Inhibitory Effects of Traditional Chinese Medicine Shimotsu-to and Its Included Crude Fractions on Adjuvant-Induced Chronic Inflammation of Mice

Satoru KOJIMA, Kazuhiko INABA, Shinjiro KOBAYASHI,* and Masayasu KIMURA

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan. Received June 13, 1995; accepted September 13, 1995

Effects of a traditional Chinese medicine, Shimotsu-to (a combined prescription of cnidium rhizome, peony root, angelica root and rehmannia root), and its included crude fractions were investigated on an adjuvant-induced chronic inflammation model of mice. The aqueous extract (30, 100 and 300 mg/kg, i.p.) of Shimotsu-to reduced the carmine content, granuloma weight, inflammation cell count and pouch fluid weight in the inflammation model, respectively. The extract of Shimotsu-to without cnidium at the same doses did not produce significant changes in these four inflammatory parameters. The same doses of extracts of Shimotsu-to without peony, and without angelica, weakly reduced these parameters, except for pouch fluid weight. The extract (30, 100 and 300 mg/kg) of cnidium significantly reduced these four parameters. The same doses of peony extract reduced carmine content, granuloma weight and pouch fluid weight, but less than those of the cnidium extract. The extract of cnidium and peony at the same doses reduced in an additive manner these inflammatory parameters in their combination. These results demonstrated that the Shimotsu-to extract reduced angiogenesis, granuloma formation, inflammatory cell migration and pouch fluid exudation in the adjuvant-induced chronic inflammation model. Cnidium represented the main ingredient for producing the anti-chronic inflammatory effects of Shimotsu-to extract. Cnidium and peony exhibited additive anti-inflammatory effects in combination.

Key words Shimotsu-to; cnidium; peony; anti-chronic inflammation; anti-angiogenesis; adjuvant-induced mouse chronic inflammation model

Shimotsu-to (Si-Wu-Tang) is a traditional Chinese medicine consisting of four crude fractions: cnidium rhizome, peony root, angelica root and rehmannia root in a preparation of the same dry weight. It has been used clinically for improving abnormal blood coagulation, fibrinolysis, atherosclerosis and chronic inflammation in traditional Chinese medicine. The effects of the extract of Shimotsu-to have been investigated from the aspect of blood diseases. We have reported that an aqueous extract of Shimotsu-to improves reduced levels of red blood cells, hemoglobin and hematocrit in an experimental anemic rat.¹⁾ However, there have been few studies concerning the effect of Shimotsu-to extract on blood vessels. We have previously reported that the Shimotsu-to extract inhibited abnormal proliferation of vascular smooth muscle cells in primary culture and indicated that the inhibitory effect of Shimotsu-to depends on the effects of cnidium and angelica.²⁾ The extract of peony has been reported to inhibit carrageenin-induced hind paw edema, but not cotton pellet granuloma formation.³⁾ The extract of angelica also inhibits capillary permeability in acute inflammation.⁴⁾ The present study was focused on their effects on angiogenesis in the chronic inflammatory process.

We have reported the pouch granuloma in an adjuvant-induced chronic inflammation model.⁵⁾ Pathohistological study of the pouch granuloma demonstrated that liquefaction necrosis was formed in the early stage, followed by coagulation necrosis accompanied by inflammatory cell infiltration. The newly sprouted capillaries were well-developed and then fibrous tissue was formed along with the capillaries.⁵⁾ For simultaneous determination of four inflammatory parameters: angiogenesis, granuloma formation, migration of inflammatory cells and exudation of

pouch fluid, a quantitative method was developed in the adjuvant-induced chronic inflammatory model.^{5–8)} The aim of our present study was to investigate the effects of the Shimotsu-to extract on granuloma angiogenesis, granuloma formation, inflammatory cell migration and pouch fluid exudation in a chronic inflammation model. We further examined the anti-inflammatory effects of the crude fractions included in Shimotsu-to to search a main ingredient.

MATERIALS AND METHODS

Animals Male ddY mice (5–6 weeks of age, weighing 26–30 g) were purchased from Japan Shizuoka Laboratory Center (Hamamatsu). The mice were maintained under a constant temperature ($23 \pm 1^\circ\text{C}$) and humidity ($55 \pm 5\%$) with lights on from 6 a.m. to 6 p.m., and were fed the usual laboratory diet (MF, Oriental Yeast, Tokyo) and tap water freely.

Adjuvant-Induced Pouch Granuloma Air pouch granuloma was prepared by injection of Freund's complete adjuvant (FCA) with 0.1% croton oil as reported.^{6–8)} Three ml of air was injected subcutaneously into the dorsum of mouse under ether anesthesia to produce a regular oval air pouch. The FCA emulsion was prepared using 2 mg heat-killed *M. tuberculosis* (from Professor I. Azuma, Hokkaido University, Hokkaido) per ml of Freund's incomplete adjuvant. The FCA emulsion (0.5 ml) containing 0.1% croton oil (Nacalai Tesque, Kyoto) was injected into the air pouch under ether anesthesia. Mice were killed by injection of 1 ml of 10% carmine solution (Merck, Darmstadt, Germany) containing 5% gelatin (Nacalai Tesque), which was kept warm at 40°C , into the tail vein on day 5 after FCA injection.

* To whom correspondence should be addressed.

The dead mice were cooled at below 4 °C for several hours. The granuloma tissues were excised, separated from the surrounding loose connective tissue and weighed. All the exudative pouch fluid was harvested and weighed. The inflammatory cells in the pouch were counted with a haemocytometer. The carmine content of granuloma tissues was measured as follows: The granuloma tissues were cut, solubilized with 3N NaOH and acidified with 36% HCl. After centrifugation, the supernatant was filtered. The carmine content in the filtrate was determined by measuring the optical density at 490 nm. The carmine content is an index of newly formed blood vessels in pouch granuloma.⁶⁾

Preparation of Extract of Crude Drugs Cnidium rhizome (Hokkaido), peony root (Sichuan, China), angelica root (Hokkaido) and rehmannia root (Henan, China) were used. Shimotsu-to was prepared by mixing above four crude fractions at the dry weight ratio of 1:1:1:1. The aqueous extracts of Shimotsu-to, Shimotsu-to without its included crude fractions, a mixture of cnidium and peony at the same dry weight and each fraction were prepared by being heated at 96–98 °C in 6 volumes of ion-exchanged water for 40 min with an automatic extractor Torobi (Tochimoto, Osaka), filtered through a mesh (No. 42, Sanpo, Tokyo), and lyophilized with a freeze drier (DF-03G, ULVAC, Tokyo). The dry weight yields of extract were presented in Table 1. These dried extracts (30, 100 and 300 mg/kg) were suspended homogeneously in saline containing 1% Avicel (Asahi Chemical Industry, Tokyo) and injected intraperitoneally at 2 h after FCA injection, and then subsequently once a day for 4 d. Since these three doses of the extracts of Shimotsu-to, Shimotsu-to without its included crude fractions, a mixture of cnidium and peony, as well as each fraction separately, were administered into mice, amounts of fractions in these extracts were overlapped.

Statistical Analyses The data are expressed as the means $\pm$ S.E. and analyzed by one-way analysis of variance. The significance of the difference between the data was assessed by the test of Scheffe or Tukey at the level of $p=0.05$ or 0.01 , respectively.

RESULTS

Inhibitory Effects of Shimotsu-to on Angiogenesis, Granuloma Formation, Migration of Inflammatory Cells and Exudation of Pouch Fluid in Adjuvant-Induced Chronic

Table 1. Extract Yields of Shimotsu-to, Shimotsu-to without Its Included Crude Fraction, and Individual Crude Fractions

Extract	Yield % (w/w)	Extract	Yield % (w/w)
Shimotsu-to	32.2	Cnidium + peony	24.9
Shimotsu-to –cnidium	32.0	Cnidium	24.0
–peony	31.4	Peony	26.2
–angelica	30.2	Angelica	29.0
–rehmannia	21.6	Rehmannia	47.1

These extracts were prepared by being heated at 96–98 °C in 6 volumes of ion-exchanged water for 40 min, and were then filtered through a mesh and lyophilized with a freeze-drier.

Inflammation The effect of Shimotsu-to on granuloma angiogenesis was compared with that on granuloma formation, inflammatory cell migration and fluid exudation in adjuvant-induced chronic inflammation. The extract (30, 100 and 300 mg/kg) of Shimotsu-to reduced in a dose-dependent manner the carmine content, granuloma weight, inflammatory cell number and pouch fluid weight (Fig. 1 left). The intensity of inhibition in these parameters were in the order: pouch fluid weight > inflammatory cell count > granuloma weight $\geq$ carmine content. The anti-inflammatory effect of Shimotsu-to extract was compared with that of a Japanese Sino-medicine, Keishi-ka-jutsu-bu-to, as reported previously.⁹⁾ The extract of Keishi-ka-jutsu-bu-to (50, 100 and 200 mg/kg), prepared at 50 °C, reduced dose-dependently all four parameters. The potencies were in the order: pouch fluid weight > carmine content $\geq$ inflammatory cell count > granuloma weight (Fig. 1 right). These results demonstrated that the anti-inflammatory effects of the Shimotsu-to extract were different from those of Keishi-ka-jutsu-bu-to in the adjuvant-induced chronic inflammation model.

Effects of Shimotsu-to without Its Included Crude Fraction on Angiogenesis, Granuloma Formation, Inflammatory Cell Migration and Pouch Fluid Exudation

To investigate the crucial role of fractions included in Shimotsu-to, the effects of Shimotsu-to without its included crude fractions were compared with the effect of Shimotsu-to. The extract (30, 100 and 300 mg/kg) of Shimotsu-to without cnidium did not affect all four inflammatory parameters. The reducing effects of the extract of Shimotsu-to without cnidium at 100 and 300 mg/kg on these parameters were significantly weaker than those of complete Shimotsu-to extract at the same doses (Figs. 2–5 left). The extract (30, 100 and 300 mg/kg) of Shimotsu-to without peony significantly reduced granuloma weight, inflammatory cell count and pouch fluid weight in a dose-dependent manner, and tended to

Fig. 1. Inhibitory Effects of Shimotsu-to and Keishi-ka-jutsu-bu-to on Carmine Content ($\square$), Granuloma Weight ($\bullet$), Inflammatory Cell Count ($\blacktriangledown$) and Pouch Fluid Weight ($\blacktriangle$) in Adjuvant-Induced Chronic Inflammation of Mouse

An extract (30, 100 and 300 mg/kg) of Shimotsu-to was suspended in 1% Avicel-saline and injected intraperitoneally once a day for 5 d. The control values of carmine content, granuloma weight, inflammatory cell count and pouch fluid weight without any extract were 0.368 ± 0.015 mg ($n=14$), 357 ± 8 mg ($n=14$), $(2.8 \pm 0.4) \times 10^7$ cells ($n=14$) and 146 ± 17 mg ($n=14$), respectively. The data are expressed as the means $\pm$ S.E. of % relative amounts ($n=14-16$) to the control value without any extract. The data of Keishi-ka-jutsu-bu-to were replotted from Reference 9).

Fig. 2. Inhibitory Effects on Carmine Content of Shimotsu-to (○), Shimotsu-to without Its Included Crude Fraction (— Crude Fraction: Left; ●) and Each Included Fraction (Right; ●)

The extracts (30, 100 and 300 mg/kg) of Shimotsu-to, Shimotsu-to without cnidium (—1), without peony (—2), without angelica (—3), without rehmannia (—4), and cnidium (1), peony (2), angelica (3) and rehmannia (4) were injected intraperitoneally. The control value of carmine content without any extract was presented in the legend of Fig. 1. The data are expressed as the means $\pm$ S.E. of % relative amounts ($n=7-16$) to the control value without any extract. The data of Shimotsu-to were replotted from Fig. 1. *a*) $p < 0.05$, *b*) $p < 0.01$: significantly different from without extract. *c*) $p < 0.01$: significantly different from Shimotsu-to.

Fig. 3. Inhibitory Effects on Granuloma Weight of Shimotsu-to (○), Shimotsu-to without Its Included Crude Fraction (— Crude Fraction: Left; ●) and Each Included Fraction (Right; ●)

The extracts (30, 100 and 300 mg/kg) of Shimotsu-to, Shimotsu-to without cnidium (—1), without peony (—2), without angelica (—3), without rehmannia (—4), and cnidium (1), peony (2), angelica (3) and rehmannia (4) were injected intraperitoneally. The control value of granuloma weight without any extract was presented in the legend of Fig. 1. The data are expressed as the means $\pm$ S.E. of % relative amounts ($n=7-16$) to the control value without any extract. The data of Shimotsu-to were replotted from Fig. 1. *a*) $p < 0.05$, *b*) $p < 0.01$: significantly different from without extract. *c*) $p < 0.05$, *d*) $p < 0.01$: significantly different from Shimotsu-to.

reduce the carmine content (Figs. 2—5 left). The 50% inhibitory dose values (ID_{50}) were estimated from dose-dependent data below 50% and a little over 50% of the relative amount by the method of least-squares (Tables 2, 3). The potencies of the extract of Shimotsu-to without peony on the carmine content, granuloma weight and pouch fluid weight were smaller than those of the Shimotsu-to extract, and the potency on inflammatory cell count was similar to that of Shimotsu-to extract (Table 2). The extract (30, 100 and 300 mg/kg) of Shimotsu-to without angelica significantly reduced these four parameters (Figs. 2—5 left). The reducing effects of the extract of Shimotsu-to without angelica at 300 mg/kg were

Fig. 4. Inhibitory Effects on Inflammatory Cell Count of Shimotsu-to (○), Shimotsu-to without Its Included Crude Fraction (— Crude Fraction: Left; ●) and Each Included Fraction (Right; ●)

The extracts (30, 100 and 300 mg/kg) of Shimotsu-to, Shimotsu-to without cnidium (—1), without peony (—2), without angelica (—3), without rehmannia (—4), and cnidium (1), peony (2), angelica (3) and rehmannia (4) were injected intraperitoneally. The control value of inflammatory cell count without any extract was presented in the legend of Fig. 1. The data are expressed as the means $\pm$ S.E. of % relative amounts ($n=7-16$) to the control value without any extract. The data of Shimotsu-to were replotted from Fig. 1. *a*) $p < 0.05$, *b*) $p < 0.01$: significantly different from without extract. *c*) $p < 0.05$, *d*) $p < 0.01$: significantly different from Shimotsu-to.

Fig. 5. Inhibitory Effects on Pouch Fluid Weight of Shimotsu-to (○), Shimotsu-to without Its Included Crude Fraction (— Crude Fraction: Left; ●) and Each Included Fraction (Right; ●)

The extracts (30, 100 and 300 mg/kg) of Shimotsu-to, Shimotsu-to without cnidium (—1), without peony (—2), without angelica (—3), without rehmannia (—4), and cnidium (1), peony (2), angelica (3) and rehmannia (4) were injected intraperitoneally. The control value of pouch fluid weight without any extract was presented in the legend of Fig. 1. The data are expressed as the means $\pm$ S.E. of % relative amounts ($n=7-16$) to the control value without any extract. The data of Shimotsu-to were replotted from Fig. 1. *a*) $p < 0.05$, *b*) $p < 0.01$: significantly different from without extract. *c*) $p < 0.05$, *d*) $p < 0.01$: significantly different from Shimotsu-to.

significantly smaller than those of Shimotsu-to extract on granuloma formation and inflammatory cell count, but were similar to those on carmine content and pouch fluid weight (Figs. 2—5 left). Since the amounts of each crude fraction in the extract of Shimotsu-to without angelica were contained in an amount 1.3-fold greater than in the same dose of the Shimotsu-to extract, the significant difference between the effects of these extracts was underestimated. The extract (30, 100 and 300 mg/kg) of Shimotsu-to without rehmannia significantly reduced four inflammatory parameters in a dose-dependent manner,

Table 2. Fifty % Inhibitory Doses (ID_{50}) of Shimotsu-to and Shimotsu-to without Its Included Crude Fraction for Angiogenesis, Granuloma Formation, Inflammatory Cell Migration and Pouch Fluid Exudation in Adjuvant-Induced Air Pouch Granuloma of Mice

Extract	ID_{50} (mg/kg)			
	Carmine content	Granuloma weight	Inflammatory cell count	Pouch fluid weight
Shimotsu-to	450	336	116 (84.5—158)	67.8 (50.6—90.9)
Shimotsu-to—cnidium	No effect ^{a)}	No effect	No effect	No effect
—peony	No effect	Weak	233 (129—421)	217 (109—433)
—angelica	Weak ^{b)}	Weak	Weak	80.2 (46.5—138)
—rehmannia	509	Weak	118 (67.1—206)	55.0 (21.5—141)

The values in parenthesis represent 95% confidence limits ($n=7-16$). a) No significant effect. b) Significant effect but impossible to estimate ID_{50} .

and presented similar effects on these parameters to those of Shimotsu-to extract (Figs. 2—5 left, Table 2). These results suggested that cnidium induced the inhibitory effects of Shimotsu-to on these inflammatory parameters. Peony may weakly cause an anti-angiogenic effect and angelica may also produce inhibitory effects on granuloma formation and inflammatory cell migration.

Effects of Crude Fractions Included in Shimotsu-to on Angiogenesis, Granuloma Formation, Migration of Inflammatory Cells and Exudation of Pouch Fluid To confirm the results of Shimotsu-to without its included crude fractions, the effects of these fractions were investigated on the four inflammatory parameters. The extract (30, 100 and 300 mg/kg) of cnidium significantly reduced these four parameters in a dose-dependent manner (Figs. 2—5 right). The potencies of cnidium extract for the carmine content and granuloma weight were weaker than those of complete Shimotsu-to extract, but its potencies for inflammatory cell count and pouch fluid weight were similar to those of Shimotsu-to extract (Table 3). The extract (30, 100 and 300 mg/kg) of peony reduced the carmine content, granuloma weight and pouch fluid

Table 3. Fifty % Inhibitory Doses (ID_{50}) of Shimotsu-to and Its Included Crude Fraction for Angiogenesis, Granuloma Formation, Inflammatory Cell Migration and Pouch Fluid Exudation in Adjuvant-Induced Air Pouch Granuloma of Mice

Extract	ID_{50} (mg/kg)			
	Carmine content	Granuloma weight	Inflammatory cell count	Pouch fluid weight
Shimotsu-to	450	336	116 (84.5—158)	67.8 (50.6—90.9)
Cnidium	Weak ^{a)}	Weak	128 (61.8—264)	124 (75.6—205)
Peony	Weak	Weak	No effect	397
Angelica	No effect ^{b)}	No effect	No effect	No effect
Rehmannia	No effect	No effect	No effect	No effect

The values in parenthesis represent 95% confidence limits ($n=7-16$). a) Significant effect but impossible to estimate ID_{50} . b) No significant effect.

weight in a dose-dependent manner, but not the inflammatory cell count. These effects of peony extract on all parameters were weaker than those of cnidium extract (Figs. 2—5 right, Table 3). The extracts (30, 100 and 300 mg/kg) of angelica and rehmannia did not significantly affect these four parameters, respectively (Fig. 2—5 right). These results confirmed that cnidium was the main ingredient involved in the inhibitory effects of Shimotsu-to on four inflammatory parameters. Peony also had a minor role in the effect of Shimotsu-to on angiogenesis, granuloma formation and exudation of pouch fluid.

Effects of Cnidium and Peony in Combination on Angiogenesis, Granuloma Formation, Migration of Inflammatory Cells and Exudation of Pouch Fluid The effects of a mixture of cnidium and peony at the same dry weight on these four inflammatory parameters were compared with those of cnidium and peony, separately. The extract (30, 100 and 300 mg/kg) of the mixture of both crude drugs together inhibited all parameters in a dose-dependent manner (Fig. 6). Its inhibitory effects on the four parameters did not differ significantly from those of cnidium and peony extracts, respectively. These results demonstrated that the inhibition of cnidium and peony

Fig. 6. Comparison of Inhibitory Effects on Four Inflammatory Parameters between the Extract of a Mixture (1:1) of Cnidium and Peony, the Mixed Extract (1:1) of Cnidium and Peony, the Cnidium Extract and the Peony Extract

The extracts (30, 100 and 300 mg/kg) of cnidium (1), peony (2), cnidium and peony (1+2) and a mixture of cnidium and peony (3) were injected intraperitoneally. The control values of carmine content, granuloma weight, inflammatory cell count and pouch fluid weight without any extract were presented in the legend of Fig. 1. The data are expressed as the means $\pm$ S.E. of % relative amounts ($n=6-8$) to the control value without any extract. The data of extracts of cnidium and peony were replotted from Figs. 2—5. a) $p < 0.05$, b) $p < 0.01$: significantly different from without extract.

was additive on these chronic inflammatory parameters.

To investigate whether the additive effects of a mixture of cnidium and peony were induced by their extraction process, the effects of a mixed extract of cnidium and peony at the same concentration on these parameters was compared with those of the extract of a mixture of cnidium and peony. The mixed extract (30, 100 and 300 mg/kg) also inhibited these parameters in a dose-dependent manner and its inhibitory effects were not significantly different from those of the extract of their mixture (Fig. 6). These results showed that the additive inhibitory effects of cnidium and peony depended on their actions on these parameters, but not on their extraction process.

DISCUSSION

Shimotsu-to, a traditional Chinese medicine, has been used clinically for improving syndromes involving deficiencies of the blood.¹⁰⁾ The syndromes mainly occur in females and are recognized as a stagnancy of blood, including blood coagulation, fibrinolysis, atherosclerosis and chronic inflammation.^{11,12)} The effects of Shimotsu-to on these syndromes have been investigated from the aspect of blood diseases. In the present study, the effects of Shimotsu-to were investigated pharmacologically on newly formed micro blood vessels in adjuvant-induced chronic inflammation in mice. The Shimotsu-to extract inhibited not only angiogenesis but also granuloma formation, inflammatory cell migration and pouch fluid exudation. The inhibitory effect of Shimotsu-to extract on angiogenesis paralleled that on granuloma formation. This inhibitory pattern was similar to that of hydrocortisone and indomethacin.^{7,13)}

To study the main fractions in Shimotsu-to for their anti-inflammatory effects, the potency of the extract of Shimotsu-to without its included crude fraction (Table 2), and each included fraction (Table 3), was compared with that of Shimotsu-to. The positive results of these extracts

on these inflammatory parameters in Tables 2 and 3 are summarized in Fig. 7. The extract of Shimotsu-to without rehmannia had similar effects on three inflammatory parameters, except granuloma formation, to those of the Shimotsu-to extract, demonstrating no effect of rehmannia. The extract of Shimotsu-to without peony presented weaker inhibition of these four parameters than the Shimotsu-to extract. The peony extract significantly inhibited three parameters, except for inflammatory cell count (Figs. 2—5). However, the inhibitory effects of the peony extract on these four parameters were weaker than those of the Shimotsu-to extract (Fig. 7). The inhibitory effects of the cnidium extract on the four inflammatory parameters were greater than those of the other three fractions in Shimotsu-to (Figs. 2—5). The potencies of the cnidium extract were similar to those of the Shimotsu-to extract for pouch fluid exudation and inflammatory cell migration, and were smaller for angiogenesis and granuloma formation (Fig. 7). These results demonstrated that cnidium was the main fraction participating in the anti-chronic inflammatory effects of Shimotsu-to. Butylidenephthalide (120 mg/kg), which was contained in the essential oil of cnidium rhizome,²⁾ also significantly reduced the carmine content, granuloma weight and pouch fluid weight to $50.1 \pm 3.4\%$, 58.6 ± 3.5 and $33 \pm 6.4\%$ of the relative amount ($n=11$), respectively. These results supported the inhibitory effect of cnidium on the inflammatory parameters.

The inhibitory effects of Shimotsu-to on angiogenesis and granuloma formation were not completely explained by just the effects of cnidium, because the effects of cnidium extract on these parameters were weaker than those of Shimotsu-to extract. The combined effects of cnidium and peony, therefore, were investigated by examining the inhibitory effects of an extract of their mixture. Several lines of our evidence demonstrated that the combination of these fractions additively inhibited those four parameters, and the additive effects depended on their individual actions on these parameters, but were

Fig. 7. Intersected Axis Expressing log 50% Inhibitory Dose (ID₅₀, g/kg) of Shimotsu-to and Shimotsu-to without Rehmannia, Shimotsu-to without Peony, and Peony and Cnidium for Carmine Content (CC), Granuloma Weight (GW), Pouch Fluid Weight (PFW) and Inflammatory Cell Count (ICC)

These values were estimated from the data in Tables 2 and 3.

not induced by their extraction process. First, the inhibitory effects of the extract from the mixture of cnidium and peony at the same dry weight were similar to those of mixture of their individual extracts at the same concentration. Second, the yield of extract from a mixture of cnidium and peony was similar to those of the individual extracts.

The anti-angiogenic effect of cnidium extract was smaller than the effect in the previous experiment under the same inflammatory model.¹⁴⁾ The inhibitory effects of peony extract on the migration of inflammatory cells and the exudation of pouch fluid were also smaller than those reported previously.⁹⁾ This difference may be due to different extracting conditions of crude fraction. In the present investigation, the extracts were prepared by heating at 96–98 °C without a water-cooler, which is different from the extraction at 50 °C with a water-cooler in previous studies.^{9,14)} These results demonstrated that volatile substances such as essential oils and/or heat-unstable substances in cnidium and peony may induce the anti-angiogenic effect and also the inhibitory effects on cell migration and fluid exudation, respectively. The diminution of anti-angiogenic effect of angelica extract might also depend on these different extract conditions.²⁾ These results under various extraction conditions are supported by the different results of Keishi-ka-jutsu-bu-to, a Japanese Sino-medicine, extracted at 50 and 100 °C.⁹⁾

The inhibitory pattern of Shimotsu-to on the chronic inflammation model was different from Keishi-ka-jutsu-bu-to and Kakkon-to-ka-senkyu-shin'i, which have been used clinically to improve chronic inflammatory disease such as rheumatoid arthritis and nasal inflammation, respectively, as reported previously.^{9,14)} Shimotsu-to presented similar inhibitory effects on angiogenesis and granuloma formation in chronic inflammation. However, Keishi-ka-jutsu-bu-to and Kakkon-to-ka-senkyu-shin'i exhibit weaker inhibitory effects on granuloma formation.^{9,14)} In traditional Chinese medicine, Keishi-ka-jutsu-bu-to and Kakkon-to-ka-senkyu-shin'i belong to a different classification from Shimotsu-to. The different effects between

Shimotsu-to and Keishi-ka-jutsu-bu-to or Kakkon-to-ka-senkyu-shin'i on granuloma formation depended on the different prescriptions.

In conclusion, Shimotsu-to inhibited all angiogenesis, granuloma formation, inflammatory cell migration and pouch fluid exudation in adjuvant-induced chronic inflammation. Cnidium appeared to be the main ingredient in the anti-chronic inflammatory effects of Shimotsu-to. Also, cnidium and peony exhibited additive anti-inflammatory effects in combination.

Acknowledgment The authors would like to thank Central Research Laboratories, Yomeishu Seizo Co., Ltd., Nagano, for kind gifts of Shimotsu-to-related extracts.

REFERENCES

- 1) Kojima S., Ezaki N., Inoue M., Yanagisawa K., Sugawara Y., *J. Traditional Med.*, **11**, 231–235 (1994).
- 2) Kobayashi S., Mimura Y., Notoya K., Kimura I., Kimura M., *Jpn. J. Pharmacol.*, **60**, 397–401 (1992).
- 3) Sugishita E., Amagaya S., Ogiwara Y., *J. Pharmacobio-Dyn.*, **7**, 427–435 (1984).
- 4) Tanaka S., Kano Y., Tabata M., Konoshima M., *Yakugaku Zasshi*, **91**, 1098–1104 (1971).
- 5) Kimura M., Suzuki J., Amemiya K., *J. Pharmacobio-Dyn.*, **8**, 393–400 (1985).
- 6) Kimura M., Amemiya K., Yamada T., Suzuki J., *J. Pharmacobio-Dyn.*, **9**, 442–446 (1986).
- 7) Kimura M., Kobayashi S., Luo B., Kimura I., *Int. Arch. Allergy Appl. Immunol.*, **93**, 365–370 (1990).
- 8) Kobayashi S., Kimura I., Kimura M., *Folia Pharmacol. Jpn.*, **105**, 357–363 (1995).
- 9) Kimura M., Kimura I., Luo B., Kobayashi S., *Phytother. Res.*, **5**, 195–200 (1991).
- 10) Chen S. (ed.), “Zengguan Taipinghuimin Hejijufang,” Enterprise Co., Ltd., Tokyo, 1988, p. 150.
- 11) Terasawa K., Kimura M., Sakuragawa N., Uchiyama Y., Toriizuka K., Ueno M., *Yakugaku Zasshi*, **103**, 313–318 (1983).
- 12) Yakazu D., *J. Japan Soc. Oriental Med.*, **25**, 167–187 (1975).
- 13) Luo B., Kobayashi S., Kimura I., Kimura M., *Phytother. Res.*, **5**, 231–233 (1991).
- 14) Kimura M., Kimura I., Guo X., Luo B., Kobayashi S., *Phytother. Res.*, **6**, 209–216 (1992).