mediated biphasic cutaneous reaction. Interestingly, Sho-seiryu-to, Byakko-ka-ninjin-to and Tokaku-joki-to at the dose of 2.0 g/kg inhibited biphasic cutaneous reaction more markedly than prednisolone. In our experiment, Shofu-san inhibited IgE-mediated biphasic cutaneous reaction with

Table IIIb Effect of Kampo formulations on IgE-mediated biphasic skin reactions in passively sensitized mice

	dose (g/kg)	IPR				LPR			
		Ear swelling (μm)	SD	% inhibition	P value	Ear swelling (μm)	SD	% inhibition	P value
control		48	4			48	4		
	0.5	48	3	0 %		39	5	19 %	*
Shimotsu-to	1	47	4	2 %		38	3	21 %	**
TJ-71	2	47	2	2 %		36	3	25 %	***
Prednisolone	0.01	39	4	19 %	*	38	1	21 %	***
control		46	3			39	1		
	0.5	42	2	9 %		29	5	26 %	*
Ogi-kenchu-to	1	47	2	-2 %		30	3	23 %	***
TJ-98	2	42	2	9 %		28	4	28 %	***
Prednisolone	0.01	34	3	26 %	***	24	2	38 %	***
control		46	5			44	3		
	0.5	51	8	-11 %		40	4	9 %	
Ji-zuso-ippo	1	42	3	9 %		37	3	16 %	*
TJ-59	2	39	6	15 %		33	4	25 %	***
Prednisolone	0.01	39	6	15 %		40	2	9 %	*
control		48	3			47	2		
	0.5	53	4	-10 %		42	2	11 %	*
Gorei-san	1	56	3	-17 %		42	2	11 %	*
TJ-17	2	53	2	-10 %		43	2	9 %	*
Prednisolone	0.01	38	3	21 %	***	38	3	19 %	***
control		44	1			49	3		
	0.5	49	4	11 %		45	5	8 %	
Unsei-in	1	45	3	2 %		39	1	20 %	***
TJ-57	2	47	6	-7 %		41	4	16 %	**
Prednisolone	0.01	36	4	18 %	***	38	3	22 %	***

Mice were given i.v. injection of 1.0 ml of anti-DNP IgE mAb preparation 24 h before skin testing with 0.1 % DNFB in 100 % ethanol.
Kampo formulation at doses of 0.5, 1.0 and 2.0 g/kg were administered orally 2 h before the challenge.
Prednisolone was administered i.p. 2 h before DNFB challenge. Ear swelling was assessed 1 h (IPR) and 24 h (LPR) after the challenge.
Each value represents the mean±S.D. of 3 mice. *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$; by Mann-Whitney's U test.

IPR rather than LPR in mice. Tsunematsu *et al.*[5] reported that Shofu-san inhibited both IPR and LPR. A plausible explanation of the difference in the inhibitory effect between them may be included the difference in the degree of ear swelling of control mice.

The second group (Table. IIIb) inhibited mainly LPR, but not IPR (-/+ group). It was composed of Gorei-san, Unsei-in, Shimotsu-to, Ogi-kenchu-to and Ji-zuso-ippo. Interestingly, these Kampo formulations showed comparatively selective inhibition of LPR. Ogi-kenchu-to is especially used for infantile patients with dermatitis, and there are some clinically effective adult cases with atopic dermatitis. Unsei-in consists of Oren-gedoku-to and Shimotsu-to. Unsei-in as well as Shimotsu-to significantly inhibited LPR but not IPR (Table. IIIb). In contrast, Oren-gedoku-to did not affect either IPR or LPR (Table. IIIc). Some clinical cases of the condition of atopic dermatitis getting worse by the treatment of Unsei-in have sometimes been observed.[12] This clinical observation may be partly associated with the enhancement of IPR by containing of Oren-gedoku-to in Unsei-in. Gorei-san as well as Toki-shakuyaku-san and Sho-seiryu-to are the representative Kampo medicines for improving the stasis of body fluids which is referred to as "water", a concept that includes overall water metabolism and various functions such as the defense system. Gorei-san enhanced IPR and inhibited LPR (Table. IIIb), while Toki-shakuyaku-san and Sho-seiryu-to inhibited both IPR and LPR (Table. IIIa). Although these formulations are recognized to be water-improving drugs in Kampo medicine, their

Table IIIc Effect of Kampo formulations on IgE-mediated biphasic skin reactions in passively sensitized mice

	dose (g/kg)	IPR				LPR			
		Ear swelling (μm)	SD	% inhibition	P value	Ear swelling (μm)	SD	% inhibition	P value
control		48	3			47	2		
	0.5	49	3	-2 %		43	3	9 %	*
Saiko-keishi-to	1	46	5	4 %		43	1	9 %	**
TJ-10	2	50	3	-4 %		44	3	6 %	
Prednisolone	0.01	38	3	21 %	***	38	3	19 %	***
control		44	3			39	3		
	0.5	41	2	7 %		35	2	10 %	
Saiko keishi kankyo-to	1	42	3	5 %		37	4	5 %	
TJ-11	2	34	2	23 %	***	39	2	0 %	
Prednisolone	0.01	31	1	30%	***	34	2	13 %	*
control		44	1			49	3		
	0.5	45	2	-2 %		53	3	-8 %	
Oren-gedoku-to	1	47	2	-7 %		49	3	0 %	
TJ-15	2	46	5	5 %		51	5	-4 %	
Prednisolone	0.01	36	4	18 %	***	38	3	22 %	***
control		46	4			44	3		
	0.5	45	2	2 %		40	1	9 %	*
Juzen-taiho-to	1	53	4	-15 %		43	2	2 %	
TJ-48	2	44	4	4 %		38	3	14 %	*
Prednisolone	0.01	37	3	20 %	***	40	4	9 %	
control		46	4			44	3		
	0.5	46	3	0 %		40	3	9 %	*
Keigai rengyo-to	1	50	4	9 %		40	3	9 %	
TJ-50	2	36	2	22 %	***	36	2	18 %	***
Prednisolone	0.01	37	3	20 %	***	40	4	9 %	
control		51	4			43	2		
	0.5	48	5	6 %		45	3	-5 %	
Yoku-kan-san	1	50	3	2 %		44	1	-2 %	
TJ 54 3	2	54	3	-6 %		44	2	-2 %	
Prednisolone	0.01	39	3	24 %	***	37	2	14 %	***
control		48	4			48	4		
	0.5	47	3	2 %		45	2	6 %	
Rokumi gan	1	46	5	4 %		44	4	8 %	
TJ-87	2	47	4	2 %		46	4	4 %	
Prednisolone	0.01	39	4	19 %		38	1	21 %	***
control		49	5			43	2		
	0.5	47	2	4 %		46	2	-7 %	
Inchinko-to	1	58	9	18 %		44	2	-2 %	
TJ-135	2	73	2	-49 %		46	3	-7 %	
Prednisolone	0.01	38	3	22 %	***	43	1	0 %	

Mice were given i.v. injection of 1.0 ml of anti-DNP IgE mAb preparation 24 h before skin testing with 0.1 % DNFB in 100 % ethanol.
Kampo fomulations at doses of 0.5, 1.0 and 2.0 g/kg were administered orally 2 h before the challenge.
Prednisolone was administered i.p. 2 h before DNFB challenge. Ear swelling was assessed 1 h (IPR) and 24 h (LPR) after the challenge.
Each value represents the mean±S.D. of 3 mice. *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$ by Mann-Whithey's U-test.

effects were divided into -/+ and +/+ groups. Thus, further investigations will be needed to examine the difference of the inhibitory mechanism in detail.

The third group which consisted of Oren-gedoku-

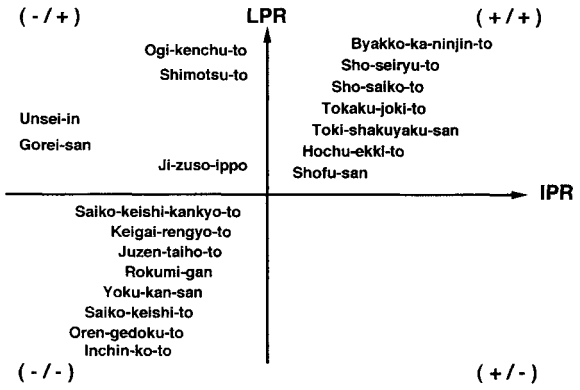

Fig. 2 Summary of the effects of 20 Kampo formulations on IgE-mediated biphasic skin reaction. Effects of Kampo formulations tested were subdivided into the four groups of +/+, -/+, +/- and -/- of IPR/LPR.

to, Yoku-kan-san, Rokumi-gan, Inchinko-to and so forth (Table. IIIc), did not induce any inhibition of IPR and LPR (-/- group). However, in some cases, the enhancement of ear swelling at IPR or LPR was observed. Inchinko-to is generally used for improving liver diseases such as hepatitis and cirrhosis, but markedly enhanced IPR in a dose-dependent manner.

Fig. 2 summarized the effects of all Kampo formulations tested. All of the Kampo formulations were again divided into the three groups of +/+, -/+ and -/- of IPR/LPR. Since the inhibitory effect of the group +/+ was similar to that of prednisolone, it is of prime interest to determine the molecular mechanisms for the effecacies of this group in comparison with prednisolone. On the other hand, some of the Kampo formulations such as Unsei-in, Gorei-san, Oren-gedoku-to and Inchinko-to tended to exacerbate the murine IgE-mediated cutaneous reaction rather than no effect. Although the detailed mechanism for the enhancement of the reaction is not yet understood, a possible reason may be that the holistic pattern of symptoms and individual pathogenic alterations, the so-called "SHO", by which the diagnosis of disease states and the ways of treatment in Kampo are determined, is due to mismatching of these medicines.

In conclusion, we have examined the effect of a total of 20 Kampo medicines on murine IgE-mediated cutaneous reaction. The pattern of the effectiveness of these formulations was mainly demonstrated in the three groups of +/+, -/+, and -/- of IPR/LPR ; "SHO" was not taken into consideration in this study. Some of the formulations such as Gorei-san, Unsei-in, Shimotsu-to and Ogi-kenchu-to resulted in comparatively selective inhibition of LPR which is clinically considered to be an important phase associated with severe chronic diseases. The detailed mechanisms of the inhibition of IgE-mediated biphasic cutaneous reaction by the administration of Kampo formulations are now under investigation in our laboratory. Although Kampo formulations in the group (-/-) actually have shown clinical effects in some cases, these findings would provide one of the useful information for the division of treatment protocols incorporating Kampo medicines for allergic diseases.

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和文抄録

20種類の漢方方剤のIgE依存性二相性皮膚反応に対する効果を検討した。BALB/cマウスに抗モノクローナルIgE抗体を注射して受動的に感作し，抗原を塗布することにより，即時型皮膚反応と遅発型皮膚反応がそれぞれ1時間後と24時間後に観察された。この二相性皮膚反応に対する抑制効果の違いにより，20種類の漢方方剤は3つの群に分けられた。第1群は小青竜湯，当帰芍薬散，白虎加人参湯，桃核承気湯などからなり，即時型反応と遅発型反応の両相を抑制した。第2群は五苓散，温清飲，四物湯，黄耆建中湯を含み，遅発相のみを抑制した。第3群は黄連解毒湯，抑肝散，六味丸，茵蔯蒿湯などからなり，両相とも抑制効果を示さなかった。以上の成績は，漢方薬によるアレルギー性疾患に対する治療選択の決定に有益であると考えられる。

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