Studies on anti-inflammatory effects of Japanese Oriental (Kampo) medicines : Inhibitory effects on experimental acute and chronic inflammatory models in rats

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Abstract

Anti-inflammatory effects of Keishi-ka-jutsubu-to (KJ), Keishi-ni-eppi-ichi-to (KE), Keishi-shakuyaku-chimo-to (KS) and Eppi-ka-jutsu-to (EJ), recognized to be effective clinically in rheumatoid arthritis (RA), were examined, first in carrageenin air-pouch inflammation in rats. Oral administration of these four Kampo prescriptions showed a tendency of inhibitory effect on granulation tissue formation, which is characteristic of chronic proliferative inflammation, and particularly that of KS and EJ suppressed significantly. Secondarily, to reveal whether the inhibitory effect of KS and EJ on chronic inflammation is followed by inhibition at the acute inflammatory stage or not, the effects of these two drugs on carboxymethyl cellulose (CMC) air-pouch inflammation in rats were investigated. At the acute stage of inflammation, oral administration of these drugs showed a tendency to inhibit leukocyte emigration, but there was no perceptible effect on exudation of plasma proteins. In conclusion, one of the mechanisms of suppression at the chronic stage of inflammation was thought to be the inhibitory effect on the proliferation of tissue fibroblasts.

Keywords rheumatoid arthritis, Kampo medicine, anti-inflammatory effect, carrageenin air-pouch method, carboxymethyl cellulose air-pouch method, collagen, fibroblast proliferation.

Abbreviations CMC, carboxymethyl cellulose ; Hyp, hydroxyproline ; RA, rheumatoid arthritis ; EJ, Eppi-ka-jutsu-to (Yue-Bi-Jia-Shu-Tang), 越婢加朮湯 ; KE, Keishi-ni-eppi-ichi-to (Gui-Zhi-Er-Yue-Bi-Yi-Tang), 桂枝二越婢一湯 ; KS, Keishi-shakuyaku-chimo-to (Gui-Zhi-Shao-Yao-Chih-Mu-Tang), 桂枝芍薬知母湯 ; KJ, Keishi-ka-jutsubu-to (Gui-Zhi-Jia-Shu-Fu-Tang), 桂枝加朮附湯.

Introduction

In the treatment of rheumatoid arthritis (RA) nowadays, progress has been achieved with the use of several kinds of nonsteroidal anti-inflammatory drugs and immunomodulating agents. However, not a few patients, who can no longer stand their adverse side effects, visit our department of Japanese Oriental (Kampo) Medicine, Toyama Medical and Pharmaceutical University. One of the authors reported previously on the therapeutic effect of Kampo medicine, such as Keishi-shakuyaku-chimo-to and Keishi-ka-ryo-jutsubu-to, on RA,[1] and the efficacy of Kampo treatment has also been reported by others.[2-4]

RA is thought to be a disease which typifies chronic proliferative inflamation based on autonomic immunological disorder. Therefore, for the purpose of evaluating anti-rheumatic drugs, it is necessary to approach it from both sides of the anti-inflammatory effect and immunomodulating effect.

However, there have been but a few reports

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about the mechanisms of that action of Kampo prescriptions.[5,6] The present study was undertaken in an attempt to clarify the anti-inflammatory effects of Kampo prescriptions separated from immunological property, used with carrageenin air-pouch inflammation and carboxymethyl cellulose (CMC) air-pouch inflammation in rats. Four prescriptions, such as Keishi-shakuyaku-chimo-to and Eppi-ka-jutsu-to were chosen from previous reports[1-3] concerned with Kampo treatment on RA.

Materials and Methods

Preparation of Kampo extracts : Four Kampo prescriptions, Keishi-ka-jutsubu-to (KJ), Keishi-ni-eppi-ichi-to (KE), Keishi-shakuyaku-chimo-to (KS) and Eppi-ka-jutsu-to (EJ) were chosen as the experimental medicines in this study. The components of the Kampo prescriptions are listed in Table I. All the medicines were the same as those used daily in our university hospital. Each decoction was made from Kampo medicines after boiling for one hour with 400 ml of water to exactly 300 ml. To give an example of EJ, each medical plant (c, d, e, f, g and h in Table I) was mixed (sum total 24 g), 400 ml of water added, the mixture then boiled for one hour, filtrated while warm, and water adjusted to exactly 300 ml. For the purpose of experimenting with 3 times or 5 times a higher dose, three times or five times (×3, ×5 each) the amount of the decoction was made after evaporation. In the case of EJ×3, the decoction was made after evaporation to one third (100 ml) the amounts of decoction from 300 ml of EJ.

Table I Medical plants used in the Kampo prescriptions of this study (g/day).

Components / Prescriptions	a	b	c	d	e	f	g	h	i	j	k
桂枝加朮附湯 Keishi-ka-jutsubu-to (KJ) Gui-Zhi-Jia-Shu-Fu-Tang	4.5	4.5	3.0	1.0	4.5	5.0	—	—	—	—	1.0
桂枝二越婢一湯加附子 Keishi-ni-eppi-ichi-to-ka-bushi (KE) Gui-Zhi-Er-Yue-Bi-Yi-Tang-Jia-Fu-Zi	3.0	3.0	3.0	1.0	4.0	—	3.0	5.0	—	—	1.0
桂枝芍薬知母湯 Keishi-shakuyaku-chimo-to (KS) Gui-Zhi-Shao-Yao-Chih-Mu-Tang	4.0	3.0	2.0	1.0	—	5.0	3.0	—	4.0	4.0	1.0
越婢加朮湯 Eppi-ka-jutsu-to (EJ) Yue-Bi-Jia-Shu-Tang	—	—	2.0	1.0	3.0	4.0	6.0	8.0	—	—	—

a ; Keihi (Cinnamomi Cortex) from China*
b ; Shakuyaku (Paeoniae Radix) Japan*
c ; Kanzo (Glycyrrhizae Radix) China*
d ; Shokyo (Zingiberis Rhizoma) China*
e ; Taiso (Zizyphi Fructus) China*
f ; Byakujutsu (Atractylodis Rhizoma) China*
g ; Mao (Ephedrae Herba) from China*
h ; Sekko (Gypsum Fibrosum) China*
i ; Chimo (Anemarrhenae Rhizoma) China*
j ; Bofu (Ladebouriellae Radix) China*
k ; Shirakawabushi (Aconiti Tuber) Japan**

Note : Each drug was mixed with 400 ml of water and boiled to 300 ml.
*Supplied by Tochimoto-Tenkaido Co., Ltd., Osaka.
**Supplied by Uchida Wakan-Yaku Co., Ltd., Tokyo.

Chemicals : Carrageenin (Seakem #202 carrageenin) was from Marine Colloid Inc. (N.J., U.S.A.), CMC (Serogen F3H) from Daiichi Industrial Pharmaceutical Co., Ltd. (Kyoto, Japan), Dulbecco's modified Eagle's medium (DMEM) from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan), and indomethacin from Sigma Chemical Co. (St. Louis, U.S.A., Lt. No. 117F-

0595). All other reagents, including prednisolone (Lt. No. TLE7002), chloramine T, perchloric acid, *p*-dimethylaminobenzaldehyde, and amido black were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan).

Animals : Male 6 week-old Sprague-Dawley rats weighing 160－200 g were purchased from Japan SLC Co., Ltd. (Hamamatsu, Japan). They were housed in air-conditioned quarters at 22± 1℃ and 50－60% relative humidity under a 12 hr light/12 hr dark cycle (lights on 7 : 00－19 : 00). Animals were kept on a commercial pellet diet with water *ad libitum* throughout the experiments.

Carrageenin air-pouch inflammation : For the models of subacute and chronic stages of inflammation, carrageenin air-pouch inflammation was used. According to the method of Tsurufuji *et al.*,[7,8] rats were injected subcutaneously in the back with 7 ml of air to form an air pouch. On the next day, an injection of 4 ml of 2% (W/V) carrageenin solution in 0.9% NaCl into the air pouch initiated the inflammatory responses. The effects of each decoction (drug) were examined in two ways, *i.e.* the preventive and curing effects. For investigating the preventive effect, the rats were each administered orally via a catheter 1.5 ml/day of the decoction, or the same amount of water (37℃) for the control group, for 9 days, from the day before the carrageenin injection (day－1) to 8 days after (day 7), as shown in Fig. 1. Rats were administered to also in the same way : indomethacin (0.25 mg/day) for the indomethacin group, and prednisolone (0.25 mg/day) for the prednisolone group. After the rats were sacrificed on day 7, the amount of pouch fluid and the weight of granulation tissue were determined. Granulation tissues were frozen and used for quantitative analysis of collagen and non-collagen protein.

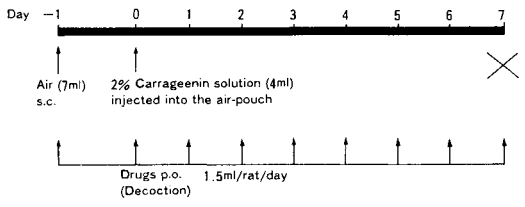

Fig. 1 Method of carrageenin air-pouch inflammation (1) for estimating preventive effects of the test drugs.

For estimating the ability of the test drugs to reduce the pre-formed granulation tissue at the chronic stage, the procedure shown in Fig. 2 was adopted. After selecting rats with a large pouch size, which were thought to have sufficiently developed formation of granulation tissue, they were separated into several groups on day 4. The rats were then orally given via catheter a 2 ml/rat/day of the decoction (KS×5 or EJ×3) or the same amounts of water (37℃) to control from day 4 to day 8. On day 9, they were sacrificed and the amount of pouch fluid, the weight of granulation tissue, and the amounts of collagen or non-collagen protein of the tissues were measured.

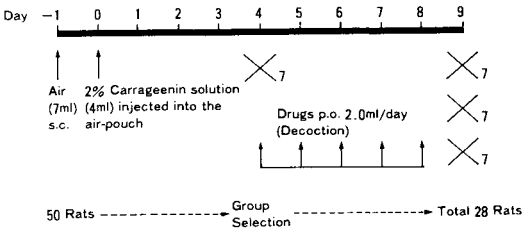

Fig. 2 Method of carrageenin air-pouch inflammation (2) for estimating curing effects of the test drugs.

CMC air-pouch inflammation : As a model of acute inflammation, CMC air-pouch inflammation was used. According to the method of Tsurufuji *et al.*,[9,10] 5 ml of air was injected subcutaneously in the dorsum of rats one day before the injection of 8 ml of 2% (W/V) CMC solution in 0.9% NaCl into the air sac to initiate the acute inflammation. They were given the decoction (×3) orally through a catheter at 37℃, 1.5 ml/rat to the medication groups or the same amount of 37℃ water to the control group. As shown in Fig. 3, they were administered using the following schedule : one day before, 1 hour before, and 1, 3 and 5 hours after the injection of CMC.

Two hundred microliters of the pouch fluid was collected in a tuberculin syringe at 3, 5, and

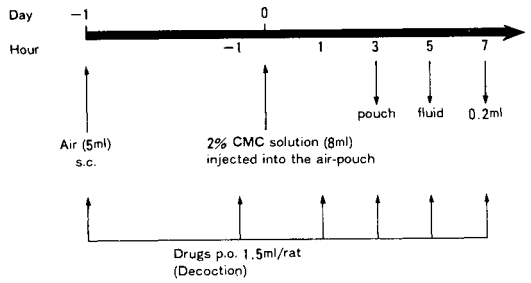

Fig. 3 Method of CMC air-pouch inflammation.

7 hours after the CMC injection. Each sample was divided into two portions : 100 μl of fluid was for the measurement of leukocytes, and the other 100 μl for that of the protein content in the CMC fluid. The leukocyte number, which means the migrated leukocytes, was counted using a Coulter counter (Model Zb, Coulter Electronics, Inc., Hialeah, Florida, U.S.A.), and the exudated protein concentration was measured by Lowry's method[11] for determination of vascular permeability.

Quantitative analysis of collagen or non-collagen protein in the granulation tissue : By using the granulation tissue of carrageenin air-pouch inflammation in rats, a comparative examination was performed on the changes both in the amounts of collagen and non-collagen protein, in medicated groups with KS and EJ. A quantitative analysis of hydroxyproline, specific amino acid of collagen, was performed after tissue collagen was gelatinized for 1 hr at 120℃, 1.0 kg/cm^2 G, and a portion was filtrated. With another part, *i.e.*, the non-gelatinized part of the tissue, quantitative determination of non-collagen protein was undertaken according to Lowry's method[11] after dissolving in 1 N NaOH solution. Then the non-collagen protein was evaluated.

Hydroxyproline assay : Samples from the gelatinized part of the tissue were made 6 N with conc-HCl and hydrolyzed for 16 hr at 105℃. After careful neutralization with 10 N NaOH, the hydroxyproline content was determined as described by Woessner.[12] Briefly, 200 μl of chloramine T solution was added to 1,000 μl of the hydrolyzed samples in duplicate. The tubes were vortexed and allowed to stand for 20 min at room temperature, and 800 μl of perchloric acid (3.15 mol) was added. After 5 min at room temperature, 800 μl of 20 % *p*-dimethylaminobenzaldehyde solution was added. The tubes were heated at 60℃ for 20 min and cooled in cold water for 5 min, and absorbance was determined at 557 nm. The hydroxyproline content was derived from the standard curve.

Evaluation of Kampo prescriptions on proliferation of fibroblasts derived from the granulation tissue : Fibroblasts derived from day 8 granulation tissue of carrageenin air-pouch inflammation were cultured in a 5% CO_2 incubator at 37℃. As standard medium, Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum was used. Then a water-soluble component of the medical decoction (KS or EJ) was added to the medium up to 5% maximum. Every three days the medium containing the test drug was changed, and at the same time the number of fibroblasts was counted until 12 days. Cell counting was performed according to the method of Vilček *et al.*[13] with a minor modification. Fibroblasts in each well were dyed with amido black, and absorbance was read at 630 nm. Cell numbers were estimated from the standard curve.

Statistical analysis : In several experiments, results were expressed as percent or percent inhibition of respective control in order to consider the results together. The results, expressed as mean ± S.E.M., were subjected to statistical analysis according to the Student's *t*-test when individual groups were compared ; when different treatments were compared with the same control group, Duncan's multiple range test was used.

Results

Anti-inflammatory effects of Kampo prescriptions on carrageenin air-pouch inflammation

The amount of pouch fluid and weight of granulation tissue on day 7 in carrageenin air-pouch inflammation (preventive effects) are shown in Fig. 4. All four prescriptions exhibited an inhibitory tendency on the weight of the granulation tissue. Further, at high doses of KS or

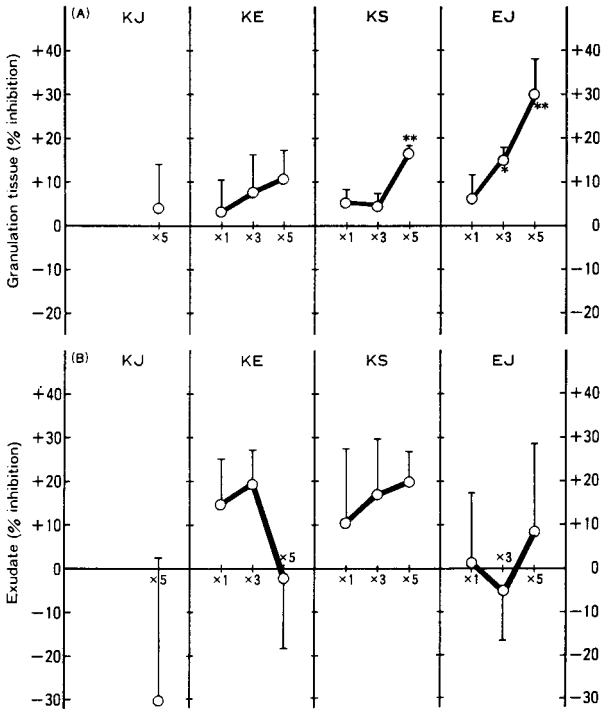

Fig. 4 Preventive effects on the amounts of granulation tissue (A) and exudate (B) of the carrageenin-induced inflammation following the method shown in Fig. 1.
Vertical line means percent inhibition of control on the amounts of granulation tissue (A) or exudate (B). Each point represents the mean±S.E.M. of six or seven rats. Values are statistically significant *versus* corresponding controls : $*p<0.05$; and $**p<0.01$.

EJ, the suppression was statistically significant. The suppressive rate was 30.1% ($p<0.01$) with EJ×5, 16.7% ($p<0.01$) with KS×5, and 14.8% ($p<0.05$) with EJ×3. However, the suppressive tendency recognized in KJ and KE could not become statistically significant even at a five times higher dose. As concerns the amounts of exudate, none of the four prescriptions showed significant suppression. Concerning indomethacin (0.25 mg/day) and prednisolone (0.25 mg/day), percent inhibitions of granulation tissue were 3.4%, 48.9% ($p<0.01$), and those of exudate were 24.0% and 42.0%, respectively. As an adverse side effect, EJ×5 induced a tendency of diarrhea.

Effects of KS and EJ on CMC air-pouch inflammation

The carrageenin air-pouch inflammation mentioned above can be conceived at subacute and chronic stages[14] ; on the other hand, the CMC air-pouch method is suitable for estimating the acute stage of inflammation. To reveal the mode of action of both KS and EJ which have an antiphlogistic effect on the carrageenin-induced inflammation model, the effects of these two drugs on CMC air-pouch inflammation in rats were investigated. There was no influence on exudate protein, while a suppressive tendency was exhibited on leukocyte emigration in the pouch 7 hr after CMC injection (Fig. 5). The suppressive rates of KS and EJ were 17.2% and 15.8%, respectively, not a statistically significant difference.

Quantitative analysis of collagen and non-collagen protein in granulation tissue

Effects of KS and EJ on collagen hydroxyproline and non-collagen protein in granulation tissue of carrageenin air-pouch inflammation are

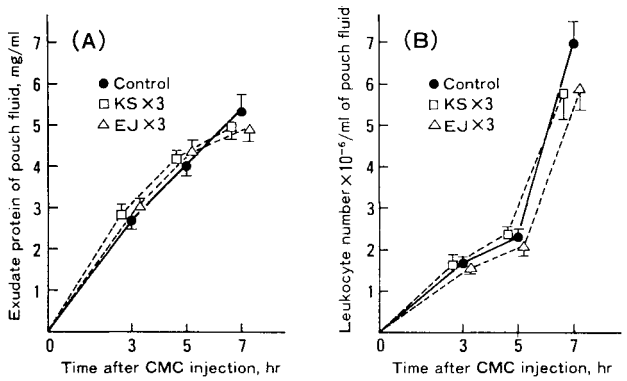

Fig. 5 Effects of KS and EJ on exudate protein (A) and leukocyte emigration (B) in CMC air-pouch inflammation in rats.

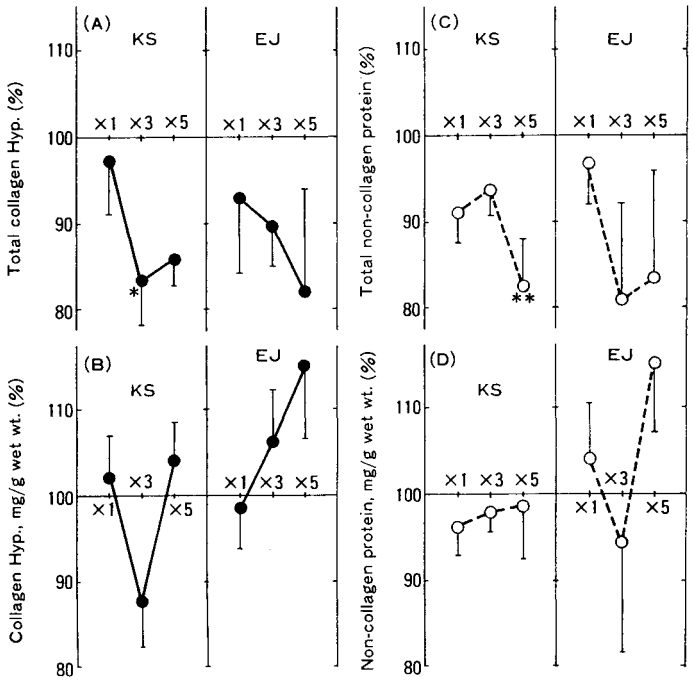

Fig. 6 Inhibitory effects on the amounts of collagen (●) and noncollagen protein (○) of the carrageenin-induced inflammation following the method shown in Fig. 1. Vertical line means percentage of amount compared with control.
Each point represents the mean±S.E.M. of six or seven rats. Values are statistically significnat *versus* corresponding controls : $*p<0.05$; and $**p<0.005$.

shown in Fig. 6. Both groups tended to suppress total collagen hydroxyproline in general, and KS×3 suppressed total collagen hydroxyproline with statistical significance ($p<0.05$). By measurement of the changes in the amount of total protein as non-collagen protein (collagen-free residue), it was demonstrated that both groups possess a suppressive tendency, and the suppres-

sion with KS×5 was seen to be significant ($p<0.005$). However, when the amount of collagen hydroxyproline or non-collagen protein was expressed as per wet weight of granulation tissue, a difference in their tendencies between the KS and EJ groups became evident, *i.e.*, EJ exhibited an increasing tendency, whereas KS had little effect on the amount of collagen hydroxyproline or non-collagen protein.

Inhibitory effects of KS and EJ on proliferation of fibroblasts derived from granulation tissue

To investigate the mechanism of reducing granulation tissue in carrageenin air-pouch inflammation by KS and EJ, effects on the proliferation of fibroblasts after adding these test drugs were examined. As shown in Fig. 7, observation up to day 12 revealed that both KS and EJ suppressed the proliferation of fibroblasts concentration-dependently ($p<0.001$).

Effect of KS and EJ on chronic stage in carrageenin air-pouch inflammation

Following the method shown in Fig. 2, the anti-inflammatory effect at the chronic stage in carrageein air-pouch inflammation of the two efficient drugs (EJ×3 and KS×5) was examined. During the procedure, any adverse effects including diarrhea were not observed. As shown in Fig. 8, both EJ×3 and KS×5 exhibited an inhibitory effect on granulation tissue weight and the amount of exudate on day 9 compared with the same-day control group. In particular, the EJ×3 group suppressed the granulation tissue weight and both the EJ×3 and KS×5 groups suppressed the amount of exudate significantly (both $p<0.01$).

The correlation between suppression of granulation tissue weight and changes of tissue collagen was analysed by comparing the amount of collagen and non-collagen protein from the granulation tissue (Fig. 9). Both the EJ×3 and KS×5 groups suppressed total collagen content compared with that of control significantly ($p<0.01$). So far as non-collagen protein was concerned, both groups displayed suppressive tendency, but it was not statistically significant. When the amount of collagen protein was expressed per wet weight of granulation tissue, the KS×5 group showed a suppressive tendency whereas the EJ×3 group showed an increasing tendency of collagen hydroxyproline levels. Concerning the

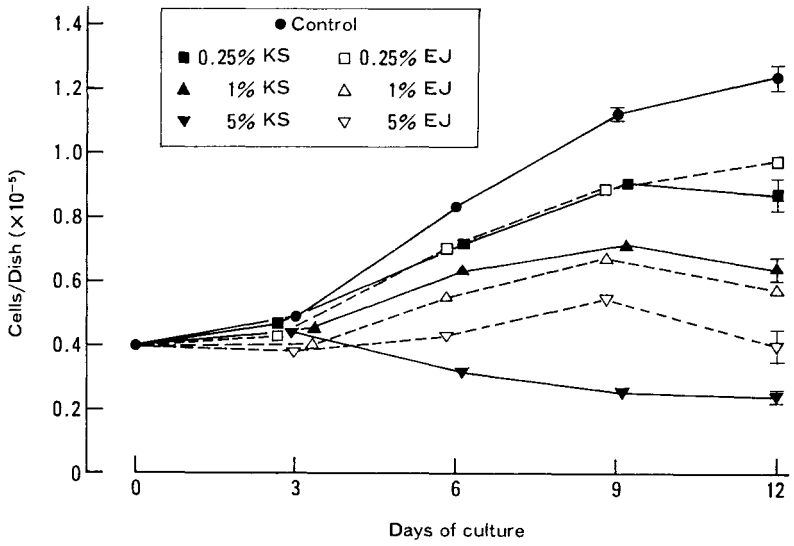

Fig. 7 Inhibitory effects of KS and EJ on the proliferation of granulation tissue-derived fibroblasts in culture.

Results are expressed as cells number per dish, mean values±S.E.M. (vertical bars); vertical bars within the size of the symbol used are omitted. All values at day 6, 9 and 12 are significant *versus* corresponding controls ($p<0.001$).

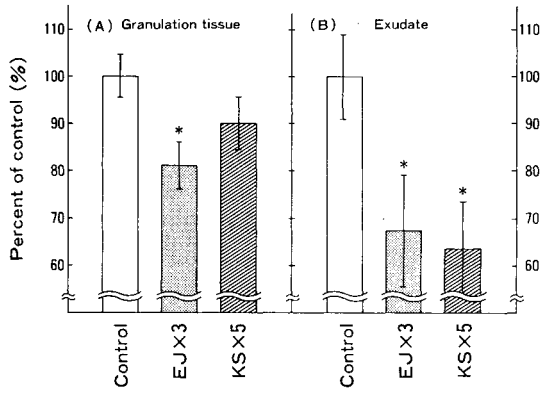

Fig. 8 Curing effects of EJ and KS on the amounts of granulation tissue (A) and exudate (B) of the carrageenin-induced inflammation following the method shown in Fig. 2.
Vertical line means percent of control. Each column and bracket represents the mean± S.E.M. of seven rats. Values significantly different from the control : $*p<0.01$.

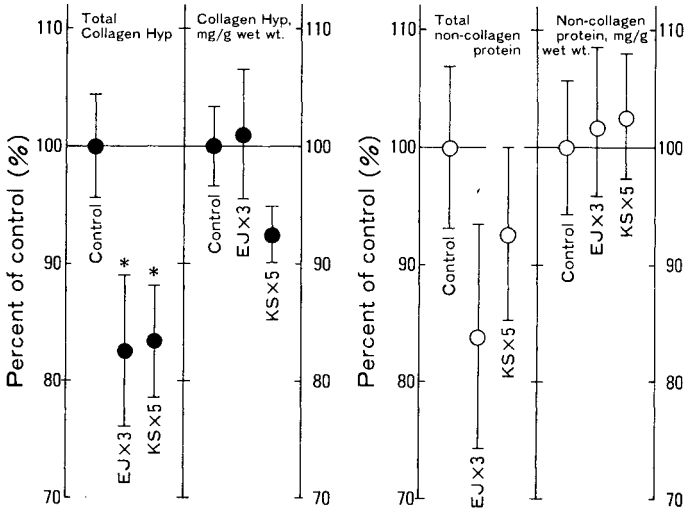

Fig. 9 Inhibitory effects on the amounts of collagen (●) and noncollagen protein (○) of the carrageenin-induced inflammation following the method shown in Fig. 2.
Vertical line means percent of control. Each point represents the mean±S.E.M. of seven rats. Values significantly different from the control : $*p<0.01$.

non-collagen protein per wet weight, there was no diversity between the two groups.

Discussion

RA is thought to be a disease which typifies chronic proliferative inflammation, and therefore animal models with proliferative inflammation are desirable for studying the pharmacological regulation of anti-inflammatory drugs for RA. In regard to the characteristics of carrageenin air-pouch inflammation which was mainly used in our study, these may be enumerated : (1) Inflammatory reactions from acute to chronic stages are extensively reproducible ; (2) Adequate materials for studying inflammation biochemically can be obtained from inflammatory tissue in a relatively short period (about one week) ; (3) It is an excellent model of chronic proliferative inflammatory diseases because of its reproducibility and general usefulness.[14] By using this inflammation model, it was revealed that KS and

EJ suppressed granulation tissue formation.

In order to elucidate the mechanism of anti-inflammatory effects of these test drugs, the collagen hydroxyproline level in granulation tissue and the inhibitory effect on the proliferation of fibroblasts from granulation tissue were also investigated. It was suggested that both KS and EJ have an inhibitory effect on the proliferation of fibroblasts from granulation tissue.

For the purpose of examining their behavior at the acute stage of inflammation (stage of increased vascular permeability and chemotaxis of leukocytes), an experiment on CMC air-pouch inflammation in rats was carried out. In this study both KS and EJ tended to inhibit leukocyte emigration, though they did not inhibit the vascular permeability expressed in the exudate protein of the pouch fluid. Therefore, it is hardly possible that the two drugs, KS and EJ, inhibited chronic inflammation from inhibition at the acute stage of the inflammation in an intermediate manner.

With regard to the inhibition of granulation tissue by high dose of effective prescriptions (KS, EJ), EJ×5, EJ×3 and KS×5 suppressed granulation tissue respectively 30.1%, 14.8% and 16.7% in the preventive effect (Fig. 4). These values are by no means inferior to that of indomethacin (3.4%), though they are inferior to that of prednisolone (48.9%). Concerning comparison of Kampo medicine in the case of KS between the usual human dosage and this experimental effective dose on rats, the usual human dosage is 5−6 ml/kg and this experimental dose was about 10 ml/kg, 30 ml/kg, 50 ml/kg in KS (×1), KS×3, KS×5, each, when they are converted into KS×1. From the result that KS×5 reduced granulation tissue and KS×3 did not reduce it, it is suggested that an effective dose for rats in this study is about ten times higher than the usual human dosage. It is conjectured that this is caused by difference in sensitivity to KS between humans and rats.

The present study revealed that both KS and EJ possess anti-inflammatory effects in the third stage of inflammation. By measuring the amount of collagen in the granulation tissue, it was demonstrated, however, that their respective anti-inflammatory activities were not the same. From the analysis of collagen formation per wet weight, it may be assumed that the EJ has the inhibitory effect of transferring free-water to inflammatory sites.

As concerns the anti-inflammatory effect of the medical plants comprising the Kampo prescriptions used in this study, it has been reported that Aconiti Tuber,[15] Ephedrae Herba,[16] Atractylodis Rhizoma[17] and Glycyrrhizae Radix[18] have anti-inflammatory effects at the early stage, such as increased vascular permeability or leukocyte migration. On the other hand, at the late stage of inflammation (third stage : granuloma formation or connective-tissue proliferation) Glycyrrhizae Radix[18] (glycyrrhizin, glycyrrhetic acid), Sinomeni Caulis et Rhizoma[19] (sinomeniae), Bupleuri Radix[20] (saikosaponin), Platycodi Radix[21] (platycodin) and Magnoliae Flos[22] (magnoshinin) have been reported as efficient medical plants. Among them, Glycyrrhizae Radix[18] is a common ingredient of the four prescriptions used in this study. However, KS and EJ showed an anti-granulation effect whereas KJ and KE did not. Therefore, it is suggested that either the pharmacological effect of Glycyrrhizae Radix is itself weak, or its effect is reduced by interaction with the other co-used medical plants.

Concerning the effects of medical plants on the third stage of inflammation, Arichi *et al.*[23] reported that a Bupleum combination such as Saiko-keishi-kankyo-to (Chai-Hu-Gui-Zhi-Gan-Jiang-Tang), Sho-saiko-to (Xiao-Chai-Hu-Tang) and Saiko-keishi-to (Chai-Hu-Gui-Zhi-Tang) has a suppressive effect on this stage. In relation to this finding, Yamamoto *et al.*[20] also indicated that saikosaponin, a main component of Bupleuri Radix, has an anti-inflammatory effect on this stage.

There have been several reports about medical plants or Kampo prescriptions and their anti-inflammatory effects in regard to adjuvant arthritis in rats. According to such reports, Angelicae Radix, Atractylodis Rhizoma and Paeoniae Radix,[24-26] Ephedrae Herba, Moutan Cortex and Ladebouriellae Radix,[24,25] as well as KJ, KE and

KS[6] have been recognized as possessing anti-inflammatory activity. There is much proof to indicate that adjuvant arthritis is closely correlated with cellular and humoral immunity in its onset and development.[27] Therefore the mechanisms of its inflammation are different from the experimental model of the present study, which is thought to be clear-cut as an inflammatory model separated from immunological property. Though KJ and KE exhibited no inhibitory effects in this carrageenin-induced model, it does not mean that KJ and KE, recognized to be effective clinically in RA, are unsuitable for treatment of RA. It is possible that these drugs are efficient clinically either in an immunological way or from the acute stage of inflammation. However, it is noteworthy that KS and EJ revealed anti-inflammatory activity in the third stage of chronic proliferative inflammation apart from differing immunological mechanism.

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和文抄録

慢性関節リウマチに臨床的に効果の認められている桂枝加朮附湯（KJ），桂枝二越婢一湯（KE），桂枝芍薬知母湯（KS），越婢加朮湯（EJ）について，まずカラゲニン空気嚢炎症ラットを用い，抗炎症効果を検討した。4方剤は経口投与にて，慢性炎症の特徴である肉芽組織の形成を抑制する傾向を認め，特にKSとEJで有意であった。次にKSとEJでの慢性炎症の抑制が急性炎症の抑制を介しているか否かをカルボキシメチルセルロース（CMC）空気嚢炎症ラットを用い検討した。KSとEJは，急性炎症反応のうち白血球浸潤に対し抑制傾向を示したものの，血漿蛋白の滲出反応は抑制しなかった。さらに，有効方剤による肉芽線維芽細胞増殖抑制実験により，慢性炎症抑制機序のひとつとして，肉芽組織線維芽細胞に対する増殖抑制機序が示唆された。

References

1) Terasawa, K. and Imadaya, A. : Therapeutic effect of Sino-Japanese (Kampoh) medicine on rheumatoid arthritis. *J. Med. Pharm. Soc. WAKAN-YAKU* **2**, 439-445, 1985.
2) Taru, A. : The Treatment of Rheumatoid Arthritis by Kampô-medicine. *J. Traditional Sino-Jap. Med.* **2**, 6-16, 1981.
3) Imadaya, A., Terasawa, K., Tosa, H., Itoh, T., Sakuragawa, N. and Takahashi, K. : A trial of the traditional herbal treatment (Kampoh medicine) to Rheumatoid Arthritis. *Proc. Symp. WAKAN-YAKU* **15**, 207-215, 1982.
4) Tanaka, H. : Kampo medicine on rheumatoid arthritis : Remission inducing tendency of Saireito. *Pharma Medica* **6** (suppl.), 70-79, 1988.
5) Cyong, J. and Otsuka, Y. : Kampoh medicines and anti-inflammation. *KAMPO-IGAKU* **6**, 12-20, 1982.
6) Kano, K., Yasuda, A., Kanamoto, I., Adati, I., Ueno, M., Horikoshi, I. and Terasawa, K. : Anti-inflammatory effects of Keisi-ka-zyutubu-tô, Keisi-ni-eppi-iti-tô and Keisi-syakuyaku-timo-tô (1) : Inhibitory effects on inflammatory models in rats. *J. Med. Pharm. Soc. WAKAN-YAKU* **1**, 249-253, 1984.
7) Tsurufuji, S. : How to assess inflammation in animals. *Drug. Exptl. Clin. Res.* **5**, 79-84, 1979.
8) Tsurufuji, S., Sato. H., Min, K.R. and Ohuchi, K. : Difference in the anti-inflammatory effect of indomethacin between acute and chronic stages of carrageenin-induced inflammation. *J. Pharm. Dyn.* **1**, 8-14, 1978.
9) Tsurufuji, S. and Utsuki, S. : Anti-inflammatory activity assayed by CMC pouch method of clonixin, indomethacine, phenylbutazone and hydrocortisone acetate. *Folia Pharmacol. Japan.* **67**, 509-513, 1971.
10) Ishikawa, H., Niinobe, S. and Tsurufuji, S. : Studies on the mode of action of anti-inflammatory agents. I : Quantitative analysis of anti-inflammatory effect by carboxymethyl cellulose pouch method. *Yakugaku Zasshi* **88**, 1472-1477, 1968.
11) Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. : Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265-275, 1951.
12) Woessner, J.F. : The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. *Arch. Biochem. Biophys.* **93**, 440-447, 1961.
13) Vilček, J., Palombella, V.J., Hendriksen-Destefano, D., Swenson, C., Feinman, R., Hirai, M. and Tsujimoto, M. : Fibroblast growth enhancing activity of tumor necrosis factor and its relationship to other polypeptide growth factors. *J. Exp. Med.* **163**, 632-643, 1986.
14) Nakagawa, H. : Regulation of chronic proliferative

inflammation by anti-inflammatory drugs. *Yakugaku Zasshi* **102**, 221-235, 1982.

15) Hikino, H., Konno, C., Takata, H., Yamada, Y., Yamada, C., Ohizumi, Y., Sugio, K. and Fujimura, H. : Anti-inflammatory principles of Aconitum Roots. *J. Pharm. Dyn.* **3**, 514-525, 1980.

16) Hikino, H., Konno, C., Takata, H. and Tamada, M. : Anti-inflammatory principles of Ephedra Herbs. *Chem. Pharm. Bull.* **28**, 2900-2904, 1980.

17) Endo, K., Taguchi, T., Taguchi, F., Hikino, H., Yamahara, J. and Fujimura, H. : Anti-inflammatory Principles of Atractylodes Rhizomes. *Chem. Pharm. Bull.* **27**, 2954-2958, 1979.

18) Kumagai, A. : Hormone-like action of licorice and glycyrrhizin. *Metabolism and Disease* **10**, 632-645, 1973.

19) Saeki, K., Yokoyama, J. and Wake, K. : Inhibition of granulation tissue growth by histamine. *J. Pharmacol. Exp. Ther.* **193**, 910-917, 1975.

20) Yamamoto, M. : Biochemistry of Bupleurum falcatum : Anti-inflammatory and metabolic actions. *Metabolism and Disease* **10**, 695-701, 1973.

21) Takagi, K. and Lee, E.B. : Pharmacological studies on platycodon grandiflorum A. DC. II. Anti-inflammatory activity of crude Platycodin, its activities on isolated organs and other pharmacological activities. *Yakugaku Zasshi* **92**, 961-968, 1972.

22) Kimura, M., Suzuki, J., Yamada, T., Yoshizaki, M., Kikuchi, T., Kadota, S. and Matsuda, S. : Anti-inflammatory effect of neolignans newly isolated from the crude drug "Shin-i" (Flos Magnoliae). *Planta Med.* **51**, 291-293, 1985.

23) Arichi, S., Okada, Y., Kobayashi, N. and Wakazono, F. : Study on Saiko (1) Kyokyokuman and Saiko. *Proc. Symp. WAKAN-YAKU* **4**, 48-57, 1970.

24) Cho, S., Takahashi, M. Toita, S. and Cyong, J. : Suppression of adjuvant arthritis on rat by Oriental herbs (I). *Shoyakugaku Zasshi* **36**, 78-81, 1982.

25) Toita, S., Takahashi, M., Shindo, M., Cyong, J., Yamada, H., Kiyohara, H. and Cho, S. : Studies on suppressive effect of adjuvant arthritis by Chinese herbs. *Proc. Symp. WAKAN-YAKU* **16**, 262-266, 1983.

26) Takahashi, M., Cyong, J., Toita, S., Shindo, M. and Cho, S. : Suppression of adjuvant arthritis by oral administration of Oriental Herbs II. *Kitasato Arch. of Exp. Med.* **55**, 34-45, 1982.

27) Kobashi, O. : Adjuvant arthritis. In "Experimental Animal Models of Intractable Diseases" (Ed. by M. Kyogoku), Soft Science Inc., Tokyo, pp. 340-350, 1984.