Studies on the antitumor-promoting activity of naturally occurring substances. VIII.[1] Inhibitory effect of coumarins isolated from Bai-Hua Qian-Hu on two stage carcinogenesis

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Abstract

Pd-Ia, Pd-II and Pd-III, angular-type pyranocoumarins, isolated from Bai-Hua Qian-Hu, *Peucedanum praeruptorum* DUNN. (*Umbelliferae*), inhibited the promotion of two stage skin tumor formation promoted by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 7, 12-dimethylbenz [*a*] anthracene (DMBA)-initiated mice. Moreover, Pd-Ia inhibited two stage lung tumor formation promoted by glycerol in 4-nitroquinoline 1-oxide (4NQO)-initiated mice. These results indicate that these angular-type pyranocoumarins may be promising as an agent for cancer prevention.

Key words Umbelliferae, Bai-Hua Qian-Hu, angular-type pyranocoumarin, antitumor-promoting activity, two stage skin carcinogenesis, two stage lung carcinogenesis.

Abbreviations Byakka-zenko (Bai-Hua Qian-Hu), 白花前胡 ; Saiko (Chai-Hu), 柴胡 ; Zenko (Qian-Hu), 前胡.

Introduction

We have reported the structural analyses[2-10] and the biological activities[11-18] on various coumarins of Chinese crude drug "Qiau-Hu" (Zenko in Japanese). The seselin-type coumarin Pd-Ia, 3'-angeloyloxy 4'-acetoxy-3', 4'-dihydroseselin (see Fig. 1), was isolated from Bai-Hua Qian-Hu (Byakka-zenko in Japanese), the root of *Peucedanum praeruptorum* DUNN. (*Umbelliferae*), by Okuyama.[2] And Pd-Ia was found to have organic calcium antagonistic action on a smooth muscle preparation of ileum and taenia coli isolated from guinea pig,[11] organic calcium antagonist-like action on anaphylactic mediator release from mast cell induced by concanavalin A.[12] The effect of anti-platelet aggregation[14] and the effect on Ca^{2+}-current and action potential duration in single ventricular cells of guinea pig hearts.[16]

Next, we have found that various types of coumar-

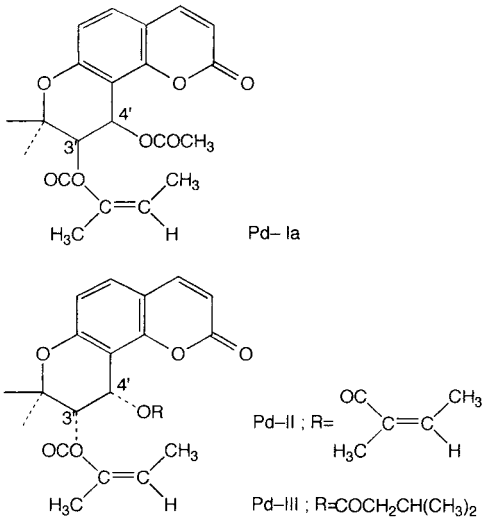

Fig. 1 Structures of Pd-Ia, Pd-II and Pb-III isolated from Bai-Hua Qian-Hu.

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Table I Inhibitory effect of coumarins obtained from Bai-Hua Qian-Hu on TPA-stimulated ^{32}Pi incorporation into phospholipids of HeLa cells (*in vitro*).

		Inhibition (%)
Pd-Ia	50 μg/ml	84.7
Pd-II	50 μg/ml	100
	20 μg/ml	73.1
	10 μg/ml	27.2
Pd-III	50 μg/ml	100
	20 μg/ml	81.6
	10 μg/ml	46.7

HeLa cells cultured in Petri dishes were incubated with one of the test compounds (final concentration was the above-mentioned). After 1h, Pi (10 μCi/culture) was added with or without TPA (50 nM). Incubation was continued for 4h, and then radioactivity incorporated in the phospholipids fraction was assayed. Data are mean values of duplicate experiments and are expressed as % inhibition.

ins showed anti-tumor-promoting activity.[17-22] In our previous studies, Pd-Ia and Pd-II inhibited two stage skin tumor formation.[1,17,21] In the present study, we wish to report the two stage carcinogenesis experiment *in vivo* of Pd-Ia against pulmonary tumors. Moreover, because Pd-III had more potent inhibitory effect on tumor-promoting *in vitro* test than Pd-Ia and Pd-II (see Fig. 1 and Table I),[21] we also report that we compared the potency of Pd-III on two stage skin carcinogenesis test with those of Pd-Ia and Pd-II.

Materials and Methods

Chemicals : 4-Nitroquinoline 1-oxide (4NQO), dimethylbenz [*a*] anthracene (DMBA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) were obtained from Sigma Chemical. The fine grade of glycerol was purchased from Nacalai Tesque, Inc, Kyoto, Japan.

Animals : 6-week-old male ddY mice and female ICR mice were obtained from Tokyo Laboratory Animals Science Corporation (Tokyo, Japan) and housed in polycarbonate cages in a temperature-controlled room with daily care.

Two stage carcinogenesis test on pulmonary tumors : 6-week-old male ddY mice were used. Each experimental group consisted of 14 mice and received the following initiation/promotion treatment. 4NQO as an initiater was dissolved in olive oil-cholesterol (20 : 1). A dose of 4NQO 0.3 mg (1.57 μmol) per mouse was given by a single subcutaneous injection at the starting time. Glycerol as a promoter was dissolved in H_2O (5 %) and given as drinking H_2O ad libitum. From four weeks after initiation, the promoting treatment was continued for 25 weeks. Mice as control group were given 5 % glycerol with 0.04 % HCO 60. Mice as Pd-Ia group were given Pd-Ia 1.25 mg (3.3 μmol) plus 0.04 % HCO 60 per 5 % glycerol 100 ml. The consumption of the solutions was measured three times a week, and the body weight of the mice was measured once a week. Each experimental group was sacrificed by cervical dislocation after 25 weeks. Each pulmonary lobe was separated, and the number of induced tumors was counted with the naked eye. The lungs were embedded in paraffin, sectioned, and stained with hematoxylin eosin by conventional methods to study the pulmonary tumors histologically.

Two stage carcinogenesis test on skin tumors : Each group of 15 ICR mice was housed and given H_2O ad libitum. The back of each mouse was shaved with surgical clippers before initiation. Each mouse was initiated with DMBA 100 μg (394 nmol) in acetone. One week after initiation, each mouse was promoted twice a week by application of TPA as a promoter in acetone. Mice in the control group were treated with TPA 2 μg (3.2 nmol) per mouse. Mice in the Pd-Ia group were treated with Pd-Ia 1 mg (2.6 μmol) plus TPA 2 μg (3.2 nmol) per mouse, mice in the Pd-II group were treated with Pd-II 1 mg (2.3 μmol) plus TPA 2 μg (3.2 nmol) per monse and mice in the Pd-III group were treated with Pd-III 1 mg (2.3 μmol) plus TPA 2 μg (3.2 nmol) per mouse. From one week after initiation, the promoting treatment was continued for 20 weeks. The number of tumors on the backs of the mice was counted once a week, and the body weight of the mice was measured once a week.

Results

The effect of Pd-Ia on two stage carcinogenesis of the lung was studied. As shown in Table II, the average number of tumors per mouse was reduced by Pd-Ia. The inhibitory effect of Pd-Ia on two stage lung carcinogenesis was 47.9 % ($p<0.05$, t-test) at 25 weeks of promotion. HCO 60 as surface active agent,

Table II Effect of Pd-Ia on pulmonary tumors formation promoted by glycerol in 4NQO-initiated mice.

Group Initiation/Promotion	Number of mice	Incidence (%)	Average number of tumors per mouse
4NQO/5% glycerol +0.04 % HCO60	11	72.7	4.36
4NQO/5 % glycerol +Pd-Ia+0.04 % HCO60	11	63.6	2.27

4NQO, 4-nitroquinoline 1-oxide.
HCO 60, surface active agent.
Pd-Ia was dissolved 1.25 mg in 5% glycerol 100 ml.

which was used to dissolve Pd-Ia, did not affect tumor formation in this experiment. The increase rate of body weight by Pd-Ia treatment was the same grade as that of control. Thus, Pd-Ia did not seem to have general toxicity.

These results suggest that Pd-Ia showed an inhibitory effect on two stage lung carcinogenesis using 4NQO as an initiater and glycerol as a promoter. Tumors of each lung were analyzed as to histopathological findings and the alveolar - bronchiolar adenoma was determined. All of adenoma were existent contact with the pleura and not the center of lung.

In the two stage skin carcinogenesis test, we compared the potency of Pd-Ia with those of Pd-II and Pd-III. Tumor bearing mice (%) are shown in Fig. 2-A and the average number of tumors per mouse is shown in Fig. 2-B. The inhibitory effect of Pd-Ia on the average number of tumors per mouse was 48.0 %, that of Pd-II was 35.6 % and that of Pd-III was 49.7 % ($p<0.05$, t-test) at 20 weeks of promotion. Pd-III, which had the strongest inhibitory effect among the three coumarins *in vitro* test (see Table I), showed the strongest inhibitory effect on the two stage skin carcinogenesis test. Thus, Pd-III may show a more potent antitumor-promoting effect than Pd-Ia and Pd-II.

Discussion

During research on the active components of the Chinese crude drug belonging to *Umbelliferae*, Okuyama *et al.* isolated a number of coumarins from "Qian-Hu" which was classified into Q-I, -II, -III and -IV types. As described previously, Pd-Ia, Pd-II and

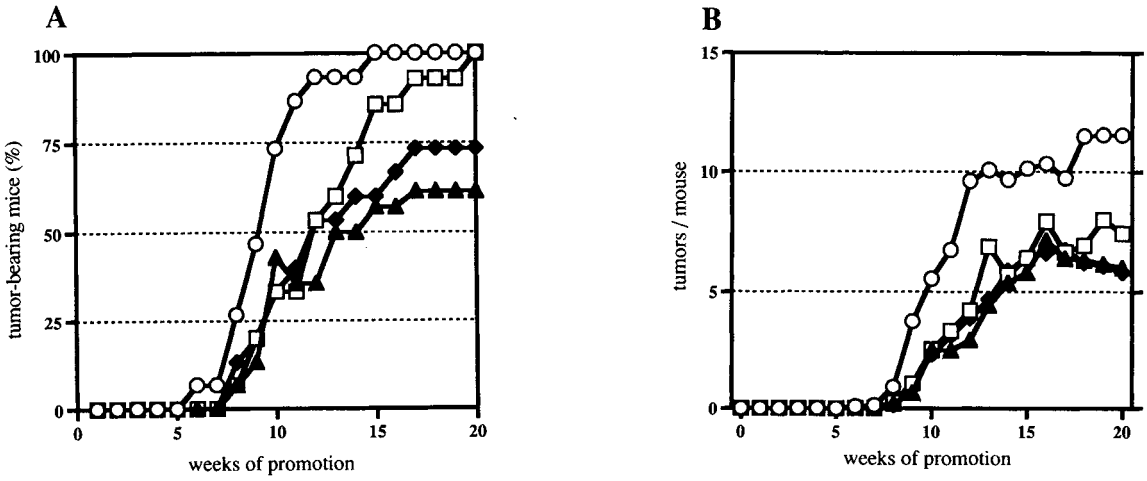

Fig. 2 Effect of coumarins isolated from Bai-Hua Qian-Hu on skin tumors formation promoted by TPA in DMBA initiated mice. All mice were initiated with DMBA (100 μg, 394 nmol). From 1 week after initiation, 2.0 μg (3.2 nmol) of TPA was applied twice a week. The test compounds were applied with each TPA application.
A : Percentage of mice with tumors.
B : Average number of tumors per mouse.

Pd-III, a seselin type coumarin, isolated from the Chinese drug "Bai-Hua Qian-Hu", the root of *Peucedanum praeruptorum* DUNN., showed antitumor promoter activity *in vitro* (see Table I). Moreover, Pd-Ia and Pd-II was found to suppress the action of TPA *in vivo* system.

Pd-Ia, which was found to have various biological activities, was proved in the present study to show an antitumor-promoting effect on two stage carcinogenesis in lung. Thus Pd-Ia, which showed inhibitory effect on two stage carcinogenesis in skin and lung, might be valuable as antitumor-promoters in chemical carcinogenesis. The investigation of inhibitory mechanisms of Pd-Ia on tumor promotion are now in progress.

Pd-III showed a more potent antitumor-promoting effect *in vitro* and *in vivo* test against two stage skin carcinogenesis than Pd-Ia and Pd-II. Pd-III might be the most potent antitumor-promoters of three. We should compare the potency of Pd-III *in vivo* test against two stage lung carcinogenesis with those of Pd-Ia and Pd-II.

Qian-Hu have been employed in Chinese medicines as antitussives, expectorants, antipyretics and stomachics, and sometimes as a remedy for chest pain. Application of it is said to be close to that of Chai-Hu. We reported Chai-Hu showed little antitumor-promoting activity.[18] Bai-Hua Qian-Hu may be a more useful Chinese drug for antitumor-promoter than Chai-Hu.

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和文抄録

漢薬白花前胡（Q-1 type）の主要成分である Pd-Ia には，*in vitro* において発癌プロモーター抑制作用として検討したリン脂質の合成亢進と，*in vivo* における発癌プロモーター抑制作用を検討した皮膚発癌2段階実験で抑制作用のあることを報告して来た。今回 *in vivo* における肺発癌2段階実験においても抑制作用の認められることがわかった。

in vitro において発癌プロモーター抑制作用の認められている白花前胡 Q-I type の成分である Pd-Ia，Pd-II，Pd-III を皮膚発癌2段階実験において抑制作用の比較を行い，*in vitro* の系で強い作用を示した Pd-III に，皮膚発癌2段階実験においても最も強い抑制作用が認められた。以上のことはこれら白花前胡の成分の化学発癌抑制の可能性を示唆しているものと考えられる。

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