Inhibitory Effect of Karoundiol on 12-O-Tetradecanoylphorbol-13-acetate-Induced Tumor Promotion

Ken Yasukawa,*a Toshihiro Akihisa,* Toshitake Tamura, and Michio Takido

College of Pharmacy, Nihon University,* 7-7-1, Narashinonodai, Funabashi-shi, Chiba 274, Japan and College of Science and Technology, Nihon University,* 1-8, Kanda Surugadai, Chiyoda-ku, Tokyo 101, Japan.

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Karoundiol [D. C-Friede-oleane-7,9(11)-diene-3z,29-diol] and 7-oxodihydrokaroundiol [7-oxo-D. C-friede-oleane-8-ene-3z,29-diol], isolated from the seeds of Trichosanthes kiriwii, were examined for the effect on 12-O-tetradecanoylphorbol-13-acetate (TPA, 1 μg/ear)-induced inflammation, following application of this tumor-promoting agent, to the ears of mice. Both compounds inhibited the inflammatory activity induced by TPA and the 50% inhibitory dose for TPA-induced inflammation was 0.3 and 0.4 μg/ear, respectively. Furthermore, at 2 μmol/mouse, karoundiol markedly suppressed the promoting effect of TPA (1 μg/mouse) on skin tumor formation in mice following initiation with 7,12-dimethylbenz[a]anthracene (50 μg/mouse).

Keywords: karoundiol; antitumor promotion; two-stage carcinogenesis; D. C-friede-oleane triterpenes; anti-inflammation; 12-O-tetradecanoylphorbol-13-acetate

The seeds of Trichosanthes kiriwii Maxim. (Cucurbitacae) have been used in traditional Chinese medicine as an anti-inflammatory agent, a cough medicine and an expectorant.1) In previous papers we reported the isolation and structural elucidation of three D. C-friede-oleane triterpenes: D. C-friede-oleane-7,9(11)-diene-3z,29-diol (karoundiol),2–3) its 3-benzoate,2) and 7-oxo-D. C-friede-oleane-8-ene-3z,29-diol (7-oxodihydrokaroundiol).3) Another D. C-friede-oleane triterpene, the potassium succinate derivative of bryononic acid (3β-hydroxy-D. C-friede-oleane-8-en-29-oic acid), isolated from Luffa cylindrica (Cucurbitacae), was reported to inhibit type I, III and IV allergies in mice.4) Our recent study of the anti-inflammatory effects of sterols and triterpenes from plants has shown that karoundiol 3-benzoate, particularly, inhibited strongly inflammation in mice induced by 12-O-tetradecanoylphorbol-13-acetate (TPA).5) The actions of inhibitors of TPA-induced inflammation seem more or less parallel their inhibitory activities on tumor promotion.5–12) This paper describes how karoundiol and 7-oxodihydrokaroundiol also strongly inhibit TPA-induced inflammation in mice, and how karoundiol, a major component of the seeds of Trichosanthes kiriwii, markedly suppressed TPA-induced tumor promotion following initiation by 7,12-dimethylbenz[a]anthracene (DMBA) in mice.

MATERIALS AND METHODS

Animals Female ICR mice were obtained 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 were available ad libitum.

Chemicals DMBA, indomethacin and hydrocortisone were purchased from Sigma Chemical Co., St. Louis, U.S.A. TPA was from Chemicals for Cancer Research Inc., Chicago, U.S.A. Dimethyl sulfoxide was obtained from E. Merck, Darmstadt, Germany. Glycyrhretinic acid was purchased from Tokyo Chemical Industry Co., Ltd.

Tokyo, Japan. Quercetin was isolated from Sophora Flos in our laboratory. D. C-Friede-oleane triterpenes (Chart 1) were isolated from the seeds of Trichosanthes kiriwii.2,3)

Assay of TPA-Induced Inflammation in Mice TPA (1 μg) dissolved in acetone (20 μl) was applied to the right ear only of ICR mice by means of a micropipette. A volume of 10 μl was delivered to both the inner and outer surfaces of the ear. The sample or its vehicle, chloroform–methanol (1:1, 20 μl) or chloroform (20 μl) alone, 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.

The ear thickness was measured before treatment (A). The edema was measured 8 h after TPA treatment (b, TPA alone; b', TPA plus sample). The inhibition ratio (IR) was calculated as follows, where

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

IR(%) = \frac{A - B}{A} \times 100

Chart 1. Chemical Structures of D. C-Friede-oleane Triterpenes

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Each value was the mean of the individual determinations from 5 mice and the 50% inhibitory dose (ID$_{50}$) values were determined by the method of probit-graphic interpolation for 4 dose levels.

**Two-Stage Carcinogenesis Experiments** A group of 15 mice underwent initiation by 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 7d after the initiation. Karoundiol (2 μmol) in acetone–dimethyl sulfoxide (9:1, 100 μl) was applied to topically 30 min before TPA treatment. The vehicle was applied topically 30 min before TPA treatment. These treatments were continued for 18 weeks.

**Statistical Analysis** Statistical analysis was by Student’s $t$-test.

**RESULTS**

**Inhibitory Effect of Karoundiol and 7-Oxodihydrokaroundiol on TPA-Induced Inflammation** When 1 μg of TPA was applied to the ears of ICR mice, maximum edema occurred at 8 h and thereafter declined, approaching control levels by 72 h. As can be seen in Table I, the TPA-induced inflammation was inhibited by D: C-friedooleanane triterpenes, the inhibition ratio being calculated at the maximum degree of edema. Application of the sample completely inhibited TPA-induced inflammation and this inhibitory activity was dose-dependent. The 50% inhibitory dose (ID$_{50}$) values of karoundiol and 7-oxo-dihydrokaroundiol for TPA-induced inflammation were 0.4 and 0.3 mg/ear, respectively. By comparison with standard drugs, these compounds were less effective inhibitors than hydrocortisone but were similar in activity to indomethacin. In addition, by comparison with known inhibitors of tumor promotion, these compounds were similar in activity to glycyrrhetinic acid and more effective than quercetin.

**Inhibitory Effect of Karoundiol on the Tumor-Promoting Activity of TPA** Figure 1A illustrates the time-course of skin tumor formation in the groups treated with DMBA plus TPA, with or without karoundiol. The first tumor appeared during week 7 in the group treated with DMBA plus TPA. In the group treated with DMBA plus TPA and 2 μmol karoundiol, the first tumor appeared during week 14. The percentage of tumor-bearing mice treated with DMBA plus TPA was 87% at week 14, whereas in the group treated with DMBA plus TPA and 2 μmol karoundiol it was 13%. Figure 1B shows the average number of tumors per mouse. The group treated with DMBA plus TPA produced 2.3 tumors per mouse at week 18, whereas in the group treated with DMBA plus TPA and 2 μmol karoundiol there were 0.3 tumors per mouse. Treatment with 2 μmol karoundiol caused an 88% reduction in the average number of tumors per mouse at week 18.

**DISCUSSION**

In recent studies of the antitumor-promoting activities of plant constituents$^{5–10}$ and plant triterpenes,$^{5,10,13–15}$ it has been demonstrated that these compounds markedly suppressed the promoting effect of TPA on skin tumor formation in mice following its initiation with DMBA. In this study, karoundiol was found to markedly inhibit TPA-induced tumor promotion in DMBA-initiated mice, to a degree corresponding to that of glycyrrhetinic acid and betulinic acid, inhibitors of tumor promotion. Application of TPA to mouse skin rapidly causes accumulation of ornithine decarboxylase (ODC), a treatment rise in the polyamine biosynthetic enzyme.$^{10}$ Sterol and triterpene derivatives markedly inhibit this TPA-induced ODC accumulation in mouse skin.$^{9}$ The inhibitory activities of TPA-induced inflammation have been shown to roughly parallel their inhibitory activities against TPA-induced tumor promotion.$^{5–12}$ Many triterpenes, widely dis-

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**TABLE I. Inhibitory Effect of D:C-Friedooleanane Triterpenes on TPA-Induced Inflammation in Mice**

<table>
<thead>
<tr>
<th>Compound</th>
<th>ID$_{50}$</th>
<th>mg/ear</th>
<th>μmol/ear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karoundiol</td>
<td>0.4</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Karoundiol 3-benoate</td>
<td>0.2</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>7-Oxodihydrokaroundiol</td>
<td>0.3</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Glycyrrhetinic acid</td>
<td>0.1</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>1.6</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.3</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>0.03</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

Compounds were applied 30 min before TPA (1 μg). Ear thickness was determined 8 h after treatment.

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Fig. 1. Inhibitory Effect of Karoundiol on the Tumor Promotion of Skin Papillomas by TPA in DMBA-Initiated Mice

From 1 week after tumor initiation by a single topical application of 50 μg of DMBA, 1 μg of TPA was applied twice weekly. Topical application of karoundiol (2 μmol) and vehicle was performed 30 min before each TPA treatment. Data are expressed as the percentages of mice with papillomas (A) and as the average number of papillomas per mouse (B). $\bullet$, +TPA with vehicle alone; $\bigcirc$, +TPA with karoundiol. $a$) $p<0.01$ using Student's $t$-test, compared with the control group.
tributed in edible plants, are now known to inhibit the tumor promoting activity of TPA in mice and this suggests that they may be important dietary additives for the chemoprevention of cancer.

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REFERENCES
