Mod Rheumatol (2003) 13:50–56

ORIGINAL ARTICLE

Yuka Ono · Yukio Ogihara · Shinobu Saijo
Yoichiro Iwakura · Makoto Inoue

Evaluation of Kampo medicines used to treat rheumatoid arthritis in collagen-induced arthritic and pX transgenic mice

Received: January 29, 2002 / Accepted: June 4, 2002

Abstract To evaluate the usefulness of Kampo medicines (traditional herbal medicines) used clinically for the treatment of rheumatoid arthritis (RA), we selected eight of them and examined their effects on collagen-induced arthritic and pX transgenic mice. Among these, Dai-bofu-to, Kanzo-bushi-to, and Makyo-yokkan-to significantly reduced the severity of arthritis in collagen-induced arthritis (CIA) mice. The onset of arthritis was delayed by three Kampo medicines, but only the effect of Makyo-yokkan-to was statistically significant. In addition, three Kampo medicines suppressed the arthropathy of pX transgenic mice, which had developed spontaneously. The onset of arthritis was delayed by 10.7, 8.3, and 15.4 days following treatment with Dai-bofu-to, Kanzo-bushi-to, and Makyo-yokkan-to, respectively. A study of the underlying mechanism showed that Kanzo-bushi-to decreased serum antitype II collagen antibody levels, suggesting that Kanzo-bushi-to possesses immunomodulating activity. This study shows that some Kampo medicines are effective in an induced or spontaneously developed arthritis animal model of human RA.

Key words Collagen-induced arthritis (CIA) · Immunoregulation · Kampo medicines · pX transgenic mouse · Rheumatoid arthritis (RA)

Introduction

Rheumatoid arthritis (RA) is a common chronic autoimmune disease characterized by synovial hyperplasia and the deformity and destruction of joints and bone. About 0.7 million people (0.6% of the population) are currently afflicted with RA in Japan. However, regardless of recent significant progress in understanding the underlying pathogenic mechanisms, the etiology still remains unknown.

Current treatments using therapeutic agents for RA such as antirheumatic and anti-inflammatory drugs are inadequate in that they only partially control established RA. They also have side-effects that limit their use early in the disease process and interfere with prolonged administration. From this viewpoint, a novel antirheumatic drug, which decreases inflammation in the joint by a disease-specific mechanism and lacks severe side-effects resulting from long-term administration, needs to be developed.

In the treatment of RA, some traditional Japanese herbal medicines (Kampo medicines) were found to be effective clinically, although the mechanisms underlying the efficacy of the Kampo medicines have not yet been fully studied. Kampo medicines usually consist of several medicinal plants, and are applied to chronic diseases depending on the degree of development of the disease and the condition of the patient. This idea is significantly different from the use of synthetic drugs, and prompted us to study the usefulness and the underlying mechanism of these medicines. To date, we have studied the pharmacological and biochemical actions of Kampo medicines using some animal models of human diseases.[1]

In this study, we used collagen-induced arthritis (CIA) and arthritic pX transgenic mice to examine the efficacy of Kampo medicines used for the treatment of RA. CIA is a model of induced autoimmunity that shares a number of pathological, immunological, and genetic features with RA, such as the presence of fibrin deposition, hyperplasia of synovial cells, mononuclear cell infiltration, pannus formation, and eventual ankylosis of one or more articular joints.[2,3] In addition, the presence of rheumatoid factor and systemic manifestations have been observed in CIA model animals.[4,5] Moreover, susceptibility to both CIA and RA is intimately associated with the expression of specific major histocompatibility complex (MHC) class II molecules.[6]

Y. Ono · Y. Ogihara · M. Inoue (✉)
Laboratory of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan
Tel. +81-52-836-3417; Fax +81-52-836-3415
e-mail: inoue@phar.nagoya-cu.ac.jp

S. Saijo · Y. Iwakura
Center of Experimental Medicine, Institute of Medical Science, University of Tokyo, Tokyo, Japan

pX transgenic mice, which carry the human T-cell leukemia virus type I (HTLV-I) *env-pX* region under its own long terminal repeat, were produced to elucidate the pathological roles of the virus in RA, and were found to develop chronic inflammatory arthropathy.[7] The pathology of the lesions closely resembled that of RA in humans, indicating marked synovial and periarticular inflammation with articular erosion caused by the invasion of granulation tissue.[8,9] In addition, structural changes in the *N*-linked sugar chains of serum immunoglobulin G were observed in pX transgenic mice, as are found in patients affected with RA.[10] Thus, CIA and pX transgenic mice with induced and spontaneously developed arthritis provide useful animal models, for RA in humans, and allow us to study the efficacy of therapeutic agents and their underlying mechanisms. In this study, we selected ten types of Kampo medicine which are frequently used to treat RA in Japan, and examined their usefulness for RA treatment.

Materials and methods

Animals

Male DBA/1J mice were purchased from Nippon Charles River (Kanagawa, Japan). pX transgenic mice carrying the HTLV-1 *env-pX* region were provided by Dr. Iwakura of the University of Tokyo. The transgene was detected through dot-plot hybridization using DNA prepared from mouse tails, and littermates were used as controls. All mice were housed in a temperature-controlled room (23 ± 1°C), with lighting from 6 A.M. to 6 P.M., under specific-pathogen-free conditions, at the Laboratory Animal Center of Nagoya City University. They were fed a sterilized commercial diet with a low protein content (Nippon Crea, Shizuoka, Japan) and given water ad libitum. All DBA/1J mice were used at 8 weeks of age, and pX transgenic mice were used at 4–6 weeks of age.

Preparation of Kampo medicines

Kampo medicines (dose per person per day) were prepared as follows. All the crude drugs shown in Table 1 were weighed, added to 700 ml water, decocted for 1 h, and then concentrated to 300 ml. This decoction was lyophilized to give a powdered extract (Makyo-yokkan-to, 1.9 g; Eppi-ka-jutsu-to, 5.4 g; Keishi-ni-eppi-ichi-to, 4.6 g; Kami-shimotsu-to, 11.3 g; Keishi-shakuyaku-chimo-to, 6.0 g; Daibofu-to, 10.5 g; Kanzo-bushi-to, 2.7 g; Keishi-kajutsubu-to, 6.9 g).

Induction and clinical evaluation of CIA

Bovine type II collagen (CII) was dissolved in 0.01 M acetic acid at a concentration of 3 mg/ml and emulsified in an equal volume of complete Freund's adjuvant (Wako, Osaka, Japan) containing 4 mg/ml mycobacterium butyricum (Difco Laboratories, Detroit, MI, USA). Then 100 μl emulsion (150 μg CII) was injected intradermally into the root of the limbs of male DBA/1J mice. Twenty-one days after the first immunization, a booster intraperitoneal injection with 75 μg CII dissolved in 0.01 M acetic acid was given. Kampo medicines, dissolved in the daily intake of drinking water at a concentration of 10 times a human daily dose, were given to the mice from 3 days before the first immunization to 3 weeks after the second immunization. FK506 (provided by Fujisawa Pharmaceutical), which was used as a positive control drug, was administered orally every day, except Sunday, from the day of the first immunization. The onset of arthritis was considered to be the day when erythema and/or swelling were first observed. The severity of the arthritis was evaluated for each paw by a scoring method according to the degree of inflammation, where grade 0 = normal, grade 1 = swelling of one finger, grade 2 = swelling of more than two fingers, grade 3 = swelling of the heel, and grade 4 = joint deformity with ankylosis, resulting in a maximum score of 16 per animal.

Clinical evaluation of pX transgenic mice

Kampo medicines dissolved in the daily intake of drinking water at a concentration of 10 times a human daily dose were given to the pX transgenic mice from the day when they became 4 weeks old for 10 weeks. The onset of arthritis was considered to be the day when erythema and/or swelling were first observed. The severity of the arthritis was judged macroscopically every other day by a scoring method according to the degree of inflammation. The severity scores were graded as follows: grade 0 = normal, grade 1 = light swelling of the joint and/or redness of the foot pad, grade 2 = obvious swelling of the joint, and grade 3 = fixation of the joint, resulting in a maximum score of 12 per animal.

Measurement of anti-CII antibody

Serum anti-CII antibody levels in pooled sera of CIA mice were measured by enzyme-linked immunosorbent assay (ELISA). ELISA multiplates (H type, Sumitomo Bakelite, Tokyo, Japan) were coated with soluble bovine CII at a concentration of 5 μg/ml in phosphate-buffered saline (PBS) by incubation for 24 h at 4°C. Plates were washed with PBS containing 0.05% Tween 20 (PBS–Tween) and incubated with 1.5% bovine serum albumin containing PBS at 4°C overnight. Plates were thoroughly washed three times with PBS–Tween, and then incubated with serum diluted 1:40 000 in PBS for 24 h at 4°C. After washing with PBS–Tween, peroxidase-conjugated rabbit antimouse IgG antibody (Zymed Laboratories, South San Francisco, CA, USA) diluted 1:4000 in PBS was added to each well and incubated for 20 h at 4°C. Then the plates were washed with PBS–Tween, and incubated with 100 μl orthophenylenediamine (OPD; Nacalai Tesque, Kyoto, Japan) solution, which was prepared as follows: 40 mg OPD was dissolved in 100 ml citrate–phosphate buffer, pH 5.0, to which 5.6 μl 30% H_2O_2 was added immediately before use. The absorbance was determined by using a microplate reader (Model 450,

Table 1. Constituent crude drugs of various Kampo medicines

Kanzo-bushi-to KB		Keishi-ka-jutsubu-to KJ		Keishi-shakuyaku-chimo-to KST		Keishi-ni-eppi-ichi-to KE		Eppi-ka-jutsu-to EJ		Makyo-yokkan-to MY		Daibofu-to DB		Kami-shimotsu-to KSM	
Glycyrrhizae R.	(2.0)	Glycyrrhizae R.	(2.0)	Glycyrrhizae R.	(1.5)	Glycyrrhizae R.	(2.5)	Glycyrrhazae R.	(1.5)	Glycyrrhizae R.	(2.0)	Glycyrrhizae R.	(1.5)		
Atractylodis Lanceae Rh.	(4.0)	Atractylodis Lanceae Rh.	(4.0)	Atractylodis Lanceae Rh.	(4.0)			Atractylodis Lanceae Rh.	(3.0)			Atractylodis Lanceae Rh.	(3.0)	Atractylodis Lanceae Rh.	(3.0)
		Zingiberis Rh.	(4.0)	Zingiberis Rh.	(3.0)	Zingiberis Rh.	(3.5)	Zingiberis Rh.	(3.5)			Zingiberis Rh.	(1.5)		
		Paeoniae R.	(4.0)	Paeoniae R.	(2.5)	Paeoniae R.	(2.5)					Paeoniae R.	(3.0)	Paeoniae R.	(3.0)
Cinnamomi C.	(4.0)	Cinnamomi C.	(4.0)	Cinnamomi C.	(3.0)	Cinnamomi C.	(2.5)								
		Zizyphi F.	(4.0)			Zizyphi F.	(3.0)	Zizyphi F.	(3.0)			Zizyphi F.	(1.5)		
Aconiti T.	(0.5)	Aconiti T.	(0.5)	Aconiti T.	(0.5)							Aconiti T.	(0.5)		
				Ephedrae H.	(3.0)	Ephedrae H.	(2.5)	Ephedrae H.	(4.0)	Ephedrae H.	(4.0)				
						Gypsum fibrosum	(3.0)	*Gypsum fibrosum*	(1.5)						
				Glehniae R. cum Rh.	(3.0)							Glehniae R. cum Rh.	(3.0)		
				Anemarrhenae R.	(3.0)									Anemarrhenae R.	(1.5)
												Angelicae R.	(3.0)	Angelicae R.	(3.0)
												Rehmanniae R.	(3.0)	Rehmanniae R.	(3.0)
												Cnidii Rh.	(2.0)	Cnidii Rh.	(3.0)
												Ginseng R.	(1.5)	Ginseng R.	(2.0)
												Achyranthis R.	(1.5)	Achyranthis R.	(2.0)
												Eucommiae C.	(3.0)	Eucommiae C.	(1.5)
												Notopterygii Rh.	(1.5)		
												Astragali R.	(3.0)		
										Coicis S.	(10.0)			Coptidis Rh.	(1.5)
										Armeniacae S.	(3.0)			Ophiopogonis T.	(5.0)
														Phellodendri C.	(1.5)
														Schizandrae F.	(1.5)

R, Radix; Rh, Rhizoma; H, Herba; C, Cortex; T, Tuber; F, Fructus; S, Semen
The weight (g) of the crude drug is shown in parentheses

Bio Rad, Hercules, CA, USA) at a wavelength of 490 nm and a reference wavelength of 405 nm.

Preparation of thioglycollate-eliciting peritoneal macrophages

Male DBA/1J mice were given Kampo medicines at 10 times a human daily dose for 1 week, and were injected intraperitoneally with 2 ml 3% thioglycollate 4 days before the last day of the experiment. Peritoneal macrophages were harvested from the abdominal cavity and maintained in RPMI1640 supplemented with 10% FCS. The resulting macrophages were seeded at a concentration of 2×10^6 cells/ml and incubated in the presence or absence of lipopolysaccharide (LPS) 10 μg/ml for 24 h. Nitric oxide (NO) production was determined by measuring the accumulation of nitrite in the incubation medium using Griess reagent.[11]

Statistical analysis

Data are represented as mean ± SE of the number of animals described in the figure legends. Statistical significance was determined by nonpaired Student's *t*-test, Mann–Whitney *U*-test, or Dunnett's test using StatLight software. *P* values less than 0.05 were considered to be significant.

Results

To evaluate the usefulness of Kampo medicines used clinically for treatment of rheumatoid arthritis, we selected the eight described in Table 1 and examined their effects on collagen-induced arthritic and pX transgenic mice. Eppi-ka-jutsu-to (EJ), Makyo-yokkan-to (MY), and Kanzo-bushi-to (KB) are used to treat relatively active RA; Keishi-ni-eppi-ichi-to (KE), Keishi-shakuyaku-chimo-to (KST), and Keishi-ka-jutsubu-to (KJ) are used for patients with relatively inactive RA but with progress in the disease; Kami-shimotsu-to (KSM) and Daibofu-to (DB) are used for patients exhausted from prolonged disease. The efficacy of these Kampo medicines were first examined using CIA mice by evaluating the severity and the day of onset 6 weeks after the first injection. Data from two separate experiments are given in Table 2. MY, KJ, and DB were found to suppress the severity of arthritis significantly. KB also reduced it more than MY and KJ, and delayed the day of onset by about 8 days, but not significantly. In a further investigation, we selected MY and DB in addition to KB, which consists of simple crude drugs, instead of KJ that has six out of seven crude drugs in common with DB. We therefore investigated the effects of three Kampo medicines, MY, DB, and KB, in detail throughout the experiments. The incidence of arthritis in the three groups did not vary from that in the control group, although FK506 treatment markedly decreased the incidence (Fig. 1A). The onset of arthritis was significantly delayed in the MY- and FK506-treated groups relative to the control group (Table 3). When the severity of arthritis was evaluated by scoring the degree of inflammation, KB was found to have suppressed the arthritis from day 28 to day 40 after the first injection; MY and DB effectively reduced the severity in the early stage of the development of arthritis (Fig. 1B). During the course of the experiment, the change in body weight of the Kampo medicine-treated groups was not different from that of the control group (data not shown).

Table 2. Severity and onset of Collagen-induced arthritis (CIA) in control and Kampo medicine-treated groups

Kampo medicines	Score[a]	Onset[b]
My	7.0 ± 1.9*	27.3 ± 2.6
KJ	8.0 ± 1.2*	25.8 ± 1.1
KST	10.2 ± 1.1	26.0 ± 1.8
EJ	11.2 ± 0.8	23.8 ± 1.3
CTRL	12.2 ± 0.8	23.7 ± 1.4
DB	5.0 ± 1.4*	28.2 ± 2.5
KB	5.2 ± 2.5	30.5 ± 3.8
KSM	7.2 ± 1.9	26.4 ± 1.8
KE	9.5 ± 1.7	23.0 ± 1.6
CTRL	9.5 ± 0.9	22.5 ± 1.1

CIA in DBA/1J mice was caused as described in Materials and methods. The severity and time of onset of arthritis were assessed 6 weeks after the first injection. The number of mice used in each group was: MY, 6; KJ, 6; KSN, 6; EJ, 6; CTRL (upper table), 6; DB, 7; KB, 6; KSM, 5; KE, 6; CTRL (lower table), 6

[a] Severity score of arthritis 6 weeks after the first injection ± SE

[b] Onset of arthritis (days after first injection) ± SE

* $P < 0.05$ versus control group

Table 3. Effect of various Kampo medicines on the onset of arthritis in collagen-induced arthritic mice

Kampo medicines	Onset
MY	26.9 ± 1.5*
KB	23.6 ± 2.1
DB	23.7 ± 1.5
FK506	36.7 ± 3.2*
CTRL	21.6 ± 0.9

The onset of arthritis in CIA mice in Fig. 1 was taken as the day when erythema and/or swelling were first observed. Values represent mean ± SE of the average day of onset (days after first injection)

* $P < 0.05$ versus control group

In CIA mice, it is known that serum anti-CII antibody levels increase with the development of arthritis. We therefore examined the effects of three Kampo medicines on serum IgG anti-CII antibody in CIA mice. As shown in Fig. 2, anti-CII antibody levels were reduced in the KB-treated and FK506-treated groups. To assess the anti-inflammatory effect of these Kampo medicines, ten times the human daily dose of each Kampo medicine was administered to normal DBA/1J mice for 1 week. Thioglycolate-elicited peritoneal macrophages were prepared, and nitric oxide (NO) production by macrophages was measured after 24 h stimulation with lipopolysaccharide. MY, DB, and KB did not influence NO production, indicating that they appeared not to possess any inhibitory that activity against macrophage function.

To further evaluate the efficacy of these Kampo medicines agaist RA, we examined the effect on another animal model of RA, pX transgenic mice, which develop arthritis spontaneously. In this study we used female pX transgenic mice, because they develop arthritis easily and with high

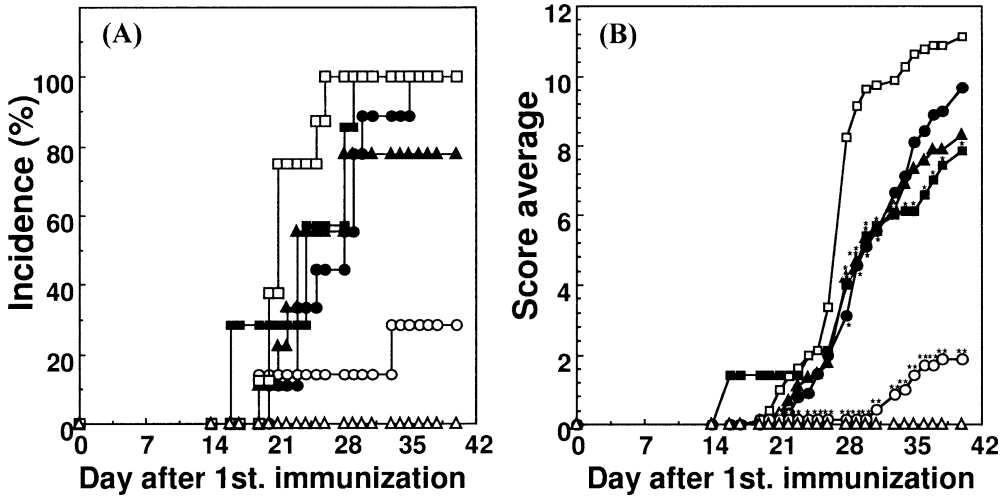

Fig. 1. Effect of various Kampo medicines on **A** incidence and **B** severity of arthritis in collagen-induced arthritic mice. DBA/1J mice were immunized intradermally with CII on day 0 and given a booster by intraperitoneal injection with CII on day 21. Kampo medicines were given to the mice from 3 days before the first immunization. *Solid circles*, MY-treated group (n = 9); *solid squares*, KB-treated group (n = 7); *solid triangles*, DB-treated group (n = 9); *open circles*, FK506-treated group (n = 6); *open squares*, control group (n = 6); *open triangles*, normal group (n = 4). $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group

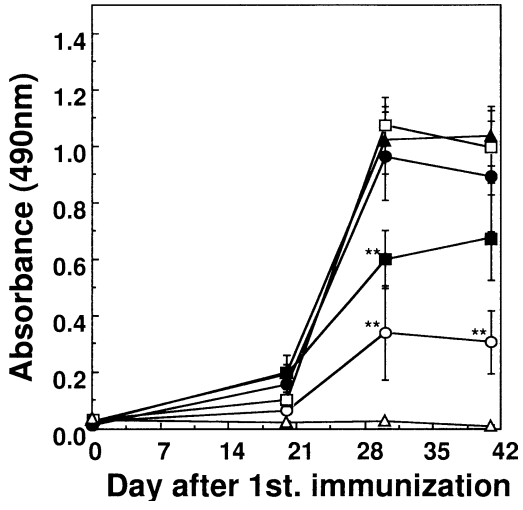

Fig. 2. Effect of various Kampo medicines on serum anti-CII antibody levels in collagen-induced arthritic mice. Anti-CII antibody levels in the sera which were prepared from the CIA mice in Fig. 1 were determined by ELISA, as described in Materials and methods. *Solid circles*, MY-treated group (n = 9); *solid squares*, KB-treated group (n = 7); *solid triangles*, DB-treated group (n = 7); *open circles*, FK506-treated group (n = 6); *open squares*, control group (n = 6); *open triangles*, normal group (n = 4). $^{**}P < 0.01$ versus control group

frequency. Kampo medicines were given to pX transgenic mice from 4 weeks after birth, and their effects on arthritis were examined. The final incidence of arthritis in the Kampo medicine-treated groups did not vary from that in the control group (Fig. 3A). However, the MY treatment strikingly delayed the onset of arthritis by 15.4 days relative to the control group (Table 4), while the KB and DB treatments also delayed the onset, but not significantly. In addition, although the arthritic score was suppressed in the Kampo medicine-treated groups, the MY treatment showed the clearest inhibitory effect among the three Kampo medicines used soon after the first administration (Fig. 3B). The pX region of the transgene encodes a protein termed tax that is a causal protein of HTLV-1-induced diseases and regulates the function of the NF-κB and cAMP

Table 4. Effect of various Kampo medicines on the onset of arthritis in pX transgenic mice

Kampo medicines	Onset
MY	34.0 ± 6.8*
KB	26.9 ± 7.5
DB	29.3 ± 5.9
CTRL	18.6 ± 3.4

The onset of arthritis in pX mice in Fig. 3 was taken as the day when erythema and/or swelling were first observed. Values represent mean ± SE of the average day of onset (days after first administration)
$^{*}P < 0.05$ versus control group

responsive element binding protein. Furthermore, NK-κB is known to mediate the production of inflammatory cytokines such as IL-1 and TNF-α. Thus, when the effects of MY, DB, and KB on the expression of tax mRNA were examined by RT–PCR, they were found to have had no influence in the inflammatory joints (data not shown).

Discussion

Many Kampo medicines are considered to be clinically effective for RA patients according to classical medical books, although there is no basic scientific evidence. RA develops slowly for a long time, and its progress depends on the patient or the condition of the patient, including physical strength and constitution. Kampo medicines are usually applied to patients based on the stage of RA and the condition of the patient, and this idea is peculiar to Oriental medicine. In this study, we selected eight Kampo medicines with different applications and different constituents of crude drugs and assessed their efficacy using two animal models for human RA: CIA and pX transgenic mice.

The arthritis of CIA and pX transgenic mice is caused by different mechanisms. In CIA mice, CII-reactive $CD4^{+}$ T cells as the primary mediators of disease induction, and anti-CII autoantibody production by B cells, both appear to be implicated in the induction of arthritis.[4,12,13] Recent stud-

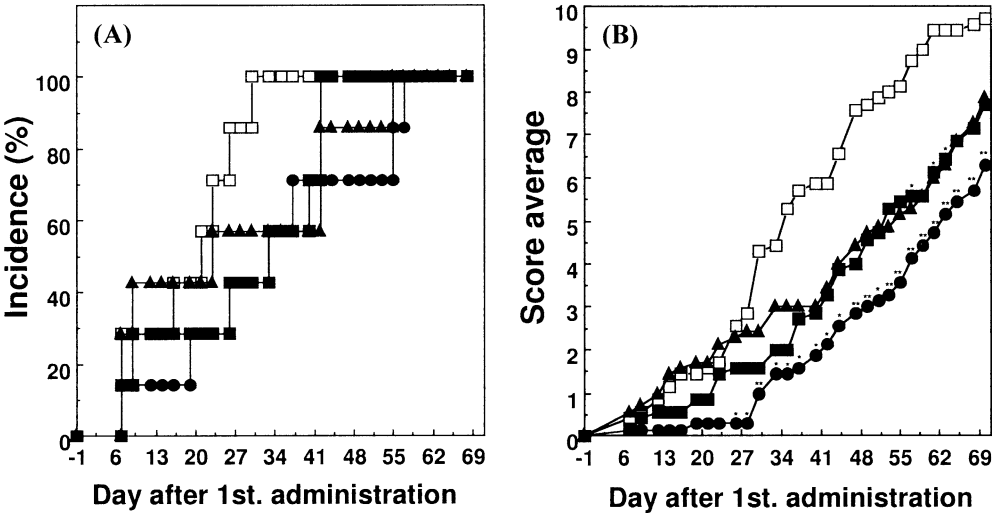

Fig. 3. Effect of various Kampo medicines on **A** incidence and **B** severity of arthritis in pX transgenic mice. Kampo medicines were given to female pX transgenic mice from 4 weeks of age for 10 weeks. The severity and incidence of spontaneously developed arthritis were examined from the first day after administration. *Solid circles*, MY-treated group ($n = 7$); *solid squares*, KB-treated group ($n = 7$); *solid triangles*, DB-treated group ($n = 7$); *open squares*, control group ($n = 4$). $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group.

ies suggest that binding of the complement activating IgG autoantibodies to CII on articular cartilage, and the subsequent cross-linking of Fc-receptors, is the primary immune mechanism leading to joint inflammation.[14,15] In this study, the finding that MY, DB, and KB effectively suppress the development of arthritis in CII-injected mice suggests that these Kampo medicines modify the immune response to CII. In fact, KB treatment decreased serum IgG anti-CII antibody levels significantly, suggesting that KB shows an immunosuppressive effect. However, MY and DB did not reduce serum IgG levels, indicating that they did not influence humoral immunity. It is known that the total amount of anti-CII antibody is not always correlated with the development of arthritis, whereas the IgG_{2a} anti-CII antibody that recognize a single causal epitope plays an important role in the development of arthritis.[16] Therefore, the effect of KB on a subtype of anti-CII antibody production should be determined. Recent evidence also suggests that NO is generated in the inflamed joints of patients with RA, and macrophages are likely to generate considerable amounts of intraarticular NO.[17] In fact, the inhibitor of inducible NO synthase was shown to prevent paw swelling and histopathological changes in the ankle joint in adjuvant-induced arthritis.[18] In this study, none of the three medicines used showed any inhibitory activity on NO production by macrophages of DBA/1J mice. This result suggests that the effects of these Kampo medicines are not due to the inhibition of macrophage function.

pX transgenic mice, another animal model, express various inflammatory cytokines, including IL-1, IL-2, IL-6, TNF-α, TGF-β1, and IFN-γ, in the joints, and further upregulate MHC class I and class II antigens.[9] Although the precise mechanism of autoimmunity induction in these transgenic mice is not yet known, overexpressed inflammatory cytokines, as well as class I and class II MHC antigens, appear to be involved in the pathogenesis of arthritis in pX transgenic mice. Because it is known that TNF-α expression in the joints leads to chronic inflammatory polyarthritis,[19] and IL-1 injection into the joint space also induces arthritis in rabbits,[20] an elevated expression of MHC molecules in the joints may be implicated in the development of autoimmunity by increasing the antigen-presenting efficiency of synovial cells. The Kampo medicines selected in this study effectively prevent the development of arthritis in pX transgenic mice, but they did not influence the expression of tax mRNA. The production of inflammatory cytokines such as IL-1 and TNF-α by macrophages is mediated by NF-κB in a similar manner to NO synthesis, and NF-κB is also partly regulated by tax. This indicates that MY, DB, and KB seem likely to act downstream of the gene regulation by tax, but not by NF-κB, to suppress the arthritis.

To assess the relationship between the suppressive activity and constituent crude drugs of Kampo medicines, we classified the medicines used in this study into three groups, based on their constituent crude drugs: group 1, KB, KJ, KST, KE, EJ; group 2, MY; group 3, DB, KSM. In the Kampo medicines in group 1, Glycyrrhizae R., Atractylodis Lanceae Rh., Zingiberis Rh., Paeoniae R., Cinnamomi C., Zizyphi F., and Aconiti T. are included as the main constituent crude drugs regardless of their efficacy against CIA. Ephedrae H., *Gypsum fibrosum*, Glehniae R. cum Rh., and Anemarrhenae R. are the constituent crude drugs in KST, KE, and EJ, which were ineffective against CIA. However, Ephedrae H. is a common crude drug in Kampo medicines used to treat rheumatoid arthritis, and it is also included in MY (group 2), that was shown to be effective against CIA. In addition, Glehniae R. cum Rh. was contained in both KST and DB. Taken together, *Gypsum fibrosum* and Anemarrhenae R. may counteract the effect of effective crude drugs for CIA. Interestingly, Anemarrhenae R. is included in KSM, which showed no strong activity. The general characteristics of Kampo medicines are that specific combinations of crude drugs exert additive, synergetic, or antagonistic effects. This indicates that the precise mechanism underlying the pharmacological action of each crude drug remains to be clarified.

In this study, MY and DB were effective in the suppression of arthritis in CIA and pX transgenic mice, although no reduction in serum anti-CII antibody levels or NO production by macrophages was observed. The production of self-

antibodies and the activation of macrophages is considered to be implicated in the development of arthritis. However, taking account of the fact that MY and DB did not suppress either serum anti-CII antibody levels or NO production, they appear not to inhibit the early phase of arthritis. Instead, they may suppress synovium hyperplasia, pannus formation, ankylosis, or joint deformity, which all occur following the activation of an inflammatory reaction.

In conclusion, this study has demonstrated that three Kampo medicines, MY, DB, and KB, are able to suppress the development of arthritis in CIA and in pX mice. The findings suggest that these Kampo medicines could be useful to treat RA. We are presently attempting to clarify the precise mechanism by which these Kampo medicines have a suppressive effect on arthritis.

References

1. Wakabayashi K, Inoue M, Ogihara Y. The effect of Keishi-bushito on collagen-induced arthritis. Biol Pharm Bull 1997;20:376–80.
2. Trentham DE, Townes AS, Kang AH. Autoimmunity to type II collagen: an experimental model of arthritis. J Exp Med 1977;146: 857–68.
3. Courtenay JS, Dallman MJ, Dayan AD, Martin A, Mosedaale B. Immunisation against heterologous type II collagen induces arthritis in mice. Nature 1980;283:666–8.
4. Holmdahl R, Andersson M, Goldschmidt TJ, Gustafsson K, Jansson L, Mo JA. Type II collagen autoimmunity in animals and provocations leading to arthritis. Immunol Rev 1990;118:193–232.
5. Stuart JM, Townes AS, Kang AH. Type II collagen-induced arthritis. Ann NY Acad Sci 1985;460:355–62.
6. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis: an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 1987;30: 1205–13.
7. Iwakura H, Tosu M, Yoshida E, Takiguchi M, Sato K, Kitajima I, et al. Induction of inflammatory arthropathy resembling rheumatoid arthritis in mice transgenic for HTLV-1. Science 1991;253: 1026–8.
8. Yamamoto H, Sekiguchi T, Itagaki K, Saijo S, Iwakura Y. Inflammatory polyarthritis in mice transgenic for human T cell leukemia virus type I. Arthritis Rheum 1993;36:1612–20.
9. Iwakura Y, Saijo S, Kioka Y, Nakayama-Yamada J, Itagaki K, Tosu M, et al. Autoimmunity induction by human T cell leukemia virus type I in transgenic mice that develop chronic inflammatory arthropathy resembling rheumatoid arthritis in humans. J Immunol 1995;155:1588–98.
10. Endo T, Iwakura Y, Kobata A. Structural changes in the N-linked sugar chains of serum immunoglobulin G of HTLV-1 transgenic mice. Biochem Biophys Res Commun 1993;192:1004.
11. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Anal Biochem 1982;126:131–8.
12. Seki N, Sudo Y, Yoshioka T, Sugihara S, Fujitsu T, Sakuma S, et al. Type II collagen-induced murine arthritis. I. Induction and perpetuation of arthritis require synergy between humoral and cell-mediated immunity. J Immunol 1988;140:1477–84.
13. Holmdahl R, Jansson L, Larsson A, Jonsson R. Arthritis in DBA/1 mice induced with passively transfered type II collagen immune serum. Immunohistopathology and serum levels of anti-type II collagen auto-antibodies. Scand J Imunol 1990;31:147–57.
14. Stuart JM, Tomoda K, Yoo TJ, Townes AS, Kang AH. Serum transfer of collagen-induced arthritis. II. Identification and localization of autoantibody to type II collagen in donor and recipient rats. Arthritis Rheum 1983;26:1237–44.
15. Kleinau S, Martinsson P, Heyman B. Induction and suppression of collagen-induced arthritis is dependent on distinct Fcγ receptors. J Exp Med 2000;1611–6.
16. Watson WC, Townes AS. Genetic susceptibility to murine collagen II autoimmune arthritis: proposed complement C5, major histocompatibility complex (MHC) and non-MHC loci. J Exp Med 1985;162:1878–91.
17. Farrell AJ, Blake DR, Palmer RMJ, Moncada S. Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. Ann Rheum Dis 1992;51:1219–22.
18. Stefanovic-Racic M, Meyers K, Meschter C, Coffey JW, Hoffman RA, Evans CH. N-monomethyl arginine, an inhibitor of nitric oxide synthase, suppresses the development of adjuvant arthritis in rats. Arthritis Rheum 1994;37:1062–9.
19. Keffer J, Probert L, Cazlaris H, Georgopoulos S, Kaslaris E, Kioussis D, et al. Transgenic mice expressing human tumor necrosis factor: a predicitive genetic model of arthritits. EMBO J 1991;10:4025–31.
20. Feige U, Karbowski A, Rordorf-Adam C, Pataki A. Arthritis induced by continuous infusion of hr-interleukin-1α into the rabbit knee joint. Int J Tissue React 1989;11:225–38.