Inhibitory Effect of Curcumin, Chlorogenic Acid, Caffeic Acid, and Ferulic Acid on Tumor Promotion in Mouse Skin by 12-O-Tetradecanoylphorbol-13-acetate

Mou-Tuan Huang, Robert C. Smart, Ching-Quo Wong, and Allan H. Conney


ABSTRACT

The effects of topically applied curcumin, chlorogenic acid, caffeic acid, and ferulic acid on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced epidermal ornithine decarboxylase activity, epidermal DNA synthesis, and the promotion of skin tumors were evaluated in female CD-1 mice. Topical application of 0.5, 1, 3, or 10 µmol of curcumin inhibited by 31, 46, 84, or 98%, respectively, the induction of epidermal ornithine decarboxylase activity by 5 nmol of TPA. In an additional study, the topical application of 10 µmol of curcumin, chlorogenic acid, caffeic acid, or ferulic acid inhibited by 91, 25, 42, or 46%, respectively, the induction of ornithine decarboxylase activity by 5 nmol of TPA. The topical application of 10 µmol of curcumin together with 2 or 5 nmol of TPA inhibited the TPA-dependent stimulation of the incorporation of [3H]thymidine into epidermal DNA by 49 or 29%, respectively, whereas lower doses of curcumin had little or no effect. Chlorogenic acid, caffeic acid, and ferulic acid were less effective than curcumin as inhibitors of the TPA-dependent stimulation of DNA synthesis. Topical application of 1, 3, or 10 µmol of curcumin together with 5 nmol of TPA twice weekly for 20 weeks to mice previously initiated with 7,12-dimethylbenz[a]anthracene inhibited the number of TPA-induced tumors per mouse by 39, 77, or 98%, respectively. Similar treatment of mice with 10 µmol of chlorogenic acid, caffeic acid, or ferulic acid together with 5 nmol of TPA inhibited the number of TPA-induced tumors per mouse by 60, 28, or 35%, respectively, and higher doses of the phenolic acids caused a more pronounced inhibition of tumor promotion. The possibility that curcumin could inhibit the action of arachidonic acid was evaluated by studying the effect of curcumin on arachidonic acid-induced edema of mouse ears. The topical application of 3 or 10 µmol of curcumin 30 min before the application of 1 µmol of arachidonic acid inhibited arachidonic acid-induced edema by 33 or 80%, respectively.

INTRODUCTION

The ground dried rhizome of the plant Curcuma longa Linn has been used for centuries as a naturally occurring medicine for the treatment of inflammatory and other diseases (1, 2), and it has also been used as a coloring agent and spice in many foods. The powdered rhizome is commonly called turmeric. Curcumin (diferuloylmethane; Fig. 1) has been identified as the major pigment in turmeric, and this substance has also been used as a spice, food preservative, and yellow coloring agent (3-5). Curcumin, which is widely used in curry, is responsible for the yellow color of curry. Turmeric contains 1-5% curcumin while the curcumin content of turmeric oleoresin is approximately 40% (6).

Recent studies have indicated that several compounds that possess antioxidant or antiinflammatory activity inhibit TPA1-induced tumors on mouse skin (7-12). Since curcumin has been reported to possess both antioxidant and antiinflammatory activity (13-17), we have evaluated the effect of curcumin on TPA-induced tumor promotion on mouse skin, and we have also evaluated the effects of the related compounds chlorogenic acid, caffeic acid, and ferulic acid as potential inhibitors of tumor promotion.

MATERIALS AND METHODS

Materials. TPA was purchased from CRC Inc., Chanhassen, MN. DL-[3H]thymidine (5 Ci/mmol) and [3H]thymidine (5 Ci/mmol) were purchased from Amersham Corp., Arlington Heights, IL. trans-Retinoic acid was obtained from Hoffmann-La Roche Inc., Basle, Switzerland. Fluocinolone acetonide, nonradioactive dl-ornithine, curcumin, chlorogenic acid, ferulic acid, and caffeic acid were purchased from the Sigma Chemical Co., St. Louis, MO, and DMBA was purchased from Calbiochem-Behring, San Diego, CA. Aquasol was purchased from New England Nuclear, Boston, MA. Ace tone and dimethyl sulfoxide were purchased from Burdick & Jackson Laboratories, Muskegon, MI. Arachidonic acid was purchased from Nu-Chek Prep Inc., Elysian, MN.

Animals. Seven-week-old female CD-1 mice were purchased from Charles River Laboratories, Kingston, NY, and kept in our animal facility at least 1 week before use. Mice were fed a Purina Laboratory Chow 5001 diet ad libitum (Ralston-Purina Co., St. Louis, MO) and kept on a 12-h light, 12-h dark cycle. Mice were provided drinking water ad libitum. The dorsal region of each mouse was shaved with electric clippers at least 2 days before treatment with TPA or DMBA. Only mice that did not show signs of hair regrowth were used. All compounds were applied to the dorsal shaved area of 8- to 9-week-old mice in 200 µl acetone or 200 µl of acetone:DMSO (90:10).

Ornithine Decarboxylase Assay and Preparation of Epidermal Homogenates. The preparation of epidermal homogenate and the determination of ornithine decarboxylase activity were done as described previously (18). Mice were treated topically with 200 µl of acetone, with TPA in acetone, or with curcumin and TPA together in acetone. In experiments with chlorogenic acid, caffeic acid, and ferulic acid, mice were treated with 200 µl of acetone:DMSO (90:10), with TPA in acetone:DMSO, or with the test compound and TPA together in acetone:DMSO. Five h after treatment, the mice were sacrificed by cervical dislocation, and the dorsal area of the skin was removed. In order to remove the epidermis from the dermis, the skins were plunged into a 58°C water bath for 30 s, and then the skins were immediately submerged in an ice water bath as described by Slaga et al. (19). The epidermis was removed from the dermis by gentle scraping and placed in 1 ml of 50 mM potassium phosphate, pH 7.7, buffer containing 2 mM diethiothreitol and 0.1 mM EDTA. The epidermis was homogenized on ice for 30 s with a Tekmar polytron tissumizer at full setting. The epidermal homogenate was centrifuged at 11,000 × g for 30 min at 4°C and the supernatant fraction was removed and stored overnight at −20°C prior to the determination of ornithine decarboxylase activity as described earlier (18). Protein was determined by the method of Lowry et al., using bovine serum albumin as the standard (20).

Quantitation of Epidermal DNA Synthesis. Mice were treated topically with 200 µl acetone, TPA in acetone, or curcumin and TPA together in acetone. Acetone:DMSO (90:10) was the solvent for experiments with chlorogenic acid, caffeic acid, and ferulic acid. At 18 h after treatment, 10 µl of [3H]thymidine solution (5 Ci/ml, 1 mCi/ml) were injected i.p. into each mouse, and 40 min later the mice were sacrificed by cervical dislocation. The isolation of epidermal DNA and determination of the incorporation of [3H]thymidine into DNA was done as previously described (18).

Tumor Studies on Mouse Skin. The dorsal region of 7-week-old...
female CD-1 mice was shaved with an electric clipper. Two days later
groups of 30 mice were treated topically with 200 nmol DMBA in 200
µl acetone, and control mice received 200 µl of acetone alone. After 1
week, mice were treated topically with 200 µl of acetone, 5 nmol TPA,
or 5 nmol TPA applied simultaneously with curcumin in 200 µl of
acetone twice weekly for 20 weeks. In another tumor study designed to
evaluate the effect of chlorogenic acid, caffeic acid, and ferulic acid on
TPA-induced tumor formation, acetone:DMSO (90:10) was used as the
solvent because chlorogenic acid at the doses examined was not
completely soluble in acetone. Tumors of at least 1 mm in diameter
were counted and recorded once every 2 weeks, and the results are
expressed as the average number of tumors per mouse and percentage
of tumor-bearing mice. Control mice that were first initiated with 200
nmol DMBA and then treated with 3 µmol curcumin in acetone or
acetone alone twice weekly for 20 weeks also were examined. Likewise,
control mice treated with acetone alone in place of DMBA and then
treated twice weekly with 5 nmol TPA in 200 µl acetone for 20 weeks
were also included in the study.

RESULTS

Effect of Curcumin, Chlorogenic Acid, Caffeic Acid, and Ferulic Acid on TPA-induced Ornithine Decarboxylase Activity in Mouse Epidermis. The effect of topically applied curcumin, chlorogenic acid, caffeic acid, or ferulic acid on TPA-induced ornithine decarboxylase activity in mouse epidermis was examined and the results are shown in Table 1. Curcumin strongly inhibited the TPA-induced increase in epidermal ornithine decarboxylase activity in a dose-dependent manner. Topical application of 0.5, 1, 3, or 10 µmol of curcumin with 5 nmol of TPA inhibited the TPA-induced increase in epidermal ornithine decarboxylase activity by 31, 46, 84, or 98%, respectively. Application of trans-retinoic acid (1.3 nmol), a known inhibitor of TPA-induced ornithine decarboxylase activity (21), inhibited the TPA-induced increase in epidermal ornithine decarboxylase activity by 88%. The effects of several naturally occurring phenolic acids that are structurally related to curcumin were studied (Table 1, experiment 2). Topical application of 10 µmol of chlorogenic acid, caffeic acid, or ferulic acid inhibited TPA-induced increases in ornithine decarboxylase activity by 25, 42, or 46%, respectively, and higher doses of the phenolic acids resulted in greater inhibition. The results of these studies indi-
cate that curcumin is about 10-fold more active than the phe-
nolic acids. The results of an additional study indicated that an i.p. injection of curcumin could inhibit the induction of ornithine decarboxylase activity in mouse epidermis that occurred after the topical application of TPA. The i.p. injection of 40 or 120 µmol of curcumin (dissolved in dimethyl sulfoxide) into mice 1 h before the topical application of 5 nmol TPA inhibited the induction of epidermal ornithine decarboxylase activity by 46 or 86%, respectively (data not shown). During the course of this study, it was found that curcumin in the vehicle precipitated out of solution in the abdominal cavity and thus may not have been completely bioavailable. Curcumin could be seen as yellow flecks in the abdominal cavity throughout the experimental period of 6 h.

Effect of Curcumin, Chlorogenic Acid, Caffeic Acid, and Ferulic Acid on TPA-induced DNA Synthesis in Mouse Epidermis. Topical application of 1, 3, or 10 µmol of curcumin together with 2 nmol of TPA inhibited the TPA-induced increase in DNA synthesis by 4, 14, or 49%, respectively (Table 2, experiment 1). The topical application of fluorocinolone acetonide (2 nmol), a known inhibitor of TPA-induced DNA synthesis, inhibited the TPA-induced increase by 86%. Topical application of 1, 3, or 10 µmol of curcumin simultaneously with 5 nmol of TPA inhibited the TPA-induced increase in the incorporation of [³H]thymidine into epidermal DNA by 3, 4, or 29%, respectively (Table 2, experiment 2). Topical application of 20 µmol of chlorogenic acid, caffeic acid, or ferulic acid together with 5 nmol of TPA inhibited the TPA-induced increase in the incorporation of [³H]thymidine into epidermal DNA by 7, 33, or 15%, respectively.

Effect of Curcumin, Chlorogenic Acid, Caffeic Acid, and Ferulic Acid on TPA-induced Tumor Promotion in Mouse Epidermis. CD-1 mice initiated with 200 nmol of DMBA and promoted with 5 nmol of TPA twice weekly for 20 weeks developed an average of 16.4 tumors/mouse. Topical application of 1, 3, or 10 µmol of curcumin with 5 nmol of TPA twice a week for 20 weeks inhibited the number of skin tumors per mouse by 39, 77, or 98%, respectively, and the percentage of animals with tumors was decreased by 21, 66, or 82%, respectively (Fig. 2). Additional groups of mice were initiated with DMBA and then treated with acetone or 3 µmol of curcumin twice weekly for 20 weeks. None of these animals developed tumors, indicating that curcumin was not a tumor promoter. In a second experiment, mice that were initiated with 200 nmol of DMBA and promoted with 5 nmol of TPA twice weekly for 19 weeks developed an average of 6.2 tumors/mouse. In this experiment, topical application of 10 µmol of curcumin, chlorogenic acid, caffeic acid, or ferulic acid together with 5 nmol of TPA twice a week for 19 weeks inhibited the number of tumors per mouse by 100, 60, 28, or 35%, respectively, and the percentage of animals with tumors was inhibited 100, 31, 28, or 7%, respectively (Table 3). The topical application of 20 µmol chlorogenic acid, 20 µmol caffeic acid, or 50 µmol ferulic acid inhibited the average number of tumors per mouse by 91, 82, or 97%, respectively, and the percentage of animals with tumors was inhibited 61, 43, or 80%, respectively (Table 3).

Effect of Curcumin on TPA- and Arachidonic Acid-induced Edema of Mouse Ears. The possibility that curcumin could inhibit TPA- and arachidonic acid-dependent inflammation was evaluated by studying the effects of curcumin on TPA- and arachidonic acid-induced edema of mouse ears. The application of 0.4 or 1 µmol of curcumin to an ear of the mouse 30 min before 0.5 nmol of TPA inhibited TPA-induced edema by 79 or 97%, respectively (Table 4, experiment 1). In additional
Forty min later the mice were sacrificed, the epidermis was removed and radioactivity in epidermal DNA was determined. Data are expressed as the mean ± SE of 16 mice.

studies, the application of 3 or 10 μmol of curcumin to an ear of the mouse 30 min before 1 μmol of arachidonic acid inhibited arachidonic acid-induced edema by 33 or 80%, respectively (Table 4, experiment 2). In another experiment, the topical application of 1.5 or 5 μmol of curcumin 30 min before 1 μmol of arachidonic acid inhibited arachidonic acid-induced edema by 32 or 58%, respectively (Table 4, experiment 3). Phenidone and nordihydroguaiaretic acid are known inhibitors of arachidonic acid metabolism (22, 23), and both of these compounds and chlorogenic acid, caffeic acid, and ferulic acid are normally less effective than curcumin. Curcumin is widely used as a yellow coloring agent and spice in commonly ingested foods, and chlorogenic acid, caffeic acid, and ferulic acid are normally occurring constituents of many human foods and beverages.

Application of TPA to mouse skin results in the rapid accumulation of inflammatory cells such as neutrophils and macrophages (24) and an increase in the release of active oxygen species (25, 26). As shown in Table 4, curcumin markedly inhibited TPA- and arachidonic acid-induced inflammation. The results could be explained by a possible effect of curcumin to inhibit the infiltration of inflammatory cells and/or to inhibit the release and action of reactive oxygen species from these cells.

It has been suggested that reactive oxygen species and other free radicals play an important role in tumor promotion (24–27). Free radical-generating compounds such as benzoyl peroxide, lauroyl peroxide, and chloroperbenzoic acid have tumor-promoting activities.
promoting activity on mouse skin (27-29), and application of TPA to mouse skin has been shown to stimulate the release and metabolism of arachidonic acid (30-33) and to increase the formation of reactive oxygen species (25, 26). Superoxide dismutase is an important enzymatic defense for the removal of superoxide anion, and the biomimetic copper(II) (3,4-diisopropylsalicylic acid), which possesses superoxide dismutase activity, has been demonstrated to inhibit TPA-induced ornithine decarboxylase activity and tumor promotion in mouse skin (34, 35). Several inhibitors of TPA-dependent tumor promotion possess antioxidant activity. Butylated hydroxytoluene (10), butylated hydroxyanisole (10, 25), sodium selenite (36), quercetin (37), nordihydroguaiaretic acid (38), a-tocopherol (39), ascorbic acid (12), and ascorbyl palmitate (12) are examples of compounds that possess antioxidant or reactive oxygen-scavenging activity and that inhibit tumor promotion and/or certain biochemical events associated with tumor promotion in mouse skin. Although the antioxidant activity of chlorogenic acid has not been compared with that of curcumin, the relative antioxidant activities of curcumin, caffeic acid, and ferulic acid roughly parallel the effects of these compounds as inhibitors of tumor promotion (see Ref. 13 and Table 3).

Several inhibitors of arachidonic acid metabolism inhibit TPA-dependent tumor promotion in mouse skin (37, 38, 40, 41), and it is thought that products of arachidonic acid metabolism may play an important role in the tumor promotion process. Mouse epidermis has been reported to possess \( \Delta^2 \), \( \Delta^5 \), \( \Delta^{12} \), and \( \Delta^{15} \)-lipoxygenase activity (37, 42-44). HPETEs and HETEs, leukotrienes, and prostaglandins are among the arachidonic acid metabolic products that are believed to play a role in TPA-induced inflammation and tumor promotion (45-47). It was found that TPA stimulates cell proliferation and increases the production of HETEs and prostaglandins in epidermal cell culture (44). In addition, inhibition of arachidonic acid metabolism results in lowered levels of HETEs and prostaglandins in human platelets (48). These products of arachidonic acid metabolism may be important in cell proliferation since high levels of arachidonic acid, HPETEs, and HETEs are found in the hyperproliferative disease psoriasis (49). Fluocinolone acetonide and other glucocorticoids represent another class of potent inhibitors of TPA-dependent tumor promotion (7), and these steroids are inhibitors of epidermal phospholipase \( A_2 \) activity in vivo and inhibit the release of arachidonic acid from cellular membranes (50). Nordihydroguaiaretic acid and quercetin are examples of \( \Delta^2 \)-lipoxygenase inhibitors that also inhibit the TPA-dependent induction of ornithine decarboxylase activity and tumor promotion in mouse skin (23, 37, 41). As indicated above, arachidonic acid metabolism is believed to give rise to reactive oxygen species and other free radicals, and studies on the TPA-induced generation of reactive oxygen species in mouse epithelial cells have indicated that inhibitors of arachidonic acid metabolism can diminish the chemiluminescence response that is associated with the generation of free radicals (51). It is possible that arachidonic acid metabolism may provide a source of free radicals that play a role in tumor promotion. The inhibitory effect of curcumin on arachidonic acid-dependent ear edema (Table 4) suggests that curcumin may block arachidonic acid metabolism by inhibiting epidermal cyclooxygenase and/or lipoxygenase activity, or that curcumin may serve as a scavenger of reactive free radicals which are produced during the metabolism of arachidonic acid. It is also possible that curcumin can interfere with the activity of epidermal phospholipase \( A_2 \) and/or inhibit TPA-induced release of arachidonic acid from membranes, since curcumin appears to be a more potent inhibitor of TPA-induced mouse ear edema than arachidonic acid-induced ear edema (Table 4). Additional studies are needed to determine the possible inhibitory effects of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on

### Table 3 Effect of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on TPA-induced tumor promotion in mouse skin

Female mice were treated topically with 200 nmol of DMBA in 200 \( \mu \)l of acetone. One week later the mice were treated topically with 5 nmol of TPA alone or together with curcumin, chlorogenic acid, caffeic acid, or ferulic acid in 200 \( \mu \)l of acetone:DMSO (90:10) twice a week for 19 weeks. The tumors per mouse are expressed as the mean \( \pm \) SE from 30 mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>11 wk</th>
<th>15 wk</th>
<th>19 wk</th>
<th>11 wk</th>
<th>15 wk</th>
<th>19 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone:DMSO (90:10)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TPA (5 nmol)</td>
<td>48.3</td>
<td>60.7</td>
<td>67.9</td>
<td>5.53 ( \pm ) 1.84</td>
<td>6.18 ( \pm ) 1.84</td>
<td>0°</td>
</tr>
<tr>
<td>TPA (5 nmol) + chlorogenic acid (10 ( \mu )mol)</td>
<td>23.3</td>
<td>40.0</td>
<td>46.6</td>
<td>0.91 ( \pm ) 0.44°</td>
<td>2.50 ( \pm ) 0.75</td>
<td>2.50 ( \pm ) 0.78</td>
</tr>
<tr>
<td>TPA (5 nmol) + chlorogenic acid (20 ( \mu )mol)</td>
<td>6.7</td>
<td>6.7</td>
<td>26.7</td>
<td>0.07 ( \pm ) 0.05°</td>
<td>0.53 ( \pm ) 0.10°</td>
<td>0.53 ( \pm ) 0.05°</td>
</tr>
<tr>
<td>TPA (5 nmol) + caffeic acid (10 ( \mu )mol)</td>
<td>24.7</td>
<td>43.3</td>
<td>46.7</td>
<td>3.47 ( \pm ) 0.09</td>
<td>4.43 ( \pm ) 1.53</td>
<td>4.43 ( \pm ) 1.89</td>
</tr>
<tr>
<td>TPA (5 nmol) + caffeic acid (20 ( \mu )mol)</td>
<td>11.5</td>
<td>23.0</td>
<td>38.5</td>
<td>0.31 ( \pm ) 0.21°</td>
<td>1.12 ( \pm ) 0.49°</td>
<td>1.12 ( \pm ) 0.67°</td>
</tr>
<tr>
<td>TPA (5 nmol) + ferulic acid (10 ( \mu )mol)</td>
<td>34.5</td>
<td>44.8</td>
<td>63.5</td>
<td>2.21 ( \pm ) 0.96</td>
<td>4.00 ( \pm ) 1.02</td>
<td>4.00 ( \pm ) 1.19</td>
</tr>
<tr>
<td>TPA (5 nmol) + ferulic acid (50 ( \mu )mol)</td>
<td>0</td>
<td>3.3</td>
<td>13.3</td>
<td>0°</td>
<td>0.17 ( \pm ) 0.08°</td>
<td>0.17 ( \pm ) 0.03°</td>
</tr>
<tr>
<td>TPA (5 nmol) + curcumin (10 ( \mu )mol)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0°</td>
<td>0°</td>
<td>0°</td>
</tr>
</tbody>
</table>

* Statistically different from TPA alone (\( P < 0.05 \)).
Table 4 Effect of curcumin on TPA- and arachidonic acid-induced edema of mouse ears

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>W/punch (mg)</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetone</td>
<td>12.78 ± 0.53</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>TPA (0.5 nmol)</td>
<td>7.35 ± 0.17°</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>TPA (0.5 nmol) + curcumin (0.4 μmol)</td>
<td>7.49 ± 0.19°</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>TPA (0.5 nmol) + curcumin (1 μmol)</td>
<td>6.78 ± 0.09°</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>TPA (0.5 nmol) + curcumin (3 μmol)</td>
<td>7.08 ± 0.20°</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Acetone</td>
<td>7.11 ± 0.24°</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Arachidonic acid (1 μmol)</td>
<td>11.57 ± 0.69</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Arachidonic acid (1 μmol) + curcumin (3 μmol)</td>
<td>10.11 ± 0.35</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Arachidonic acid (1 μmol) + curcumin (10 μmol)</td>
<td>7.99 ± 0.28°</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Arachidonic acid (1 μmol) + phenidone (2 mg)</td>
<td>8.45 ± 0.36°</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>Acetone</td>
<td>7.30 ± 0.14°</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Arachidonic acid (1 μmol)</td>
<td>10.85 ± 0.63</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Arachidonic acid (1 μmol) + curcumin (1.5 μmol)</td>
<td>9.70 ± 0.72</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Arachidonic acid (1 μmol) + curcumin (5 μmol)</td>
<td>8.80 ± 0.29°</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Arachidonic acid (1 μmol) + nordihydrourgaeric acid (1.5 μmol)</td>
<td>9.07 ± 0.31°</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Arachidonic acid (1 μmol) + nordihydrourgaeric acid (5 μmol)</td>
<td>8.98 ± 0.30°</td>
<td>53</td>
</tr>
</tbody>
</table>

*Statistically different from TPA or arachidonic acid alone (P < 0.05).

epidermal phospholipase A2, cyclooxygenase, and lipoxygenase activity. Since protein kinase C is believed to be involved in the regulation of cellular proliferation and is a receptor for TPA (52), additional studies are also needed to determine the effects of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on protein kinase C activity in the presence and absence of TPA.

Studies on the absorption and metabolism of curcumin indicate that it is absorbed after p.o. administration to rodents and that it is rapidly metabolized to glucuronide and sulfate conjugates that are excreted primarily in bile and to a lesser extent in urine (53–55). Low or undetectable blood levels of unchanged curcumin were observed after p.o. administration (54, 55), possibly because of its rapid metabolism during absorption (53–55).

Toxicity studies with turmeric or curcumin in animals indicated no histopathological changes when these substances were fed to rats, dogs, guinea pigs, or monkeys (0.5 to 2 g/kg) for 8–60 weeks (56). In addition, studies with turmeric and curcumin in rats for three generations did not show any teratogenic or carcinogenic effects (54). However feeding turmeric oleoresin to pigs at a dose of 296 to 1551 mg/kg for 16 weeks decreased body weight gain and concomitantly increased the weight of the liver and thyroid gland (56). Hyperplasia of the thyroid and epithelial changes in kidney, urinary bladder, and liver were also observed in the pigs fed turmeric oleoresin (56). Further studies are needed to determine whether these adverse effects were caused by curcumin per se or by other components in the turmeric oleoresin. Recent studies have shown that feeding caffeic acid, chlorogenic acid, or ferulic acid (2% in the diet) to rats for 4 weeks resulted in hyperplasia throughout the forestomach epithelium in the animals fed caffeic acid, but little or no effect was observed when the rats were fed chlorogenic acid or ferulic acid (57).

Extracts of turmeric were reported to cause chromosomal aberrations in several mammalian cell lines (58) and in plant cells (59). In vivo studies with both turmeric and curcumin did not show any genotoxic effects in the micronuclei test in mice, the bone marrow chromosome analysis test in mice and rats, or the dominant lethal test in mice (60, 61). Turmeric and curcumin were inactive and evaluated for mutagenicity in Salmonella typhimurium test systems in the presence of hepatic enzymes (62, 63). Curcumin was reported to possess cytotoxic (64) and antibacterial activity (65) and to inhibit the microsome-mediated mutagenicity of benzo[a]pyrene, 7,12-dimethylbenz[a]anthracene, capsaicin, chili extract, and cigarette smoke condensate (66, 66). Curcumin was also shown to inhibit the growth of tumor cells in vitro and to increase the survival of animals with lymphomas (64).

The present study, which demonstrates an inhibitory effect of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on tumor promotion by TPA in the two-stage initiation-promotion model in mouse skin, suggests a need for additional studies with these compounds in several other experimental carcinogenesis models. These studies are particularly important because of the widespread dietary ingestion of curcumin, chlorogenic acid, caffeic acid, ferulic acid, and other plant phenolics in varying proportions and amounts by the human population.

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INHIBITION OF TPA-INDUCED TUMOR PROMOTION


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