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Inhibitory effect of oral administration of Rikkunshi-to on tumor promotion in two-stage carcinogenesis in mouse skin[1)]

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Abstract

Oral administration of Rikkunshi-to (Liu-Jun-Zi-Tang, 六君子湯) inhibited tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) on skin tumor formation in mice initiated with 7,12-dimethylbenz[α]anthracene (DMBA). The delayed-type hypersensitivity reaction to 2,4-dinitrofluorobenzene was suppressed to as low as 60 % that of normal mice after DMBA plus TPA treatment. This immune suppression by DMBA plus TPA was relieved by oral administration of Rikkunshi-to. Oral administration of Rikkunshi-to canceled immunosuppression during carcinogenesis by DMBA plus TPA.

Key words Rikkunshi-to, 六君子湯, traditional Chinese herbal prescription, inhibition of tumor promotion, two-stage carcinogenesis, delayed-type hypersensitivity.

Abbreviations DMBA, 7,12-dimethylbenz[α]anthracene ; DNFB, 2,4-dinitrofluorobenzene ; DTH, delayed-type hypersensitivity ; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

Introduction

Cancer prevention is now a most urgent priority in the field of public health. Chemotherapy is important on precancer in the chemoprevention of cancer. Recently, Oka *et al.* reported that Sho-saiko-to (Xiao-Chai-Hu-Tang, 小柴胡湯), a traditional Chinese herbal prescription, suppressed the pathological change from cirrhosis to liver cancer.[2)]

Our earlier studies demonstrated that the topical application of methanol extracts from Rikkunshi-to, a traditional Chinese herbal prescription, inhibited tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) the formation of skin tumor in mice initiated with 7,12-dimethylbenz[α]anthracene (DMBA).[3)] In addition, oral administration of Juzen-taiho-to (Shi-Quan-Da-Bu-Tang, 十全大補湯), a traditional Chinese herbal prescription, has suppressed tumor promotion in two-stage carcinogenesis by DMBA plus TPA.[4)] And, the methanol extracts of Atractylodis Rhizoma and Hoelen, the formative crude drugs in Rikkunshi-to and Juzen-taiho-to, have inhibited tumor promotion by TPA in two-stage carcinogenesis in mouse skin.[5,6)] The active components, atractylon and lanostane-type triterpene acids have been isolated from these extracts of Atractylodis Rhizoma and Hoelen against TPA-induced inflammatory ear edema, respectively.[5,6)] On the other hand, it is known that the immune response was suppressed in tumor promotion during the two-stage carcinogenesis in mouse skin.[7-11)] This immune suppression was canceled by mauritianin and myricitrin, flavonol glycosides.[10)]

In this study, the oral administration of Rikkunshi-to slightly suppressed the tumor promotion by TPA following initiation with DMBA in mouse skin. The immune response was tested on delayed-type hypersensitivity (DTH) reaction with 2,4-dinitrofluorobenzene (DNFB). The immune suppression by DMBA plus TPA was relieved by oral administration of

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Rikkunshi-to.

Materials and Methods

Materials : The crude drugs were purchased from Kinokuniya Kan-Yaku Kyoku Co., Tokyo, Japan. Rikkunshi-to was formed, 4.0 g each of Atractylodis Rhizoma, Ginseng Radix, Pinelliae Tuber, Hoelen, and 2.0 g each of Zizyphi Fructus, Aurantii Nobilis Pericarpium, 1.0 g Glycyrrhizae Radix, 0.5 g Zingiberis Rhizoma. Aqueous extract of Rikkunshi-to was prepared by extraction with 5 times the weight of water for 3 h. The extract was then freeze-dried.

Chemicals : DMBA and dimethyl sulfoxide were purchased from Sigma Chemical Co., St. Louis, U.S.A. TPA from Chemicals for Cancer Research, Inc., Chicago, U.S.A. DNFB and acetone were obtained from Tokyo Chemical Industries Co., Tokyo, Japan.

Animals : Female ICR mice were purchased from Japan SLC, Shizuoka, Japan. The animals were housed in an air-conditioned specific pathogen free room (22-23°C) lit from 08 : 00 to 20 : 00. Food and water available *ad libitum*.

Two-stage carcinogenesis experiments : A group of 20 mice underwent initiation by a single application of 50 μg DMBA in acetone (100 μl) to the dorsal skin. Promotion with 1 μg of TPA in acetone (100 μl), applied twice weekly, was begun 7 days after the initiation. TPA treatment was continued for 20 weeks. Mice were treated with 0.675 % Rikkunshi-to extract (about 1,000 mg/kg/day) as their sole source of drinking water starting 1 day after the initiation by DMBA and continuing throughout the tumor promotion regimen until the end of the study.

Cell-mediated immune response : Primary sensitization was induced by DNFB as follows : DNFB was dissolved in acetone at 0.5 % (w/v) and 50 μl was applied to the dorsal skin. After 4 days, delayed-type hypersensitivity was elicited by painting 50 μl of 0.5 % DNFB solution in acetone onto the right hind paw. After 24 hours, the edema resulting from elicitation in the hind paw was measured with a dial caliper. The thickness of the footpad swelling due to delayed-type hypersensitivity minus that of the opposite footpad was expressed in 0.1 mm units.

Each group of 10 mice underwent treatment by topical application of 50 μg DMBA in acetone (100 μl) to the dorsal skin, and on the following and subsequent days the mice were given water containing 0.675 % Rikkunshi-to.

Experiment I : Topical application of 1 μg of TPA in acetone (100 μl), applied 7 days after the initiation by DMBA. Primary sensitization by DNFB was applied at 12, 24, 48, 72 and 96 hours after TPA treatment.

Experiment II : TPA was applied to the skin twice a week that was begun 7 days after the initiation by DMBA. Primary sensitization by DNFB was applied at 72 hours after the second treatment of TPA in respective week. Experiments were done at the weeks of 1-8, 11, 14, 17 and 20 on tumor promotion, respectively.

Statistical analysis : Statistical analysis was by Student's t test.

Results

Inhibitory effect of oral administration of Rikkunshi-to on tumor promotion

Since Rikkunshi-to (2.0 mg/mouse) has been shown to have antitumor promoting activity in two-stage carcinogenesis when applied topically to mouse skin treated with DMBA plus TPA,[3] we next examined its effect when given in the tap water. In the group given the tap water, the first tumor appeared at week 4 and all mice had tumors at week 11, whereas in the group given the water containing 0.675 % Rikkunshi-to appeared at week 7, and all mice had tumors at week 14, respectively (Fig. 1A). Figure 1B shows the average number of tumors per mouse at week 20. The group given the tap water produced 9.8 tumors per mouse, whereas the group given the water containing 0.675 % Rikkunshi-to had 5.8 tumors per mouse. The treatment with Rikkunshi-to caused 41 % reduction in the average number of tumors per mouse at week 20. Thus, on oral administration, Rikkunshi-to (about 1,000 mg/kg/day) slightly suppressed tumor promotion by TPA in DMBA-initiated mice.

Effect of oral administration of Rikkunshi-to on cell-mediated immunosuppression by DMBA plus TPA (Experiment I)

Figure 2 shows, the group treated with DMBA

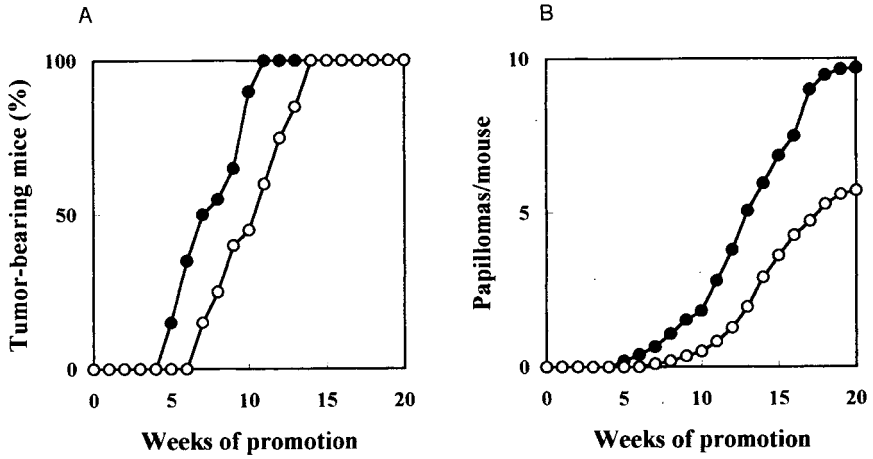

Fig. 1 Inhibitory effect of oral administration of Rikkunshi-to on tumor promotion by TPA following initiation with DMBA in mice. From 1 week after initiation by a single topical application of 50 μg of DMBA, 1 μg of TPA was applied twice weekly. Mice were treated with 0.675 % (about 1,000 mg/kg/day) Rikkunshi-to extract as their sole souse of drinking water starting 1 day after initiation by DMBA and continuing throughout the tumor promotion regimen until the end of the study. Data are expressed as percentage of mice bearing papillomas (A) and as average numbers of papillomas per monse (B), ●, DMBA+TPA with tap water ; ○, DMBA+TPA with Rikkunshi-to in water. The Rikkunshi-to treated group was statistically different from the control group by Student's t-test ($p<0.05$: week 17-20). (n=20).

plus TPA showed a suppressed DNFB-DTH, 58-68 % of that of the non-treated group through times 12, 24, 48, 72 and 96 hour. Oral administration of Rikkunshi-to canceled the suppression of DTH reaction by DMBA plus TPA at 24, 48 and 72 hour after TPA treatment.

Effect of oral administration of Rikkunshi-to on cell-mediated immunosuppression during two-stage carcinogenesis by DMBA plus TPA (Experiment II)

Figure 3 shows, the group treated with DMBA

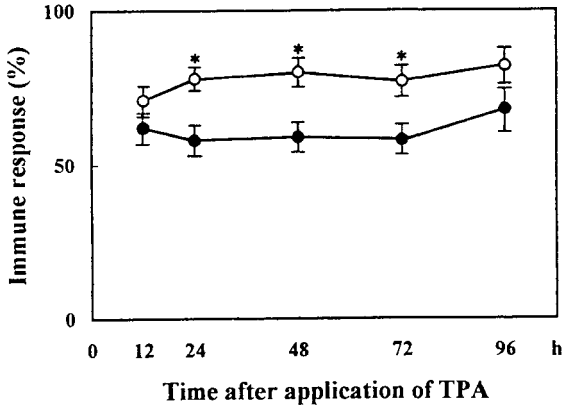

Fig. 2 Effect of oral administration of Rikkunshi-to on the immunosuppression by DMBA plus TPA in mouse skin. The immune response was showed by the percentage to the normal mouse. ●, DMBA+TPA with tap water, ○, DMBA+TPA with Rikkunshi-to in water. * $p<0.05$: The DMBA plus TPA and Rikkunshi-to treated group was statistically different from DMBA plus TPA group by Student's t-test. (n=10).

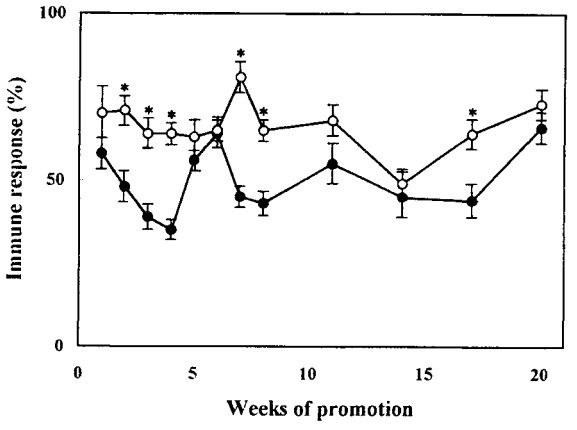

Fig. 3 Effect of oral administration of Rikkunshi-to on the immunosuppression during two-stage carcinogenesis by DMBA plus TPA in mouse skin. The immune response was showed by the percentage to the normal mouse. ●, DMBA+TPA with tap water, ○, DMBA+TPA with Rikkunshi-to in water. * $p<0.05$: The DMBA plus TPA and Rikkunshi-to treated group was statistically different from DMBA plus TPA group by Student's t test. (n=10).

plus TPA showed a suppressed DNFB-DTH, 35-66 % of that of the non-treated group through weeks 1-8, 11, 14, 17 and 20. Oral administration of Rikkunshi-to canceled the immunosuppression during carcinogenesis by DMBA plus TPA, the significant effects were observed at 2-4, 7, 8 and 17 weeks of promotion, respectively.

Discussion

In our recent study, topical application of Rikkunshi-to, a traditional Chinese herbal prescription, has been used to inhibit tumor promotion by TPA in two-stage carcinogenesis in mouse skin.[3] This is the first report that the oral administration of Rikkunshi-to inhibits tumor promotion by TPA in mice following initiation by DMBA.

The DNFB-DTH reaction has been suppressed by the tumor promoter to 30 % of that of normal mice in TPA-treated skin.[4, 7, 10] They were as low as 58-68 % of that of normal mice through times 12, 24, 48, 72 and 96, but this suppression was relieved by oral administration of Rikkunshi-to after 24, 48 and 72 hours from TPA treatment. The oral administration of Rikkunshi-to proved to have the immune activation on immunosuppression in tumor promotion during two-stage carcinogenesis. The oral administration of Rikkunshi-to had no effect on the immune system of normal mice (data not shown). DMBA has been also known to depleted Langerhans cells in treated skin,[12] Langerhans cells have been associated with local cutaneous DTH.[13] However, a part of the effect of Rikkunshi-to may be attributed to the activation of immune responses against tumors, in which Langerhans cells are involved, suggesting the reappearance of Langerhans cells associated with tumor regression.

In our previous studies, the traditional Chinese herbal prescriptions, Juzen-taiho-to and Rikkunshi-to, inhibited the tumor-promoting effect of TPA in DMBA-initiated mice. In studies by other researchers, Sho-seiryu-to (Xiao-Qing-Long-Tang, 小青竜湯), Kijitsu-gaihaku-keishi-to (Zhi-Shi-Xie-Bai-Gui-Zhi-Tang, 枳実薤白桂枝湯) and Tokaku-joki-to (Tao-He-Cheng-Qi-Tang, 桃核承気湯), traditional Chinese herbal prescriptions, suppressed tumor promotion by TPA in two-stage carcinogenesis in mouse skin.[14, 15] In addition, Chorei-to (Zhu-Ling-Tang, 猪苓湯) suppressed tumor promotion by sodium saccarin and triptophan on rat urinary bladder carcinogenesis.[16, 17] Sho-saiko-to has prevented liver cancer.[2] These results suggested that the traditional Chinese herbal prescriptions are important for the treatment of precancer and recurrence of cancer.

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和文抄録

六君子湯の水抽出エキスの経口投与は，7,12-dimethylbenz[α]anthracene (DMBA) でイニシエートしたマウスの腫瘍発現における 12-*O*-tetradecanoylphorbol-13-acetate (TPA) によるプロモーションを抑制した。2,4-dinitrofluorobenzene による遅延型過敏症反応は，DMBA と TPA 投与の後，無投与マウスの約 60 % に抑制された。この DMBA と TPA による免疫抑制効果は，六君子湯の水抽出エキスの経口投与により緩和された。六君子湯の水抽出エキスの経口投与は，発癌過程における免疫抑制効果も同様に緩和した。

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