Expression of the Anti-metastatic Effect Induced by Juzen-taiho-to is Based on the Content of Shimotsu-to Constituents

Yasuhiro ONISHI, Takeshi YAMURA, Katsunori TAUCHI, Takashi SAKAMOTO, Kazuhiro TSUKADA, Shinyu NUNOME, Yasuhiro KOMATSU, and Ikuo SAKI

Department of Pathogenic Biochemistry, Research Institute for Wakan-Yaku, Second Department of Surgery, Faculty of Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan and Kampo and Pharmacognosy Laboratory, Tsumura Central Laboratories, Tsumura & Co., Ibaraki 300–1155, Japan.

Received February 2, 1998; accepted April 3, 1998

We investigated the inhibitory effect of oral administration of Juzen-taiho-to, a Kampo Japanese herbal medicine, and its related formulations on the experimental liver and lung metastasis of tumor cells in vivo. Oral administration of Juzen-taiho-to for 7 d before tumor inoculation significantly reduced the number of liver metastatic colonies of colon 26-L5 carcinoma cells and attenuated the increase of liver weight in a dose-dependent manner ranging from 4 to 40 mg/d. Its oral administration for this same period before tumor inoculation also significantly inhibited lung metastasis of B16-BL6 melanoma cells. Juzen-taiho-to originally consisted of 8 crude drugs derived from Shimotsu-to and Shikunshu-to prescriptions together with two crude drugs (Cinnamomi Cortex and Astragali Radix). Oral administration of Shimotsu-to as well as Juzen-taiho-to for 7 d before tumor inoculation resulted in a significant reduction in the number of metastatic colonies and the liver weight as compared with the control, whereas Shikunshu-to did not exhibit such an inhibitory effect. Unsei-in containing four Shimotsu-to constituents was also active in inhibiting liver metastasis. Toki-shakuyaku-san and Ninjin-yoei-to, which include all Shimotsu-to constituents except Rehmanniae Radix and Cnidii Rhizoma, respectively, did not show a significant anti-metastatic effect. Rikkunshu-to and Ninjin-yoei-to, which contain Shikunshu-to constituents, did not affect the inhibition of liver metastasis. Hooch-eiki-to treatment before tumor inoculation also led to a significant inhibition of liver metastasis, probably through an inhibitory mechanism different from Juzen-taiho-to. These results suggest that the anti-metastatic effect of Juzen-taiho-to is partly associated with its Shimotsu-to-derived constituents.

Key words Juzen-taiho-to; Shimotsu-to; tumor metastasis

Despite the advances in diagnostic techniques for the early detection of colon cancer and the significant improvements in surgical procedures, the mortality rate of colon cancer has been increasing annually, and metastasis is a frequent cause of death by cancer. The liver is the most common target of the hematogenous metastasis in gastrointestinal tract cancer, especially colon cancer, and the prognosis for cases with liver metastasis is extremely poor. If liver metastases were inhibited, then the prognosis of patients with colon carcinoma would improve. Therefore, the treatment to prevent metastasis rather than for primary tumor is a major target in the therapy of colon cancer.

Juzen-taiho-to, a Kampo Japanese herbal medicine, was introduced to Japan in the Kamakura-dynasty (1192–1333) and since then has been used as a cure for consumption, general debility, deficiency and impairment of Yin, Yang, vital energy or blood in the viscera or bowels, and lack of appetite. It is one of the nourishing agents, so-called “Ho-zai” (in Japanese), for improving disturbances and imbalances in the homeostatic condition of the body diagnosed by Kampo medicine, and is currently administered to patients weakened by long illness, fatigue, loss of appetite, might sweats, circulatory problems, and anemia. In addition, it is used for cancer patients. Several studies have shown that Juzen-taiho-to has various biological activities: enhancement of phagocytosis, cytokine induction, antibody production, induction of mitogenic activity of spleen cells, anti-tumor effect when combined with surgical excision, augmentation of tumor activities in combination with or without other drugs, and protection from the deleterious effects of anti-cancer drugs as well as radiation-induced immunosuppression and bone marrow toxicity.

We previously reported that Juzen-taiho-to effectively prevented weakly malignant tumors from growing progressively upon co-implantation with a gelatin sponge, and that it may act to induce antioxidative substances, in addition to augmenting the host-mediated immune responses. Also, our recent study demonstrated that oral administration of Juzen-taiho-to inhibited liver metastasis produced by intraperitoneal injection of the liver metastatic variant of murine colon 26 carcinoma (colon 26-L5) cells and enhanced the survival rate, possibly through the activation of macrophages and T-cells in the immune system. The active constituents contained in Juzen-taiho-to have not yet been examined in detail, however.

To extend our previous study, we investigated here the effect of oral administration of Juzen-taiho-to and its related formulations on experimental liver and lung metastases of tumor cells in vivo.

MATERIALS AND METHODS

Preparation of Kampo Medicines Kampo medicines used in this study were obtained from Tsumura & Co., Ltd., Tokyo. Kampo formulations and their crude drug composition are listed in Table 1. They were controlled for quality by Japanese Pharmacopeia XIII. A mixture of crude drugs was added to an appropriate volume of water and extracted at 100 °C for 1 h. The extracted solution was filtered and spray-dried to obtain the dry extract powder. The blended powder

© 1998 Pharmaceutical Society of Japan
was dissolved in distilled water and administered orally to mice at the appropriate dose for 7 d before tumor inoculation.

**Mice** Specific pathogen-free female BALB/c and C57BL/6 mice, 6—8 weeks old, were purchased from Japan SLC Inc., Hamamatsu. They were maintained in the Laboratory for Animal Experiments, Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University. This study was conducted in accordance with the standards established by the Guide for the Care and Use of Laboratory Animals of Toyama Medical and Pharmaceutical University.

**Cells** The liver metastatic cell line of the colon 26 carcinoma (colon 26-L5) was obtained by the *in vivo* selection method. Highly lung metastatic B16-BL6 melanoma cells, obtained by an *in vitro* selection procedure for invasion, were kindly provided by Dr. Fidler I. J., M. D. Anderson Cancer Center, Houston, TX. Colon 26-L5 cells were maintained as monolayer cultures in RPMI-1640 supplemented with 7.5% fetal bovine serum (FBS) and L-glutamine. B16-BL6 cells were maintained as monolayer cultures in Eagle’s minimal essential medium (MEM) supplemented with 7.5% FBS, vitamin solution, sodium pyruvate, nonessential amino acids and L-glutamine.

**Assay for Experimental Liver Metastasis of Colon Carcinoma Cells** Log-phase cell cultures of colon 26-L5 cells were harvested with 1 mM EDTA in phosphate-buffered saline (PBS), washed three times with serum-free RPMI, and resuspended at appropriate concentrations in PBS. BALB/c mice under ether anesthesia underwent laparotomy by an upper median incision, the duodenal loop was exposed, and an injection of colon 26-L5 (1 × 10^7/200 μl) cells was made into the portal vein through a 29-gauge needle attached to a 1-ml syringe. A sterile absorbable cotton sponge was placed over the injection site as the needle was withdrawn to prevent bleeding and peritoneal dissemination of the tumor cells. The mice were killed 14—21 d after tumor inoculation, the number of metastatic colonies in each liver was counted macroscopically, and the liver weight was recorded to evaluate the tumor metastasis as described previously. Body weights of mice were measured during experiment. The liver was also processed for histological examination in H/E (hematoxylin/eosin)-stained sections by standard techniques.

**Assay for Experimental Lung Metastasis of Melanoma Cells** Log-phase cell cultures of B16-BL6 cells were washed three times with serum-free MEM, and resuspended to a final concentration of 2.5 × 10^7/ml in PBS. C57BL/6 mice were given an intravenous injection of melanoma cells (5 × 10^9/200 μl). Fourteen days later, the mice were killed. The lungs were fixed in Bouin’s solution and tumor colonies counted under a dissecting microscope.

**Statistical Analysis** The statistical significance of differences between the groups was determined by the Student’s two-tailed t-test.

**RESULTS**

**Inhibition of Experimental Liver Metastasis by Juzen-taiho-to** We first examined the effect of oral administration of Juzen-taiho-to on liver metastasis caused by the injection of colon 26-L5 carcinoma cells into the portal vein. The number of tumor colonies in the liver and the liver weight were measured on day 14 after tumor inoculation. Figure 1 shows that the oral administration of Juzen-taiho-to for 7 d before tumor inoculation significantly reduced the number of tumor colonies in the liver and attenuated the increase of liver weight in a dose-dependent manner ranging from 4 to 40 mg/d. Furthermore, no significant loss of body weight was observed in the Juzen-taiho-to-treated group as compared with untreated control, while the administration of CDDP (cis-diamine dichloro platinum) had a side effect of loss of body weight (Fig. 1). This result is well consistent with our previous report. A histopathological study revealed that some metastatic lesions were present in the livers of untreated control mice, but not in those of Juzen-taiho-to-treated mice (Fig. 2). These results clearly indicate that the oral administration of Juzen-taiho-to is effective in prevent-

---

**Fig. 1. Dose Response of Juzen-taiho-to on Experimental Liver Metastasis Produced by Intraportal Injection of Colon 26-L5 Carcinoma Cells**

Five BALB/c mice per group were inoculated intraperitoneally with colon 26-L5 cells (2 × 10^6). Juzen-taiho-to at the indicated doses was orally administered for 7 d before tumor inoculation. Fourteen days after tumor inoculation, mice were sacrificed and the number of tumor colonies in the livers and liver weights were measured manually (left and middle panels). CDDP was injected intravenously on day 1 and 8 after tumor inoculation. Body weights of treated and untreated groups were recorded during the experiment (right panel, control: □; Juzen-taiho-to 40 mg, ○; Juzen-taiho-to 20 mg, ●; CDDP 80 μg, ▲). *p<0.05; ***p<0.001 as compared with untreated control.
Fig. 2. Histopathological Observation of the Liver Metastases
(a) control group, (b) Juzen-taiho-to treated group. Massive metastases are seen in the control group, but not in the Juzen-taiho-to group. ×200.

Fig. 3. Effect of Oral Administration of Juzen-taiho-to on Experimental Lung Metastasis Produced by the Intravenous Injection of B16-BL6 Melanoma Cells
Five C57BL/6 mice per group were orally administered Juzen-taiho-to (40 mg/d/mouse) for 7d before intravenous injection with B16-BL6 melanoma cells (5×10⁶). Fourteen days after tumor inoculation, mice were sacrificed and lungs were removed. The number of tumor colonies in the lungs was counted manually. *p<0.001 as compared with control.

Inhibition of Experimental Lung Metastasis by Juzen-taiho-to
We next examined the effect of oral administration of Juzen-taiho-to on lung metastasis produced by i.v. injection of B16-BL6 melanoma cells, in place of the intraportal injection of colon 26-L5 carcinoma cells. The number of tumor colonies in the lung was recorded 14 d after tumor inoculation. Oral administration of 40 mg/mouse of Juzen-taiho-to for 7d before tumor inoculation significantly inhibited lung metastasis of B16-BL6 cells as compared with the control (Fig. 3). The results of Figs. 1 and 3 clearly demonstrate that Juzen-taiho-to was able to significantly inhibit the lung and liver metastases of two different types of tumors.

Effect of Juzen-taiho-to, Shimotsu-to and Shikunshi-to on Experimental Liver Metastasis
Juzen-taiho-to consist of 8 crude drugs derived from Shimotsu-to and Shikunshi-to prescriptions and two other crude drugs (Cinnamomi Cortex and Astragali Radix) (Table 1). Therefore, we investigated whether treatment with Shimotsu-to and Shikunshi-to could inhibit the experimental liver metastasis caused by colon 26-L5 cells. Oral administration of Shimotsu-to as well as Juzen-taiho-to for 7 d before the tumor inoculation resulted in a significant reduction in both the number of metastatic colonies and the liver weight as compared with the control, whereas Shikunshi-to did not exhibit such an inhibitory effect (Fig. 4). These results suggest that Shimotsu-to-derived constituents are mainly responsible for the development of the anti-metastatic effect induced by Juzen-taiho-to.

Effect of Juzen-taiho-to and its Related Kampo Medicines on Experimental Liver Metastasis
To further confirm the inhibitory effect by Shimotsu-to and Juzen-taiho-to, we examined whether other Kampo formulations with or without the four crude drugs of Shimotsu-to could inhibit experimental liver metastasis. As shown in Fig. 5, Juzen-taiho-to showed the most potent anti-metastatic effect among the Kampo medicines used in this study. Oral administration of Unsei-in containing the four crude drugs of Shimotsu-to significantly inhibited the liver metastasis as compared with the untreated control, while Toki-shakuyaku-san and Ninjinyo-to, in which one crude drug among the Shimotsu-to constituents is lacking (Table 1), showed no significant inhibitory effect. Rikken-shi-to, which consists of Shikunshi-to and other two crude drugs (Table 1), was not active at inhibiting liver metastasis (Fig. 4). Hochu-ekki-to, one of the “Ho-zai” (a tonic medicine), showed a slight but significant anti-metastatic effect, despite having only the crude drug Angelicae Radix in common with Shimotsu-to (Table 1).

DISCUSSION
In the present study, oral administration of Juzen-taiho-to showed a significant inhibition of liver metastasis by colon 26-L5 carcinoma cells as well as lung metastasis by B16-BL6 melanoma cells in comparison to the untreated control (Figs. 1 and 3). Also, oral administration of Juzen-taiho-to did not cause any marked loss of the body weight. These findings are in good agreement with our previous report. Since Juzen-taiho-to inhibited liver metastasis of colon 26-L5 cells even in NK-deficient mice as well as normal mice, but not in macrophage or T-cell deficient mice, its anti-metastatic effect would be mainly mediated by the activation of macrophages and/or T-cells in the host immune system.

Juzen-taiho-to is composed of 8 crude drugs derived from two prescriptions (Shimotsu-to and Shikunshi-to) and two other crude drugs (Table 1). We therefore examined the effect
Table 1. List of Kampo Formulations and Their Composition of Crude Drugs

<table>
<thead>
<tr>
<th>Crude drug</th>
<th>Japanese medicine</th>
<th>Kampo medicine</th>
<th>Juzen-taiho-to</th>
<th>Shimotsu-to</th>
<th>Shikunshi-to</th>
<th>Rikkunshi-to</th>
<th>Unsei-in</th>
<th>Ninjin-yoei-to</th>
<th>Toki-shakuyaku-san</th>
<th>Hochu-ekki-to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astragali Radix</td>
<td>Ogi</td>
<td>(8)</td>
<td>(2)</td>
<td>(1)</td>
<td>(1.5)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Cinnamomi Cortex</td>
<td>Keihi</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(2.5)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Rehmanniae Radix</td>
<td>Jio</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Paeoniae Radix</td>
<td>Shakuyaku</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Cnidii Rhizoma</td>
<td>Senkyu</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Angelicae Radix</td>
<td>Toki</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Atractylodis Lancae Rhizoma</td>
<td>Sojutu</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Ginseng Radix</td>
<td>Ninjin</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Hoelen</td>
<td>Bukuryo</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Glycyrrhizae Radix</td>
<td>Kanzo</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Zingiberis Rhizoma</td>
<td>Shokyo</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Zizyphi Fructus</td>
<td>Taiso</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Pinelliae Tuber</td>
<td>Hange</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Bupleuri Radix</td>
<td>Saiko</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Aurantii Nobilis Pericarpium</td>
<td>Chimpi</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Polygalae Radix</td>
<td>Ongi</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Schisandrae Fructus</td>
<td>Gomishii</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Climacifugae Rhizoma</td>
<td>Shoma</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Scutellariae Radix</td>
<td>Ogon</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Phellodendri Cortex</td>
<td>Obaku</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Copidis Rhizoma</td>
<td>Oren</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Gardeniae Fructus</td>
<td>Sanshishii</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Alismatis Rhizoma</td>
<td>Takusha</td>
<td>(8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
</tbody>
</table>

* Atractylodis Rhizome (Byakujutsu) was used in place of Atractylodis Lancae Rhizoma (Sojutu). Numbers in parentheses represent the ratio of crude drugs in formulation preparations.

Fig. 4. Effect of Oral Administration of Juzen-taiho-to, Shimotsu-to and Shikunshi-to on Liver Metastasis Produced by Intraportal Injection of Colon 26-L5 Carcinoma Cells

Five BALB/c mice per group were orally administered Juzen-taiho-to, Shimotsu-to or Shikunshi-to at the dose of 40 mg/d/mouse for 7 d before intraportal vein injection of colon 26-L5 carcinoma cells (2 × 10⁶). Sixteen days after tumor inoculation, mice were sacrificed and the livers removed. The number of tumor colonies in the livers and liver weights were measured manually. * p < 0.01; ** p < 0.001 as compared with control.

of Shimotsu-to and Shikunshi-to as well as Juzen-taiho-to on experimental liver metastasis by colon 26-L5 cells, to identify which constituents in Juzen-taiho-to are responsible for development of the inhibitory effect. Oral administration of Shimotsu-to as well as Juzen-taiho-to before the tumor inoculation significantly inhibited the number of metastatic colonies and the increase in liver weight as compared with the control (Fig. 4). In contrast, Shikunshi-to did not inhibit liver metastasis effectively. Unsei-in which contains the four Shimotsu-to constituents was also active at inhibiting liver metastasis (Fig. 5). Toki-shakuyaku-san and Ninjin-yoei-to, which include the Shimotsu-to constituents lacking Rehmanniae Radix and Cnidii Rhizoma, respectively, did not show a significant anti-metastatic effect. Interestingly, although Ninjin-yoei-to contains 9 of the crude drugs (except Cnidii Rhizoma) in Juzen-taiho-to, its effect was much less than that of Juzen-taiho-to. In addition, Rikkunshi-to and Ninjin-yoei-to, which contain Shikunshi-to constituents, did not affect the inhibition of liver metastasis, as true of Shikunshi-to. These findings suggest that the four constituents in Shimotsu-to, but not those in Shikunshi-to, are primarily responsible for the development of the anti-metastatic effect induced by Juzen-taiho-to. However, since Juzen-taiho-to was more effective at inhibiting tumor metastasis than Shimotsu-to, it would seem
likely that other constituents in Juzen-taiho-to are also associated with augmentation of the anti-metastatic effect.

Hochu-ekki-to as well as Juzen-taiho-to and Ninjin-yoei-to are known to be nourishing agents (Ho-zai) with an ability to modulate the host-mediated immune responses. The former also exhibited a significant inhibition of the liver metastasis by colon 26-L5 cells similarly to Juzen-taiho-to (Fig. 5). The constituents in Hochu-ekki-to are apparently different from those of Juzen-taiho-to (Table 1). Cho et al. reported that Hochu-ekki-to was able to stimulate NK cells to induce the inhibition of tumor growth rather than macrophages or T-cells. Therefore, the mechanism responsible for its inhibition of liver metastasis may be different from that by Juzen-taiho-to. Further investigation will be needed to determine the differences in the anti-metastatic mechanisms.

In conclusion, the anti-metastatic effect induced by Juzen-taiho-to is partly associated with the Shimotsu-to-derived constituents (Rehmanniae Radix, Paoniae Radix, Cnidii Rhizoma, Angelicae Radix) contained in Juzen-taiho-to. Since these prescriptions are prepared from the combination of many crude drugs, they must have an effect that differs from the combined effect of the individual constituent drugs. Therefore, some formulations containing Shimotsu-to constituents may be effective for the inhibition of tumor metastasis. Juzen-taiho-to may thus provide a useful basis for the prevention of cancer metastasis.

Acknowledgements This work was supported in part by Grants-in-Aid for Cancer Research from the Japanese Ministry of Education, Science, Sports and Culture (No. 09254101 & 07273106). We thank Ms. Kazuko Hayashi and Ms. Sanae Hirota for their technical assistance.

REFERENCES