Atractylodis Rhizoma Extract and Its Component, Atractylon, Inhibit Tumor Promotion in Mouse Skin Two-Stage Carcinogenesis

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Summary

The methanol extract of Atractylodis Rhizoma, a traditional Chinese medicine, was found to have antitumor-promoting activity in two-stage carcinogenesis. From the active fraction of the extract, atractylon was isolated. The isolated compound showed inhibitory activity against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear inflammation in mice. Furthermore, atractylon markedly inhibited tumor promotion by TPA following 7,12-dimethylbenz[a]anthracene initiation in mice.

Key words: Atractylon; Atractylodis Rhizoma; Antitumor promotion; Two-stage carcinogenesis; Anti-inflammatory activity.

Introduction

Atractylodis Rhizoma, the rhizome of *Atractylodes japonica* Koidzumi, is used for the treatment of pain, gastritis and enteritis (Namba, 1980). The pharmacological action of Atractylodis Rhizoma includes inhibition of stress ulceration of the stomach (Kubo, et al., 1983). Atractylon, the major essential oil component of Atractylodis Rhizoma, has been shown to have a significant preventative effect against carbon tetrachloride- and D-galactosamine-induced lesions in primary-cultured rat hepatocytes (Kiso, et al., 1983; 1985).

In the present study, the methanol extract from Atractylodis Rhizoma was found to inhibit TPA-induced tumor promotion during two-stage carcinogenesis in mice. Atractylon (fig. 1) was isolated from the active fraction. Furthermore, atractylon was found to inhibit tumor promotion markedly in two-stage carcinogenesis in mice.

Materials and Methods

**Materials:** Atractylodis Rhizoma (the rhizome of *Atractylodes japonica* Koidzumi) was obtained from the market in Tokyo. This was identified as the rhizome of *Atractylodes japonica* by description, chemical analysis and purity test (Namba, 1980; Takitani, 1991).

**Extraction from Atractylodis Rhizoma:** One hundred grams of Atractylodis Rhizoma was extracted by refluxing with methanol for 3 h. The organic solvent was evaporated in vacuo to dryness. The yield of MeOH extract was 18.9 g.
One hundred grams of Atractylodis Rhizoma was extracted by refluxing with water for 3 h. The solvent was freeze-dried. The yield of water extract was 12.1 g.

Isolation of active agent from the methanol extract of Atractylodis Rhizoma: Ten grams of the methanol extract of Atractylodis Rhizoma was separated using a n-hexane-methanol-water mixture (19:19:2). Yields were: n-hexane-soluble fraction: 4.49 g; methanol-water-soluble fraction: 4.89 g. The n-hexane-soluble fraction was then subjected to CC on silica gel using n-hexane as a solvent to obtain six fractions. The inhibition ratio of the fractions were showed in Table 1 on TPA-induced inflammatory ear edema. The component active (I) was isolated from the potent inhibitory fraction (5, yield: I = 718 mg) and was identified as atractylon by standard physical and chemical procedures and comparison with an authentic sample (Hikino et al., 1964; Nishikawa et al., 1976).

Animals: Female ICR mice were obtained from Japan SLC, Shizuoka, Japan, and housed in an air-conditioned room (22–23 °C) lit from 8:00 to 20:00. Food and water were available ad libitum.

Assay of TPA-induced inflammation in mice: TPA (1 nM) dissolved in acetone (20 μl) was applied to the right ear of each mouse with a micropipette. A volume of 10 μl was delivered to both the inner and outer surfaces of the ear. Twenty microliters of the sample solution, their vehicles, methanol-water (1:1) or chloroform-methanol (1:1) (as a control) was applied topically about 30 min before each TPA treatment. For ear thickness determinations, a pocket thickness gauge (Mitsutoyo Co., Ltd., Tokyo, Japan) with a range of 0–9 mm, graduated at 0.01-mm intervals and modified so that the contact surface area was increased, thus reducing the tension, was applied to the tip of the ear.

Ear thickness was determined at before treatment (a). Edema was measured 6 h after TPA treatment (b: TPA alone; b': TPA plus sample). The following values were then calculated:

Edema A: edema induced by TPA alone (b-a).
Edema B: edema induced by TPA plus sample (b'-a).

Inhibition ratio (%) = \( \frac{\text{Edema A} - \text{Edema B}}{\text{Edema A}} \times 100 \)

Each value was the mean of individual determinations from 5 mice. The 50% inhibitory dose (ID_{50}) values were determined by probit-graphic interpolation for four dose levels.

Two-stage carcinogenesis experiments: Three groups of 15 mice underwent initiation by application of 50 μg of 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. Atractylon (2 μmol), or the methanol extract (2 mg) of Atractylodis Rhizoma and their vehicle, acetone-dimethylsulfoxide-water (18:1:1, 100 μl) were applied topically 30 min before TPA treatment. These treatments were continued for 20 weeks.

### Results

Inhibition by the methanol extract of Atractylodis Rhizoma on TPA-induced inflammatory ear edema and TPA-induced tumor promotion: The methanol extract of Atractylodis Rhizoma markedly inhibited the inflammation induced by TPA in mice (Table 1). Figure 2A illustrates the time course of skin tumor formation in the groups treated with DMBA plus TPA, with or without the methanol extract from Atractylodis Rhizoma. The first tumor appeared at week 6 and all mice had tumors by week 9 in the group treated with DMBA plus TPA. In the group treated with DMBA plus TPA and the methanol extract of Atractylodis Rhizoma, the first tumor appeared during week 10. The percentage of tumor-bearing mice treated with DMBA plus TPA was 100% at week 20, whereas that in the group treated with DMBA plus TPA and the methanol extract of Atractylodis Rhizoma was 33%. Figure 2B shows the average number of tumors per mouse. The group treated with DMBA plus TPA produced 10.4 tumors per mouse at week 20, whilst the group treated with DMBA plus TPA and the methanol extract of Atractylodis Rhizoma had 0.6 tumors per mouse. Treatment with the methanol extract of Atractylodis Rhizoma therefore caused a 94% reduction in the average number of tumors per mouse at week 20.

<table>
<thead>
<tr>
<th>Name</th>
<th>ID_{50} (mg/ear)</th>
<th>I.R.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract</td>
<td>–</td>
<td>43*1</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>–</td>
<td>80*2</td>
</tr>
<tr>
<td>Fraction 1</td>
<td>–</td>
<td>29*2</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>–</td>
<td>30*1</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>–</td>
<td>60*1</td>
</tr>
<tr>
<td>Fraction 4</td>
<td>–</td>
<td>77*1</td>
</tr>
<tr>
<td>Fraction 5</td>
<td>–</td>
<td>82*1</td>
</tr>
<tr>
<td>Fraction 6</td>
<td>–</td>
<td>9* 1</td>
</tr>
<tr>
<td>Atractylon</td>
<td>0.9</td>
<td>82*1</td>
</tr>
<tr>
<td>Quercetin</td>
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<td>40*2</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.3</td>
<td>96*2</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>0.03</td>
<td>99*2</td>
</tr>
</tbody>
</table>

The samples were applied 30 min before TPA treatment; ear edema was determined at 6 h after TPA treatment. *p < 0.01 by Student's t-test as compared to the control group. I.R.: Inhibition ratio (at 12 mg/ear; 21 mg/ear).

Investigation of atractylon from the methanol extract of Atractylodis Rhizoma for inhibitory activity against TPA-induced ear edema: The component active against TPA-induced inflammatory ear edema was separated from the methanol extract of Atractylodis Rhizoma, and was identified as atractylon (Table 1). Inhibitory effect of fractions 3
Atractylodis Rhizoma Extract and Its Component

Fig. 2. Inhibitory effect of methanol extract of Atractylodis Rhizoma on the tumor promotion of skin papillomas by TPA in DMBA-initiated mice.

From 1 week after initiation by a single topical application of 50 μg of DMBA, 1 μg of TPA was applied twice weekly. Topical application of methanol extract (2 mg) of Atractylodis Rhizoma and vehicle was performed 30 min before each TPA treatment. Data are expressed as percentage of mice bearing papillomas (A), and as average numbers of papillomas per mouse (B).

Fig. 3. Inhibitory effect of atractylon on the tumor promotion of skin papillomas by TPA in DMBA-initiated mice.

From 1 week after initiation by a single topical application of 50 μg of DMBA, 1 μg of TPA was applied twice weekly. Topical application of atractylon (2 μmol) and vehicle was performed 30 min before each TPA treatment. Data are expressed as percentage of mice bearing papillomas (A), and as average numbers of papillomas per mouse (B).

and 4 was due to atractylon in fraction. Full details of the isolation and identification, and the spectral data, are available on request from the author for correspondence.

Inhibitory effect of atractylon against TPA-induced inflammation in mice: As table 1 shows, the 50% inhibitory dose of atractylon for TPA-induced inflammatory ear edema was 0.9 mg/ear. Application of the sample completely inhibited TPA-induced inflammation and this inhibitory activity was dose-dependent. By comparison with standard drugs, this compound was a less effective inhibitor than hydrocortisone and indomethacin, but was more effective than quercetin, a known inhibitor of tumor promotion. Atractylon inhibited TPA-induced inflammation in mice to a similar extent as cepharaophine, a known inhibitor of tumor promotion and inflammation.

Inhibitory effect of atractylon on the tumor-promoting activity of TPA: Figure 3A illustrates the time course of skin tumor formation in the groups treated with DMBA plus TPA, with or without atractylon. The first tumor appeared during week 6 and all mice had tumors by week 10 in the group treated with DMBA plus TPA. In the group treated with DMBA plus TPA and 2 μmol atractylon, the first tumor appeared during week 8. The percentage of tumor-bearing mice treated with DMBA plus TPA was 100% at week 20, whereas that in the group treated with DMBA plus TPA and 2 μmol atractylon was 33%. Figure 3B shows the average number of tumors per mouse. The group treated with DMBA plus TPA produced 10.4 tumors per mouse at week 20, whereas the group treated with DMBA plus TPA and 2 μmol atractylon had 0.4 tumors per mouse. Treatment with 2 μmol atractylon therefore caused a 96% reduction in the average number of tumors per mouse at week 20.

Discussion

Our previous studies have demonstrated that extracts from edible plants and crude drugs can inhibit TPA-induced inflammatory ear edema, and have identified active components separated from hop and stevia (Yasukawa, et al., 1993b). Furthermore, plant constituents, such as flavonol glycosides (Yasukawa, et al., 1990), sterols and triterpenes (Yasukawa, et al., 1991a), lignan (Yasukawa, et al., 1992), and alkaloids (Yasukawa, et al., 1988a; Yasukawa, et al., 1989; Yasukawa, et al., 1991b; Yasukawa, et al., 1993) have been shown to inhibit tumor promotion during two-stage carcinogenesis in mouse skin.

The traditional Chinese medicines (Kampo medicines in Japanese) are today frequently used to treat human cancer as complementary drugs in Japan. The major uses of the Kampo medicines Rikkunshi-to and Zyuzen-taiho-to are in the treatment of immunosuppression caused by anti-cancer
drugs, and poor health after operations. Atractylodis Rhizoma, one of the crude drugs used, is of importance in the formulation of Kampo medicines. Oral administration of Zyuzen-taiho-to slightly suppressed TPA-induced tumor promotion in two-stage carcinogenesis in mouse skin (Haranaka, et al., 1987).

Discussion

The methanol extract of Atractylodis Rhizoma markedly suppressed tumor promotion by TPA in DMBA-initiated mice. The active compound, atracylone (one of sesquiterpenes), was isolated from the extract and was found to have an inhibitory effect against TPA-induced inflammation in mice. By comparison with standard drugs, this compound was a less effective inhibitor than hydrocortisone and indomethacin, but was more effective than quercetin, a known inhibitor of tumor promotion. In addition, atracylone markedly inhibited TPA-induced tumor promotion in mice. By comparison with known inhibitors on tumor promotion, this compound was a less effective inhibitor than hydrocortisone and poor health after operations. Atractylodis Rhizoma, one of the crude drugs used, is of importance in the formulation of Kampo medicines. Oral administration of Kampo medicines. Oral administration of Zyuzen-taiho-to slightly suppressed TPA-induced tumor promotion in two-stage carcinogenesis in mouse skin (Haranaka, et al., 1987).

Atractylodis Rhizoma is used in combined Kampo prescriptions, it is of interest from the viewpoints of both Chinese traditional medicine and pharmacognostical science that such sesquiterpenes have inhibitory activity against tumor promotion of carcinogenesis.

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References


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