

Organ selectivity of Juzen-taiho-to and Ninjin-yoei-to in the expression of anti-metastatic efficacy

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Abstract

We investigated the inhibitory effect of oral administration of Juzen-taiho-to and Ninjin-yoei-to on liver metastasis caused by intraportal vein injection of colon 26-L5 cells and lung metastasis by intravenous injection of same tumor cells. Juzen-taiho-to significantly inhibited liver metastasis but not lung metastasis. In contrast, Ninjin-yoei-to was effective at inhibiting lung metastasis but not liver metastasis. In the experimental liver and lung metastases model using same tumor in syngeneic mice system, oral administration of both formulations showed a differential pattern with organ selectivity for the expression of anti-metastatic effects. These results suggest that the different expression of the anti-metastatic effects of both Kampo medicines on tumor metastasis are partly based on the medicinal guides according to the theory of Jing and Lun (Inkei-hoshi) formed in the 13th century.

Key words Juzen-taiho-to, Ninjin-yoei-to, Metastasis, Colon carcinoma, Organ selectivity.

Abbreviations FBS, fetal bovine serum; Hochu-ekki-to (Bu-Zhong-Yi-Qi-Tang), 補中益氣湯; Juzen-taiho-to (Shi-Quan-Da-Bu-Tang), 十全大補湯; Ninjin-yoei-to (Ren-Shen-Yan-Rong-Tang), 人參養榮湯; PBS, phosphate-buffered saline; Shimotsu-to (Si-Wu-Tang), 四物湯; Toki-shakuyaku-san (Dan-Gui-Shao-Yao-San), 當歸芍藥散.

Introduction

Juzen-taiho-to, Ninjin-yoei-to and Hochu-ekki-to are nourishing agents, so-called Ho-zai (補劑) in Japanese, for improving disturbances and imbalances in the homeostatic condition of the body, diagnosed as Kyo-sho (虚証) in Japanese by Kampo medicine. Kyo-sho is a deficiency syndrome including deficiency of vital energy and blood status, *i.e.* Ki (氣) and Ketsu (血) in Japanese. These formulations are also administered to patients in various weakened conditions such as post-surgery patients with cancer and patients with chronic illness because of the ability to modulate the host-mediated immune responses. Juzen-taiho-to has also been used for the treatment of rheumatoid arthritis, atopic dermatitis, chronic fatigue syndrome, ulcerous colitis and so on. Several studies have reported that Juzen-taiho-to has various biological activities such as enhancement of immune responses and protection from the deleterious

effects of anti-cancer drugs as well as radiation-induced immunosuppression and bone marrow toxicity.¹⁻⁸⁾

We have previously reported that oral administration of Juzen-taiho-to was effective for the inhibition of liver metastasis produced by intraportal vein injection of colon 26-L5 carcinoma cells, and enhanced survival rate through the activation of macrophages and T cells rather than NK cells in the host immune system.^{9,10)} In addition, the anti-metastatic effect of Juzen-taiho-to is primarily associated with its Shimotsu-to-derived constituents.¹¹⁾ Unsei-in, a Kampo formulation containing four Shimotsu-to constituents, as well as Shimotsu-to were active in inhibiting liver metastasis of colon 26-L5 carcinoma cells, while Ninjin-yoei-to and Toki-shakuyaku-san, which do not include all Shimotsu-to constituents except Cnidii Rhizoma and Rehmanniae Radix, respectively, did not show a significant anti-metastatic effect.¹¹⁾ Recently, our preliminary study suggests that mediastinal lymph node metastasis produced by orthotopic implantation of murine Lewis lung carcinoma¹²⁾ was significantly inhibited by

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oral administration of Ninjin-yoei-to, but not by Juzen-taiho-to (data not shown).

Considering our previous study, we investigated here whether or not the difference of anti-metastatic effects of Juzen-taiho-to and Ninjin-yoei-to is due to the existence of selective organs and tissues for the expression of the efficacy.

Materials and Methods

Preparation of Kampo formulations: Juzen-taiho-to (lot No. 270048020) and Ninjin-yoei-to (lot No. 2000108020) were kindly provided from Tsumura & Co., Ltd., Tokyo, and their crude drug composition is listed in Table I. They were controlled for quality by the Japanese Pharmacopeia XIV. To assess the constancy of the formulation, the origin of each peak of Juzen-taiho-to and Ninjin-yoei-to in the HPLC profiles was identified by comparison of the retention times and UV spectra of each crude drug with those of chemically defined standard compounds, as described previously.^{1,11)} The blended powder was dissolved in distilled water before oral administration.

Animals: Specific pathogen-free female BALB/c mice, 6 weeks old, were purchased from Japan SLC, Hamamatsu. The mice were maintained in the Laboratory for Animal Experiments, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, under lamina air-flow conditions.

Tumor cells: The liver metastatic cell lines of the colon 26 carcinoma (colon 26-L5) was obtained by the

in vivo selection method of Fidler.^{13,14)} Colon 26-L5 cells were maintained as monolayer cultures in RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and L-glutamine.

Assay for experimental liver and lung metastases of colon 26-L5 carcinoma cells: Log-phase cell cultures of colon 26-L5 cells were harvested with 0.05% EDTA in phosphate-buffered saline (PBS), washed three times with serum-free RPMI, and resuspended at appropriate concentrations in RPMI. In experimental liver metastasis assay, seven BALB/c mice per group under ether anesthesia underwent laparotomy by an upper median incision, and the duodenal loop was exposed. An injection of colon 26-L5 ($2 \times 10^4/200 \mu\text{l}$) cells was administered into the portal vein through a 29-gauge needle attached to a 1-ml syringe. A sterile absorbable cotton swab was placed over the injection site as the needle was withdrawn to prevent bleeding and peritoneal dissemination of the tumor cells. The mice were sacrificed 14 days after tumor inoculation and the number of metastatic colonies in each liver was counted macroscopically to evaluate the tumor metastasis as previously described.^{15,16)} In experimental lung metastasis assay, seven BALB/c mice per group were given an intravenous injection of colon 26-L5 cells ($1.5 \times 10^4/200 \mu\text{l}$). Fourteen days later, the mice were sacrificed. The lungs were fixed in Bouin's solution and tumor colonies counted under a dissecting microscope. Juzen-taiho-to and Ninjin-yoei-to were administered orally to the mice at dose of 40 mg/day/mouse for 7 days before tumor inoculation.⁹⁾

Statistical analysis: The statistical significance of

Table 1 Kampo medicines and their constituents

Constituent	Japanese name	Juzen-taiho-to ratio	Ninjin-yoei-to ratio
Astragal Radix	Ogi	3	1.5
Cinnamomi Cortex	Keihi	3	2.5
Rehmanniae Radix	Jio	3	4
Paoniae Radix	Shakuyaku	3	2
Cnidii Rhizoma	Senkyu	3	
Angelicae Radix	Toki	3	4
Atractylodis Lanceae Rhizoma	Sojutsu	3	4a)
Ginseng Radix	Ninjin	3	3
Hoelen	Bukuryo	3	4
Glycyrrhizae Radix	Kanzo	1.5	1
Aurantii Nobilis Pericarpium	Chimpi		2
Polygalae Radix	Onji		2
Schisandrae Fructus	Gomishi		1

a) Atractylodis Rhizome (Byakujutsu) was used in place of Atractylodis Lanceae Rhizoma (Sojutsu).

*: Shimotsu-to constituents, #: Shikunshi-to constituent

differences between the groups was determined by the Student's two-tailed t-test.

Results and Discussion

We first examined the effect of oral administration of Juzen-taiho-to and Ninjin-yoei-to on liver metastasis caused by the intraportal vein (i.p.v.) injection of colon 26-L5 carcinoma cells. As shown in Fig 1A, Juzen-taiho-to significantly reduced the number of tumor colonies in the liver ($p<0.001$), but Ninjin-yoei-to did not have any effect. Furthermore, no significant change of body weight was observed in both treated groups as compared with untreated control. These results are well consistent with our previous report.¹¹⁾ To investigate the relationship between the efficacy of the formulations and its specific organs for the expression, we next examined the effect of oral administration of Juzen-taiho-to and Ninjin-yoei-to on lung metastasis produced by intrave-

nous injection of same colon 26-L5 carcinoma cells into the same syngeneic mice. Although oral administration of Ninjin-yoei-to for 7 days before tumor inoculation was not effective at inhibiting liver metastasis (Fig. 1A), it significantly inhibited lung metastasis of same tumor cells as compared with the control (Fig. 1B). In contrast, Juzen-taiho-to showed no inhibitory effects against lung metastasis, differently from the inhibition of liver metastasis. Thus, Juzen-taiho-to and Ninjin-yoei-to clearly exhibited the differential inhibitory effects for liver and lung metastases of same colon 26-L5 cells in syngeneic mice.

Although further study is necessary to examine the underlying mechanism for organ-selective expression of the anti-metastatic effects of the formulations, we attempted here to interpret our findings according to the theory of Jing and Luo, Kei-Raku (経絡) in Japanese, in traditional Chinese medicine. Jing in Chinese means route or channels and Luo means collateral net or branch

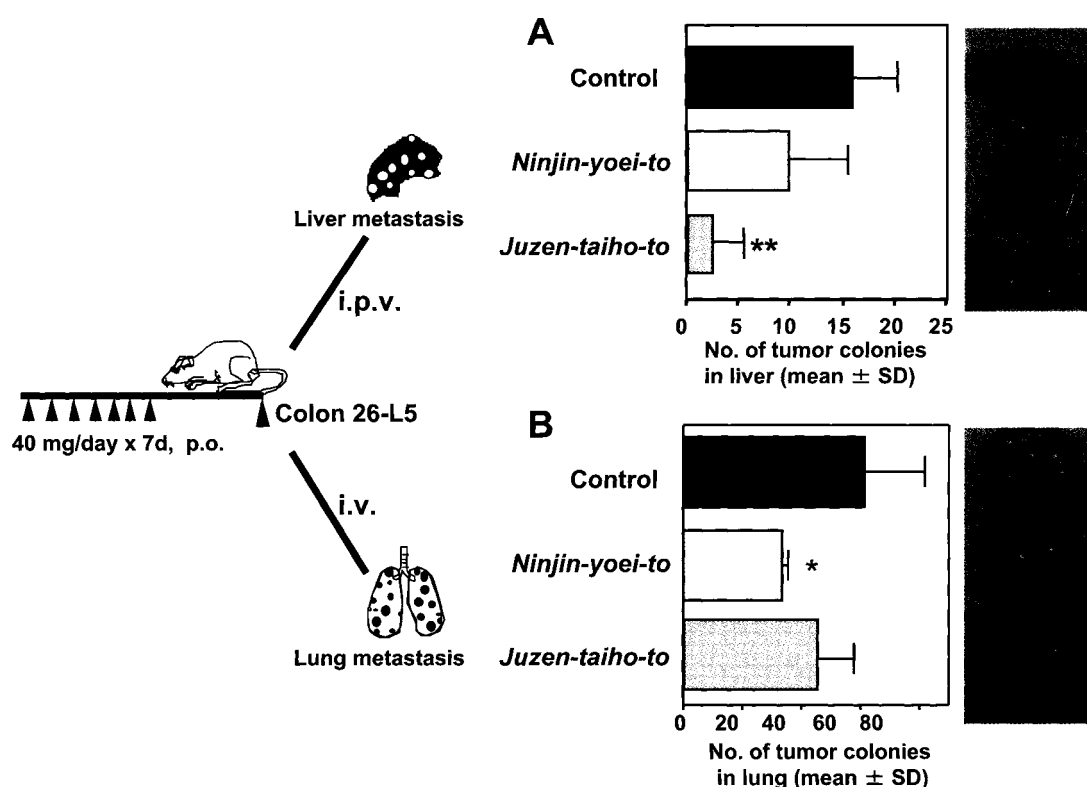


Fig. 1 Effect of oral administration of Juzen-taiho-to and Ninjin-yoei-to on liver or lung metastasis of colon 26-L5 carcinoma cells. Seven BALB/c mice per group were orally administered the indicated Kampo medicines at the dose of 40 mg/day/mouse for 7 days before intraportal vein (A) or intravenous (B) injection of colon 26-L5 cells. The mice were sacrificed 14 days after tumor inoculation. The number of tumor colonies in liver (A) or lung (B) was measured manually. * $p<0.05$, ** $p<0.001$ as compared with control.

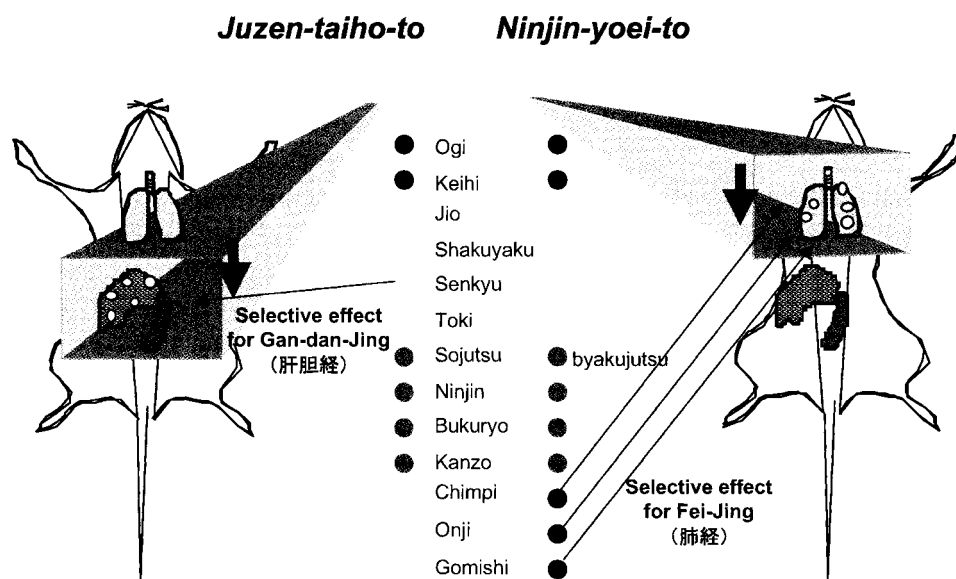


Fig. 2 Possible interpretation for organ selective effects of Kampo medicines on tumor metastasis according to medicinal guides based on the theory of Jing and Lun

of channels. It is thought that Jing and Lun connect all parts of five viscera and six bowels to regulate their functions and keep them balanced. When dysfunction occurs in some organs, as a result, the relevant pathological changes take place. Based on the medicinal guides according to this theory, so called Inkei-hoshi (引経報使) or Ki-Kei (帰経) in Japanese, traditional Chinese medicines are classified for their respective therapeutic effect on the disease of a special Jing and Lun and its pertaining organs. As illustrated in Fig. 2, Cnidii Rhizoma (Senkyu) in Juzen-taiho-to and Shimotsu-to formulation, is known to possess a selective effect for Gan-dan-Jing, Kan-Tan-Kei (肝胆経) in Japanese. Gan-Jing runs from a point on the big toe just behind the nail to a point, Qimen, Ki-Mon (期門) in Japanese, which is located about 8 cm below the nipple on either side. Gan-Jing pertains to the organ (Gan), which is considered to be regulating mind and mood, digestion and absorption function in traditional Chinese medicine, similarly to the functions of the liver in Western medicine. The indications of the Gan-dan-Jing are stuffiness in the chest, pain in the costal regions and on the top of the head. The most commonly used points, Jing-Xue, Kei-Ketsu (経穴) in Japanese, is Qimen, whose indications are pains in the chest and hypochondriac region.

On the other hand, Aurantii Nobilis Pericarpium (Chimpi), Polygalae Radix (Onji) and Schisandrae

Fructus (Gomishi) in Ninjin-yoei-to formulation, are supposed to possess a selective effect for Fei-Jing, Hai-Kei (肺経) in Japanese. Indications of Fei-Jing are cough, asthma, tightness in the chest, sore throat, and forearm where this channel passes. Physiological functions of Fei are taking charge of Ki of respiration. Therefore, Fei in traditional Chinese medicine plays the same part as the respiratory system in Western medicine. The theory is advocated by LiGao, a physician in the Jin dynasty, in Yong-yao-fa-xiang, Yo-yaku-ho-sho (用薬法象) in Japanese.

Although the conception of Gan and Fei in traditional Chinese medicine does not correspond to that of liver and lung in Western medicine, Juzen-taiho-to (containing Cnidii Rhizoma) and Ninjin-yoei-to (containing Aurantii Nobilis Pericarpium, Polygalae Radix and Schisandrae Fructus without Cnidii Rhizoma) inhibited predominantly tumor metastases to liver and lung, respectively. Thus, it is of prime interest that our pharmacological data are partly due to the theory of Jing and Lun formed in 13th century. Further study will be needed to examine the anti-metastatic effects using different types of tumors.

和文抄録

本研究では、マウス結腸癌 Colon26-L5 細胞の門脈

内移入により形成される肝転移および同細胞の尾静脈内移入により形成される肺転移に対する、十全大補湯および人参養栄湯の経口投与による抑制効果を検討した。十全大補湯は肝転移に対して有意に抑制したが、肺転移には抑制効果を示さなかった。これに対して、人参養栄湯は逆の効果、すなわち肝転移には効果を示さなかったが、肺転移に対して有意な抑制を示した。このように、同一の癌細胞と同系のマウスの転移病態モデルを用いて、二つの方剤の臓器選択的な転移抑制効果が観察された。このような効果発現の差異の解釈は、13世紀に確立された theory of Jing and Lun (引経報使) の考えに、部分的に通ずるものがあると思われる。

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References

- 1) Saiki, I.: Review, A kampo medicine "Juzen-taiho-to" -Prevention of malignant progression and metastasis of tumor cells and mechanism of action-. *Biol. Pharm. Bull.*, **23**, 677-688, 2000.
- 2) Haranaka, K., Satomi, N., Sakurai, A., Haranaka, R., Okada, N., Kobayashi, M.: Antitumor activities and tumor necrosis factor producibility of traditional Chinese medicines and crude drugs. *Cancer Immunol. Immunother.*, **20**, 1-5, 1985.
- 3) Kubota, A., Okamura, S., Shimoda, K., Harada, N., Omori, F., Niho, Y.: A traditional Chinese herbal medicine, Juzen-taiho-to, augments the production of granulocyte/macrophage colony-stimulating factor from human peripheral blood mononuclear cells in vitro. *Int. J. Immunother.*, **8**, 191-195, 1992.
- 4) Hamada, M., Fujii, Y., Yamamoto, H., Miyazawa, Y., Shui S. M., Tung Y. C., Yamaguchi, N.: Effect of a kampo medicine, zyuzentaihto, on the immune reactivity of tumor-bearing mice. *J. Ethnopharma.*, **24**, 311-320, 1988.
- 5) Kiyohara, H., Takemoto, N., Komatsu, Y., Kawamura, H., Hosoya, E., Yamada, H.: Characterization of mitogenic oepic polysaccharides from kampo (Japanese herbal) medicine "Juzen-taiho-to". *Planta Medica*, **57**, 254-259, 1991.
- 6) Maruyama, H., Takemoto, N., Maruyama, N., Komatsu, Y., Kawamura, H.: Antitumour effect of Juzentaiho-to, a Kampo medicine, combined with surgical excision transplanted Meth-A fibrosarcoma. *Int. J. Immunother.*, **9**, 117-125, 1993.
- 7) Haranaka, R., Hasegawa, R., Nakagawa, S., Sakurai, A., Satomi, N., Haranaka, K.: Antitumor activity of combination therapy with traditional Chinese medicine and OK432 or MMC. *J. Biol. Response Mod.*, **7**, 77-90, 1988.
- 8) Ohnishi, Y., Yasumizu, R., Fan, H., Liu, L., Takao-Liu, F., Komatsu, Y., Hosoya, E., Good, R. A., Isehara, S.: Effects of Juzen-taiho-toh (TJ-48), a traditional oriental medicine, on hematopoietic recovery from radiation injury in mice. *Exp. Hematol.*, **18**, 18-22, 1990.
- 9) Ohnishi, Y., Fujii, H., Hayakawa, Y., Sakukawa, R., Yamaura, T., Sakamoto, T., Tsukada, K., Fujimaki, M., Nunome, S., Komatsu, Y., Saiki, I.: Oral administration of a kampo (Japanese herbal) medicine Juzen-taiho-to inhibits liver metastasis of colon 26-L5 carcinoma cells. *Jpn. J. Cancer Res.*, **89**, 206-213, 1998.
- 10) Ohnishi, Y., Fujii, H., Kimura, F., Mishima, T., Murata, J., Tazawa, K., Fujimaki, M., Okada, F., Hosokawa, M., Saiki, I.: Inhibitory effect of a traditional Chinese Medicine, Juzen-taiho-to, on progressive growth of weakly malignant clone cell derived from murine fibrosarcoma. *Jpn. J. Cancer Res.*, **87**, 1039-1044, 1996.
- 11) Onishi, Y., Yamaura, T., Tauchi, K., Sakamoto, T., Tsukada, K., Nunome, S., Komatsu, Y., Saiki, I.: Expression of the anti-metastatic effect induced by Juzen-taiho-to is based on the content of Shimotsu-to constituents. *Biol. Pharm. Bull.*, **21** (7), 761-765, 1998.
- 12) Doki, Y., Murakami, K., Yamaura, T., Sugiyama, S., Misaki, T., Saiki, I.: Mediastinal lymph node metastasis model by orthotopic intrapulmonary implantation of Lewis lung carcinoma cells in mice. *British J. Cancer*, **79**, 1121-1126, 1999.
- 13) Ohnishi, Y., Sakamoto, T., Fujii, H., Kimura, F., Murata, J., Tazawa, K., Fujimaki, M., Sato, Y., Kondo, M., Une, Y., Uchino, J., Saiki, I.: Characterization of a liver metastatic variant of murine colon 26 carcinoma cells. *Tumor Biol.*, **18**, 113-122, 1997.
- 14) Fidler, I. J.: Selection of successive tumor lines for metastasis. *Nature (New Biol.)*, **242**, 148-149, 1973.
- 15) Komazawa, H., Saiki, I., Aoki, M., Kitaguchi, H., Satoh, H., Kojima, M., Ono, M., Itoh, I., Azuma, I.: Synthetic Arg-Gly-Asp-Ser analogues of the cell recognition site of fibronectin that retain antimetastatic and anti-cell adhesive properties. *Biol. Pharm. Bull.*, **16**, 997-1003, 1993.
- 16) Saiki, I., Matsumoto, Y., Murata, J., Makabe, T., Yoneda, J., Okuyama, H., Kimizuka, F., Ishizaki, Y., Kato, I., Azuma, I.: Recombinant fusion polypeptide with cell- and heparin-binding domains of fibronectin inhibits liver metastasis of L5178Y-ML25 lymphoma cells. *Jpn. J. Cancer Res.*, **82**, 1120-1129, 1991.