Inhibition of Tumor Promoter-induced Ornithine Decarboxylase Activity by Tannic Acid and Other Polyphenols in Mouse Epidermis in Vivo

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ABSTRACT

Naturally occurring plant phenols with antimutagenic and anticarcinogenic activities were tested for their abilities to inhibit the ornithine decarboxylase (ODC) response linked to skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate (TPA). Topical applications of tannic acid (TA) inhibit remarkably and in a dose-dependent manner TPA-induced ODC activity in mouse epidermis in vivo. This inhibitory effect of TA is dependent on the time of its administration relative to TPA. The induction of epidermal ODC activity by 8.5 nmol of TPA is inhibited maximally when 20 μmol of TA are applied topically to the skin 20 min before the tumor promoter. Gallic acid and several of its derivatives inhibit the ODC response to TPA to a lesser degree than TA. Ellagic acid is the least effective inhibitor tested. TA also inhibits the ODC-inducing activities of several structurally different tumor promoters and the greater ODC responses produced by repeated TPA treatments. The ability of TA to inhibit by 85% the ODC marker of skin tumor promotion suggests that TA and other polyphenols may be effective not only against tumor initiation and complete carcinogenesis but also against the promotion phase of tumorigenesis.

INTRODUCTION

Tumor initiation, stage 1 (conversion) and stage 2 (propagation) promotion, and progression can be induced sequentially in mouse skin (1, 2). The initiating event in skin carcinogenesis causes a genetic alteration in the program of terminal differentiation (3). The potent tumor promoter TPA1 may accelerate the differentiation and elimination of the normal keratinocytes and favor indirectly the transformation and clonal expansion of the initiated basal stem cells, which have become resistant to the TPA-induced signals of terminal differentiation (4).

The mechanisms by which TPA induces the expression of altered genes and the clonal expansion of the transformed progenitor cells may be mediated, at least in part, through Ca2+ mobilization and PKC activation (5–7). The induction of epidermal ODC activity by TPA is an excellent biochemical marker of stage 2 promotion (1, 8). Moreover, the prolonged stimulations of HPx production (9, 10) and DNA synthesis (11) in TPA-treated epidermis are required to achieve the propagation phase of skin tumorigenesis. However, the magnitude of tumor promoter-induced DNA synthesis may be linked to HPx production rather than ODC induction (12). Each of these three responses, therefore, appears to be essential but not sufficient for tumor promotion. If normal cells are more sensitive to oxidative stress than initiated cells, the increased HPx-producing activity of the epidermis may accelerate the differentiation of the noninitiated cells and trigger the prolonged compensatory stimulation of DNA synthesis required for the propagation of the tumor cells in stage 2. Because the mutagenic events of tumor initiation are irreversible, it is fundamental to the prevention of neoplasia to identify the anticancer drugs that are the most effective against the reversible propagation phase of tumorigenesis.

TA, GA, and EA have substantial potential for decreasing the risk of tumorigenicity (reviewed in Refs. 13 and 14). The naturally occurring plant phenols TA and EA are antioxidants known to inhibit skin tumor initiation and complete carcinogenesis by poly cyclic aromatic hydrocarbon but their antitumor-promoting activities have not been tested (15). EA is a candidate cancer chemopreventive agent (16) because its pharmacology is known, it is cheap and well tolerated, it is an antioxidant as effective as or better than α-tocopherol or tert-butyldihydroxy-}

MATERIALS AND METHODS

Treatment of Mice. Female CF-1 mice from Sasco, Inc. (Omaha, NE), 9 weeks old, were housed and maintained, and their dorsal skins were shaved before experimentation (29). The solutions of TPA, 12-deoxyphorbol-13-tetradecanoate, mezerein, 12-O-retinolylphorbol-13-acetate, phorbol-12,13-didecanoate, phorbol-12,13-dibenoate, (−)-indolactam V, (−)-7-octylindolactam V (all from LC Services Corp., Woburn, MA), anthralin, chrysinorbin (both from Pfizer & Bauer, Inc., Waterbury, CT), A23187, 1,2-dioctanoyl-sn-glycerol, n-dodecane (all from Sigma Chemical Co., St. Louis, MO), H2O2, and benzoyl peroxide were prepared in acetone and delivered to the shaved backs of individual mice in a volume of 0.2 ml. Multiple treatments with TPA or other tumor promoters were administered at 72-h intervals. The various doses of TSA, GA, GA lauryl ester, GA methyl ester, and α-propyl gallate (all from Sigma) tested for their abilities to inhibit the ODC responses to TPA and other tumor promoters were dissolved in acetone and applied topically in a volume of 0.4 ml at the appropriate times before or after, and to the same area of skin as, each application of tumor promoter. The various doses of EA (from Sigma) were all dissolved in a mixture of methanol:acetone (55:45) and administered topically to the skin in a single volume of 0.4 ml, except 10 μmol of EA, which were delivered...
in two consecutive applications of 0.4 ml. Controls were treated with acetone or the above mixture of vehicles only and in every experiment all mice received the same volume of solvent.

**Determination of ODC Activity.** Except as otherwise specified, epidermal ODC activities were determined in groups of six mice 5 h after the last treatment with TPA or other tumor promoters. The epidermal preparations from two mice were pooled in 3 ml of 25 mm Tris-HCl buffer, pH 7.6, containing 4 mm dithiothreitol, 1 mm EDTA, and 0.2 mm pyridoxal 5'-phosphate; homogenized with a Polytron PT-10 homogenizer for 15 s at setting 7; and centrifuged at 30,000 × g for 30 min. ODC activity was determined in 0.1-ml aliquots of the clear soluble supernatants by measuring the release of 14CO2 from L-[1-14C]ornithine-HCl (55 mCi/mmol; American Radiolabeled Chemicals, St. Louis, MO) essentially as described previously (29). Except for the time course of ODC induction by TPA in the presence or absence of TA, the results represent the mean values ± SD of two different experiments, each performed in triplicate (with two epidermises/replicate). The protein concentration of the epidermal samples was assayed with Bio-Rad dye reagent (Bio-Rad Laboratories, Richmond, CA) using crystalline bovine serum albumin as the standard.

**RESULTS**

The dose of 20 μmol of TA was selected to determine the time at which it must be applied before or after TPA treatment (time 0) in order to alter the most the induction of ODC activity caused by the tumor promoter at 5 h (Fig. 1). This TA treatment inhibits maximally TPA-induced ODC activity when it is applied 20 min before 8.5 nmol of TPA but its effectiveness is lost at treatment times further from the time of application of TPA. However, TA is able to inhibit the ODC response to TPA when it is administered over a long period of time extending from 3 h before to 1 h after the application of the tumor promoter (Fig. 1).

As shown in Fig. 2, the ability of TA to inhibit TPA-induced ODC activity when applied 20 min before the tumor promoter becomes apparent with as little as 0.5 μmol and is clearly dose dependent. The most effective dose of TA, 20 μmol, inhibits the ODC response to TPA by at least 85%.

The time course for the induction of epidermal ODC activity by a single TPA treatment is well established (8, 29). Following TPA treatment (time 0), epidermal ODC activity peaks at 5 h and returns to basal level at 12 h (Fig. 3). When applied 20 min before the tumor promoter, 20 μmol of TA inhibit remarkably TPA-induced ODC activity at all time points studied without altering the general time course observed previously.

Thus far, the studies of plant phenols as inhibitors of mutagenesis and carcinogenesis have focused on EA, which can be synthesized from GA (13, 14). Therefore, the treatments with TA, EA, GA, and several GA derivatives have been compared for their effectiveness as inhibitors of TPA-induced ODC activity (Table 1). TA is by far the most effective treatment for inhibiting the action of TPA as compared with the inhibitory effects of the other compounds tested. EA cannot be tested at 20 μmol because of its poor solubility in topically applicable solvents. Doses of 5 μmol or more are required to demonstrate the inhibitory effect of EA, which is the least effective treatment against ODC induction by TPA. Although slightly more effective than EA, GA and several of its derivatives all inhibit the ODC response to TPA to a much lesser degree than TA (Table 1).

Since the efficacy of ODC induction and skin tumor promotion is maximal when the time interval between multiple TPA treatments is about 72 h (30), it is important to compare the inhibitory effects of TA on ODC induction by single versus multiple treatments with TPA. As shown in Fig. 4, the specific activities of epidermal ODC are increased more and more by successive applications of 8.5 nmol of TPA at 72-h intervals, up to the point where they reach a plateau of maximal stimulation after the third treatment. The results indicate that TA pretreatments inhibit remarkably not only the induction of ODC activity produced by a single application of TPA but also...
the greater ODC responses elicited by each successive tumor promoter treatment.

Finally, TA was tested for its ability to inhibit the ODC responses to other phorbol ester and non-phorbol ester tumor promoters applied twice at a 72-h interval (Table 2). The ODC-inducing activities of several structurally different agents with various tumor-promoting activities have been reported previously (8, 10, 12, 29, 31–38). In general, the phorbol-related diterpene esters and the indole alkaloids with complete and/or stage 2 skin tumor-promoting activities are more potent inducers of epidermal ODC activity than other classes of non-phorbol ester tumor promoters such as the diacyl glycerols, peroxides, anthrones, Ca2+ ionophores and, n-alkanes, which must be applied at much higher doses in order to induce this enzyme activity (Table 2). Whatever their magnitudes, all these tumor promoter effects are dramatically inhibited by TA.

DISCUSSION

EA and other plant phenols, which have antineoplastic activities in various animal tumor systems, are naturally occurring diet constituents potentially very useful in cancer prevention (13, 14, 16–28). This report indicates that these antioxidants with antimutagenic and anticarcinogenic activities also inhibit remarkably a biochemical marker of tumor promotion. The induction of ODC activity, the first and rate-limiting enzyme in polyamine biosynthesis, is an event essential but not sufficient for tumor promotion (1, 2, 8). All inhibitors of ODC induction tested thus far are also effective against tumor promotion (1, 2, 8, 39). The polyphenols inhibiting the ODC-inducing activities of TPA-type tumor promoters in this study, therefore, might also inhibit skin tumor promotion. Indeed, in our first tumor experiment still in progress, we find that, after DMBA initiation and 20 weeks of promotion, the application of 5 μmol of TA 20 min before each promotion treatment with 8.5 nmol of TPA inhibits the incidence and yield of skin tumors.

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ODC activity at 5 h (nmol CO2 released/h/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td>TPA</td>
<td>6.77 ± 0.56</td>
</tr>
<tr>
<td>+ EA (2 μmol)</td>
<td>7.01 ± 0.75*</td>
</tr>
<tr>
<td>+ TA (2 μmol)</td>
<td>4.19 ± 0.31</td>
</tr>
<tr>
<td>+ EA (5 μmol)</td>
<td>6.12 ± 0.50*</td>
</tr>
<tr>
<td>+ TA (5 μmol)</td>
<td>3.26 ± 0.40</td>
</tr>
<tr>
<td>+ EA (10 μmol)</td>
<td>5.29 ± 0.38</td>
</tr>
<tr>
<td>+ GA (10 μmol)</td>
<td>3.75 ± 0.22*</td>
</tr>
<tr>
<td>+ GALE (10 μmol)</td>
<td>3.65 ± 0.20</td>
</tr>
<tr>
<td>+ GAME (10 μmol)</td>
<td>4.72 ± 0.45*</td>
</tr>
<tr>
<td>+ PG (10 μmol)</td>
<td>4.28 ± 0.36</td>
</tr>
<tr>
<td>+ TA (10 μmol)</td>
<td>2.17 ± 0.27*</td>
</tr>
</tbody>
</table>

*No significance versus TPA.
*P < 0.05, significantly smaller versus TPA.
*No significance versus TPA + GALE (10 μmol); P < 0.01, significantly smaller versus TPA + PG (10 μmol).
*GALE, gallic acid lauryl ester; GAME, gallic acid methyl ester.
*No significance versus TPA + PG (10 μmol); P < 0.05, significantly smaller versus TPA + EA (10 μmol).
*P < 0.0005, significantly smaller versus TPA + GALE (10 μmol) and TPA + TA (5 μmol).

Fig. 3. Time-response curves showing the inhibitory effect of TA on TPA-induced ODC activity in mouse epidermis in vivo. Groups of mice received topical applications to the skin of either 0.4 ml of acetone (C) or 20 μmol of TA in 0.4 ml of acetone ( ) 20 min before the application of 8.5 nmol of TPA in 0.2 ml of acetone. Mice were killed for determination of ODC activity at the indicated times following TPA treatment (time 0). The conditions of the experiment were identical with those of Fig. 1. Results are the means ± SD (bars) of duplicate determinations of enzyme activity from 3 groups of mice; each group contained the combined soluble epidermal extracts prepared from 2 mice. Basal ODC activity in control mice receiving acetone only (0.43 ± 0.05 nmol CO2 released/h/mg protein) has been subtracted from the results. a, P < 0.0005, significantly greater versus TA; b, no significance versus TA + TPA at 10 h; c, P < 0.05, significantly smaller versus control; b, no significance versus TA + TPA at 7.5 h but P < 0.025, significantly greater versus TA + TPA at 10 h; d, P < 0.005, significantly smaller versus TPA at 5 h; e, P < 0.005, significantly smaller versus TPA at 10 h and greater versus TA plus TPA at 12.5 h; f, no significance versus control or TA plus TPA at 12.5 h.

Fig. 4. Inhibitory effects of TA on the ODC responses to multiple applications of TPA in mouse epidermis in vivo. Groups of mice received topical applications to the skin of either 0.4 ml of acetone (C) or 20 μmol of TA in 0.4 ml of acetone ( ) 20 min before the application of 8.5 nmol of TPA in 0.2 ml of acetone. These treatments were repeated at 72-h intervals. The mice were killed 5 h after the indicated number of applications of TPA for enzyme assay. The conditions of the experiment and the determination of the results were identical with those of Fig. 1. Control mice treated similarly with acetone only at 72-h intervals had identical basal ODC activities (0.38 ± 0.03 nmol CO2 released/h/mg protein), which were subtracted from the results. Bars, SD; a, no significance versus third or fourth applications of acetone plus TPA; b, no significance versus third or fourth applications of TA plus TPA.
papillomas by 49 and 85%, respectively.* (-)-Epigallocatechin gallate, the main polyphenolic constituent of tannin in green tea, has also been shown to inhibit skin tumor promotion by teleocidin (40).

The mechanism by which TA, EA, GA, and GA derivatives inhibit the ODC-inducing activity of TPA is unknown. The mice show no signs of stress or discomfort following repeated applications of 20 μmol of TA. Cytotoxicity is unlikely since the inhibitory effect of TPA is rapidly reversible (Fig. 1) and similar TA and EA treatments enhance the activities of other epidermal enzymes (20, 25). Macroscopic examination of the skins collected after single or multiple TA plus TPA treatments shows that they have been very well protected from the hyperplastic and inflammatory activities of the tumor promoter as compared with the skins receiving TPA alone. Moreover, topical applications of 20 μmol of TA within 10 min of the time of sacrifice (5 h after TPA treatment) do not alter the pH of the ODC-containing supernatant and do not inhibit the activity of the TPA-induced enzyme, suggesting that the observed inhibitory effects are not simply due to traces of plant phenols remaining in the epidermal preparations (data not shown).

TPA is believed to induce ODC activity by a signal transduction mechanism involving Ca²⁺ mobilization, PKC induction, ODC mRNA expression, and protein synthesis (5, 7, 8, 41). The fact that TA is more effective against ODC induction when applied before rather than after TPA treatment (Fig. 1) suggests that this compound does not interact directly with the enzyme but interferes with the actions of the tumor promoter and/or the molecular pathways regulating enzyme activities. Antioxidants generally inhibit the ODC-inducing activity of TPA (reviewed in Refs. 15, 31, and 32). However, there is no apparent correlation between oxidant generation and ODC induction since the HPx response to TPA does not require ODC induction and is not essential for ODC induction (10, 12). Preliminary studies show that topical applications of doses of 0.5 to 2 μmol of EA, which are not sufficient to affect the ODC response to the tumor promoter (Table 1), inhibit totally TPA-induced HPx production in mouse epidermis in vivo.¹ Therefore, there is no indication that the polyphenols inhibit the induction of ODC activity by TPA because of their antioxidant activities. (-)-Epigallocatechin gallate slightly inhibits the specific binding of [³H]-TPA to mouse skin, decreases the number of phorbol ester receptors, and prevents the activation of PKC by teleocidin (40).

On an equal dose basis, TA is the most effective of the polyphenols tested as inhibitor of TPA-induced ODC activity. Comparative studies indicate that TA is also the most potent of the plant phenols at inhibiting epidermal monooxygenase activities and polycyclic aromatic hydrocarbon metabolism and covalent binding to epidermal DNA (17, 18). Moreover, the exceptional activity of TA among naturally occurring plant phenols in protecting against skin tumor initiation and complete carcinogenesis has been noted (19). There is evidence to suggest that the nature and mechanism of tumor promotion by TPA and mezerein may be different from those of the presumed promoting component of DMBA carcinogenesis (15, 31, 42). In fact, the promoting component of DMBA carcinogenesis may resemble more the promoting components of benzoyl peroxide or chrysarobin than that of TPA (34, 43). However, TA inhibits the ODC responses to both benzoyl peroxide and chrysarobin (Table 2). Therefore, it is postulated that TA may have afforded significant protection against skin tumorigenicity in earlier studies because it inhibited not only the mutagenicity but also the promoting activities of the complete carcinogens used.

TA pretreatments inhibit the ODC-inducing activity of TPA by about 85%. That TA inhibits the time course for ODC induction by TPA at each time point studied in Fig. 3 suggests that this compound does not simply delay the peak of TPA-induced ODC activity. EA is at least 50% less effective than TA at inhibiting the ODC response to TPA and higher, perhaps more effective, doses of this compound cannot be tested because they cannot be dissolved and/or spread in topically applicable solvents. The poor absorption of EA and the oxidation breakdown of its polyphenolic structure may limit the effectiveness of this treatment against tumorigenesis (14, 24). Taken together, our data and a review of the literature suggest that future studies to elucidate the chemoprotective effects of polyphenols during the multistage process of skin tumorigenesis should focus on TA.

Table 2 Inhibitory effects of TA on the ODC responses to various tumor promoters in mouse epidermis in vivo

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ODC activity at 5 h</th>
<th>% of control</th>
<th>% of respective tumor promoter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.35 ± 0.04</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>TPA</td>
<td>9.21 ± 0.82</td>
<td>2045</td>
<td>20</td>
</tr>
<tr>
<td>+ TA</td>
<td>2.64 ± 0.16</td>
<td>750</td>
<td>75</td>
</tr>
<tr>
<td>12-Dioxophorbol 13-tetradecanolate</td>
<td>9.77 ± 0.64</td>
<td>2791</td>
<td>279</td>
</tr>
<tr>
<td>+ TA</td>
<td>2.26 ± 0.12</td>
<td>645</td>
<td>64</td>
</tr>
<tr>
<td>Mezerein</td>
<td>10.89 ± 1.02</td>
<td>3111</td>
<td>311</td>
</tr>
<tr>
<td>+ TA</td>
<td>2.39 ± 0.18</td>
<td>683</td>
<td>68</td>
</tr>
<tr>
<td>12-Diacetyloxyphorbol 13-acetate</td>
<td>10.45 ± 1.18</td>
<td>29950</td>
<td>299</td>
</tr>
<tr>
<td>+ TA</td>
<td>1.82 ± 0.30</td>
<td>520</td>
<td>52</td>
</tr>
<tr>
<td>Phorbol-12,13-didecanoate</td>
<td>5.28 ± 0.52</td>
<td>15090</td>
<td>150</td>
</tr>
<tr>
<td>+ TA</td>
<td>2.16 ± 0.36</td>
<td>617</td>
<td>61</td>
</tr>
<tr>
<td>Phorbol-12,13-dibenzoate</td>
<td>1.82 ± 0.77</td>
<td>519</td>
<td>51</td>
</tr>
<tr>
<td>+ TA</td>
<td>0.81 ± 0.10</td>
<td>231</td>
<td>23</td>
</tr>
<tr>
<td>A23187</td>
<td>1.55 ± 0.03</td>
<td>157</td>
<td>15</td>
</tr>
<tr>
<td>+ TA</td>
<td>1.20 ± 0.19</td>
<td>343</td>
<td>34</td>
</tr>
<tr>
<td>1,2-Dioctanoyl-sn-glycerol</td>
<td>0.67 ± 0.14</td>
<td>191</td>
<td>19</td>
</tr>
<tr>
<td>+ TA</td>
<td>4.49 ± 0.29</td>
<td>12820</td>
<td>128</td>
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<tr>
<td>(+)-Indolactam V</td>
<td>0.83 ± 0.07</td>
<td>237</td>
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<tr>
<td>+ TA</td>
<td>7.55 ± 0.83</td>
<td>21570</td>
<td>215</td>
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<tr>
<td>+ TA</td>
<td>1.31 ± 0.22</td>
<td>374</td>
<td>37</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>1.44 ± 0.12</td>
<td>411</td>
<td>41</td>
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<tr>
<td>+ TA</td>
<td>0.85 ± 0.13</td>
<td>243</td>
<td>24</td>
</tr>
<tr>
<td>Benzoyl peroxide</td>
<td>0.63 ± 0.03</td>
<td>180</td>
<td>18</td>
</tr>
<tr>
<td>+ TA</td>
<td>1.95 ± 0.15</td>
<td>556</td>
<td>55</td>
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<tr>
<td>Chrysarobin</td>
<td>0.81 ± 0.05</td>
<td>231</td>
<td>23</td>
</tr>
<tr>
<td>+ TA</td>
<td>0.43 ± 0.03</td>
<td>123</td>
<td>12</td>
</tr>
<tr>
<td>Dodecane</td>
<td>2.82 ± 0.26</td>
<td>806</td>
<td>80</td>
</tr>
<tr>
<td>+ TA</td>
<td>2.12 ± 0.36</td>
<td>606</td>
<td>60</td>
</tr>
</tbody>
</table>

* No significance versus TPA.
* P < 0.01, significantly greater versus TPA.
* P < 0.05, significantly greater versus TPA.
* P < 0.005, significantly smaller versus TPA.
* P < 0.0005, significantly smaller versus benzoyl peroxide.
* P < 0.005, significantly greater versus control.
* P < 0.005, significantly smaller versus n-dodecane.


Because the magnitude of ODC induction almost doubles after the second TPA treatment (Refs. 12, 29, and 32; Fig. 4), it may be more accurate to compare the limited ODC-inducing activities of weak tumor promoters when they are applied twice at a 72-h interval (Table 2). Under these conditions, the abilities of several structurally different tumor promoters to induce ODC activity reflect their tumor-promoting activities. As observed after a single application (8, 29, 31, 32), the order of effectiveness of the phorbol esters as inducers of ODC activity is TPA > phorbol-12,13-didecanoate > phorbol-12,13-dibenoate. Although it is 16-fold more irritant, 12-deoxyphorbol 13-tetradecanoate induces ODC activity (Table 2) and skin tumor promotion (44) as much as TPA. 12-O-Retinoxyphorbol-13-acetate and the phorbol-related diterpene ester, mezerein, are very weak complete tumor promoters in certain strains of mice but induce ODC activity as much as TPA and are excellent stage 2 tumor promoters (1, 2). However, the PKC activator and stage 2 tumor promoter, 1,2-dioctanoyl-sn-glycerol (45), is less potent than TPA, mezerein, or 12-O-retinoxyphorbol-13-acetate in inducing ODC activity (Ref. 33; Table 2). Although the Ca2+ ionophore A23187 is a significant ODC inducer (Table 2) that stimulates HPx production and DNA synthesis almost as much as TPA and mezerein (10, 12), its tumor-promoting activity has been demonstrated only in stage 1 (I) and should be reassessed. Teleocidin, a mixture of isomers with similar tumor-promoting activities, is an indole alkaloid with TPA-type activities; it binds to the TPA receptor, induces PKC and ODC activities, and stimulates the production of O2· and H2O2 (reviewed in Refs. 15 and 46). The synthetic analogues of the teleocidin A type, (−)-indolactam V and (−)-7-octylindolactam V, which are moderate and potent activators of PKC and apparently bind to the same site on the enzyme at which the phorbol esters act, also induce remarkably ODC activity (Table 2) in relation with their binding affinities for the phorbol ester receptor (38). The free radical generator benzoyl peroxide, which induces epidermal PKC and xanthine oxidase activities, hyperplasia, and morphological changes similar to those caused by TPA, is a good tumor progressor but a weak inducer of ODC activity in relation with its weak tumor-promoting activity (15, 31, 32, 43, 47–51). The alkane n-dodecane, a weak tumor promoter active primarily in stage 2, produces no epidermal toxicity, no dark basal cells, and very little inflammation or ODC induction after a single 50-mg treatment but its hyperplastic activity after four 50-mg treatments is comparable to that of TPA or mezerein (37, 52). However, when a dose of 25 mg of n-dodecane is applied twice at a 72-h interval, it is a rather good inducer of ODC activity (Table 2). The peak of ODC induction occurs at about 56–60 h after a single application of chrysarobin but may be shifted to earlier times following multiple chrysarobin treatment; in fact, the ODC response to chrysarobin may be greater after a single application than during a twice weekly protocol (34–36). Therefore, it is possible that the ODC response measured 5 h after the last of two applications of chrysarobin at a 72-h interval does not truly reflect the ODC-inducing activity of this compound (Table 2). Since the magnitude and time course for the effects of the anthrones on epidermal ODC activity and polyamine and DNA synthesis are very different from those of TPA and mezerein, the mechanism by which the anthrones promote skin tumors may be somewhat different from that of the diterpenes (34–36). In any case, the ability of TA to inhibit all the ODC responses elicited by such different tumor promoters suggests that (a) the inhibitory effects of the polyphenols on ODC induction are not specific for the TPA-altered enzyme and (b) the plant phenols are likely to inhibit the mechanisms by which these various tumor promoters induce tumor formation.

The development of skin tumors requires repeated applications of a tumor promoter to initiated skin. Because the biochemical, histological, and morphological effects triggered by TPA in mouse skin are maximal after 3 to 5 applications of this compound (10, 12, 29, 32, 50, 53), the ability of TA to inhibit to about the same degree the greater ODC responses elicited by each successive application of TPA (Fig. 4) suggests that TA may also decrease the biological activity of chronic TPA treatment, a hypothesis verified in our current tumor promotion experiment. Since TA inhibits tumor initiation and complete carcinogenesis, our biochemical study suggests that TA and other plant phenols may be universal inhibitors of multistage carcinogenesis.

REFERENCES


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