Effect of the β-diketones diferuloylmethane (curcumin) and dibenzoylmethane on rat mammary DNA adducts and tumors induced by 7,12-dimethylbenz[a]anthracene

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Curcumin is a β-diketone constituent of the spice turmeric that possesses anticarcinogenic properties in several animal models. The present studies were conducted in order to identify β-diketones structurally-related to curcumin that would be effective dietary blocking agents toward the initiation stage of 7,12-dimethylbenz[a]anthracene (DMBA)-induced rat mammary carcinogenesis. Of the β-diketone compounds initially screened for their capacity to induce quinone-reductase (QR) activity in wild-type Hepa1c1c7 cells and a mutant subclone, curcumin (diferuloylmethane) and dibenzoylmethane were most effective. However, when added to semipurified diets fed to female rats, dibenzoylmethane (1%), but not curcumin (1%), was effective in inhibiting in vivo mammary DMBA–DNA adduct formation. This inhibitory effect on mammary adduct formation was associated with a significant increase in liver activities of glutathione S-transferase, QR and 7-ethoxyresorufin-O-deethylase activities. Female rats provided diets supplemented with dibenzoylmethane at 0.1, 0.5 and 1.0% for 14 days prior to dosing with DMBA exhibited a significant decrease in mammary tumor development, compared with controls. However, tumor development for animals fed diets containing 1.0% curcumin was not different from that of controls. Therefore, dibenzoylmethane, and possibly other structurally-related β-diketones, warrant examination as breast cancer chemopreventative blocking agents.

Introduction

Because of the relationship between diet and risk for several cancers (1), there has been considerable interest in identifying compounds, both natural and synthetic, for use as cancer chemopreventative agents (2). Curcumin (diferuloylmethane, Figure 1) is a β-diketone constituent of the spice turmeric that is prepared from the powdered rhizome of Curcuma longa Linn. This phytochemical has been reported to possess anticarcinogenic properties in several experimental models (3). Because of its lack of toxicity, its efficacy in inhibiting tumorigenesis in several models, and its multiple mechanisms of action, curcumin has been selected for further evaluation as a candidate chemopreventative agent (4). As part of ongoing investigations to identify breast cancer chemopreventative blocking agents, we have reported that curcumin inhibited mammary adenocarcinoma development and in vivo mammary DNA adduct formation when administered intraperitoneally to female rats prior to dosing with 7,12-dimethylbenz[a]anthracene (DMBA*) (5). However, it has been reported recently that dietary curcumin at levels of up to 1.0% was ineffective in inhibiting DMBA-induced rat mammary tumorigenesis (6). Therefore, we have attempted to identify other curcuminoids or other compounds structurally similar to curcumin that might be effective cancer chemopreventative agents, especially when administered in the diet. In the present report, we selected curcumin, demethoxycurcumin, bisdemethoxycurcumin, dibenzoylmethane, dibenzoylpropane and dibenzoylbutane (Figure 1) for evaluation as potential blocking agents. Demethoxycurcumin and bisdemethoxycurcumin were chosen for evaluation, since they are curcuminoids (also present in turmeric) that are identical in structure to curcumin except that they lack one or both methoxy-groups on the aromatic rings. The relative effectiveness of the three curcuminoids would provide insight into whether differences in aromatic group constituents would affect bioactivity. Dibenzoylmethane, a reported antimutagen (7), is similar to curcumin in that it possesses a β-diketone (1,3 diketone moiety linking two phenyl groups. The other two dibenzoyl compounds, dibenzoyl-propane and dibenzoylbutane, were selected for evaluation because they are diketones structurally-related to dibenzoylmethane, but do not possess the β-diketone configuration. They were evaluated in order to determine whether the length of the aliphatic chain separating the benzoyl groups affected bioactivity.

It has been reported that curcumin is an inducer of phase II detoxification enzymes (3,4) which frequently are associated with inhibition of the initiation stage of carcinogen-induced tumorigenesis. Although cancer preventative agents may act through a variety of mechanisms, many effective blocking agents are capable of stimulating the activity of phase II enzymes (8). Therefore, the compounds first used in these studies were evaluated for their capacity to induce NAD(P)H-quinone reductase (QR) activity in wild-type and mutant Hepa1c1c7 cells. This assay has been widely used to screen compounds for anticarcinogenic potential by measuring the induction of the phase II enzyme QR (9,10). However, compounds determined to be active in this assay may not necessarily be effective in inhibiting mammalian carcinogenesis. Therefore, those compounds that demonstrated inducing activity were then fed to female rats prior to dosing with the mammmary carcinogen DMBA, in order to evaluate whether each was effective in inhibiting the in vivo formation of mammary DMBA–DNA adducts and the development of DMBA-induced rat mammary tumors.

Materials and methods

Materials

Curcumin (>95%), demethoxycurcumin and bisdemethoxycurcumin were isolated from an extract of turmeric and obtained from Dr Carolyn Fisher...
beginning 5 weeks post-DMBA, were palpated weekly to determine mammary tumor development. At the termination of the tumor study, tumors were removed and classified histopathologically (11).

**Mammary adduct determination**

DNA from rat mammary tissue was isolated and the quantity of mammary DMBA–DNA adducts determined by the 32P-post-labeling procedure described by Singletary et al. (12).

**Liver glutathione S-transferase (GST), QR and cytochrome P-450 assays**

Liver samples from animals in the adduct study were homogenized in cold 0.25 M sucrose (pH 7.4) and centrifuged at 9000 x g for 20 min. The resulting supernatant was then centrifuged at 100,000 g for 1 h. The remaining cytosols were stored at −80°C, and microsomes were washed and stored at −80°C. Cytosolic GST activity toward 1-chloro-2,4-dinitrobenzene (CDNB) was determined by the method of Habig et al. (13), cystolic QR activity by the procedure of Benson et al. (14), and microsomal ethoxyresorufin-O-deethylase activity by the method of Pohl and Fouts (15).

**Statistical analyses**

Statistically significant differences in palpable mammary tumor incidence between treatment groups were determined by life-table survivor analysis using Cox’s F-test to compare differences in survival curves. Differences between means of tumor number, adducts and enzyme activities were evaluated by ANOVA. Differences between means at P < 0.05 were considered statistically significant.

**Results**

Curcumin, demethoxycurcumin and bisdemethoxycurcumin were evaluated for their inductive activity in wild-type Hepa1c1c7 murine hepatoma cells and mutant BPc1 subclone were obtained from ATCC and J.P. Whitlock (Stanford University, Palo Alto, CA), respectively.

**QR induction assay**

The screening of the compounds for QR inductive activity was performed according to methods described by Prochaska (10). The inductive activity of the compounds was determined at those concentrations that were not cytotoxic.

**Animals and diets**

Female Sprague–Dawley rats were obtained from Harlan Sprague–Dawley (Indianapolis, IN) and housed separately in wire-bottom, stainless-steel cages in rooms with controlled temperature and lighting. Animals were fed a semipurified diet containing (% by wt): vitamin-free casein (20%), DL-methionine and choline were purchased from A.E. Staley (Decatur, IL), and corn oil from Archer Daniels Midland (Decatur, IL). Edible grade sucrose, cornstarch and dihydrogen citrate (0.2%). Casein, mineral mix and vitamin mix were obtained from Sigma Chemical Co.

For the rat mammary adduct experiment, animals (n = 6/group) at 40 days of age that weighed (±SE) 120.5 ± 3.0 g were fed control diets and diets supplemented with 0.2% dibenzoylmethane, 1.0% dibenzoylmethane, 0.2% curcumin or 1.0% curcumin. At 50 days of age, animals were administered DMBA (i.g. in corn oil, 32.1 mg/kg) 24 h prior to isolation of mammary DNA, liver cytosols and liver microsomes.

For the tumor inhibition experiment, animals at 36 days of age that weighed (±SE) 103.2 ± 1.4 g were randomized into five groups and fed a control diet (n = 27) or diets supplemented with 1.0% curcumin (n = 25), 0.1% dibenzoylmethane (n = 25), 0.5% dibenzoylmethane (n = 26) or 1.0% dibenzoylmethane (n = 26). At 50 days of age, all animals were dosed with DMBA (50 mg/kg, i.g. in corn oil). After 24 h, all groups were fed the control diet for the remainder of the study. Animals were weighed weekly and,
Effect of curcumin and dibenzoylmethane

were fed to female rats for 10 days prior to determination of in vivo adduct formation, there was no significant difference in body wts among treatment groups (Table I). The feeding of curcumin at both dietary concentrations did not significantly affect animal liver weights (Table I). However, feeding dibenzoylmethane at dietary levels of 0.2% and 1.0% increased liver weights by a significant 28% and 19%, respectively. When curcumin was supplemented in the diet at concentrations of 0.2% and 1.0%, there was no significant inhibition of in vivo mammary DMBA–DNA adduct formation nor of liver GST, QR and EROD activities (Table I). However, dietary dibenzoylmethane (at 1.0%) significantly decreased total mammary adduct formation by 82% compared with controls (Table I). This was associated with a 7.9-fold increase in liver GST activity, a 6.1-fold increase in liver QR activity and a 1.6-fold increase in liver EROD activity.

In order to examine and compare the effects of dietary dibenzoylmethane and curcumin on the initiation stage of DMBA-induced mammary tumorigenesis, animals were fed diets supplemented with 0, 0.1, 0.5 and 1.0% dibenzoylmethane and 1.0% curcumin for 14 days prior to carcinogen administration. These levels of dibenzoylmethane were chosen so that a wider range of concentrations (10-fold) could be examined. Only one dietary level of curcumin (1.0%) was chosen for comparison because of the lack of effect of dietary curcumin in the previous adduct study. At the time of DMBA intubation, only rats fed the 1.0% dibenzoylmethane diet exhibited body wts significantly different from controls. Mean body wts at this time were (±SE) 163.0 ± 2.1 g and 160.2 ± 1.9 g for control and curcumin-fed rats, respectively. Body wts for animals fed 0.1, 0.5 and 1.0% dibenzoylmethane were 162.7 ± 2.6 g, 158.0 ± 2.0 g and 155.0 ± 1.9 g, respectively. During the time period after DMBA administration there were no significant differences in animal growth among treatment groups (Figure 2). With regard to tumor development, dietary curcumin (1.0%) did not inhibit cumulative mammary tumor incidence (Figure 3) or tumor multiplicity (Table II). In contrast, the cumulative mammary tumor incidence curves for animals fed 0.1, 0.5 and 1.0% dibenzoylmethane were significantly different from that of animals fed a control diet (Figure 3). Palpable tumors/rat and adenocarcinomas/rat were significantly less for animals fed the 0.5% and 1.0% dibenzoylmethane diets compared with animals fed the control diet (Table II). At a level of 1.0% in the diet, dibenzoylmethane inhibited final mammary tumor incidence and adenocarcinomas/rat by 60 and 77%, respectively.

Discussion

The screening of the six diketones using the wild-type Hepa1c1c7 cell system indicated that curcumin and dibenzoylmethane exhibited the strongest QR inductive activity. Comparison of responses to the compounds using both the wild-type and the mutant subclone BPc1 cells indicated that curcumin and dibenzoylmethane were monofunctional and bifunctional QR inducers, respectively. In contrast to curcumin, the curcuminoids, demethoxycurcumin and bisdemethoxycurcumin, were substantially less active in inducing QR activity. In addition, they were unable to induce QR activity in the mutant subclone. The reason for this lack of activity in the BPc1 cells is not known. This indicates that, at least for the latter three curcuminoids, the presence of all methoxy groups is needed for the strongest action as a monofunctional inducer in this assay system. In contrast, it has been observed that both curcumin and demethoxycurcumin were effective as inhibitors of chemically-induced skin tumor promotion (16). Therefore, the effectiveness of the individual curcuminoids as inhibitors of tumorigenesis may depend on the stage of tumor development or the specific physiological process examined. The lack of QR inductive activity of dibenzylpropane and dibenzoylbu-
tane, compared with dibenzoylmethane, suggests that the β-diketone configuration is necessary for QR induction in this assay system.

When curcumin and dibenzoylmethane were fed to rats, only dibenzoylmethane exhibited the capacity to inhibit in vivo mammary DMBA–DNA adduct formation and to modify GST, QR and EROD activities. This lack of effect of dietary curcumin partly may be caused by poor absorption and bioavailability as observed by other investigators (17–19). Nonetheless, it has been reported that dietary curcumin is associated with inhibition of liver carcinogen–DNA adduct formation (20) and of chemically-induced skin tumorigenesis (21). We observed that feeding dibenzoylmethane to rats was associated with an increase in liver GST, QR and EROD activities, which is consistent with the Hepa 1c1c7 cell data that identify dibenzoylmethane as a bifunctional inducer.

However, it is of interest to note that, at least in the liver, dibenzoylmethane was a much more effective inducer of phase II enzyme activities (GST and QR) than of the phase I EROD activity.

Because dibenzoylmethane was a bifunctional inducer, it was important to establish that its incorporation in the diet prior to DMBA administration did indeed result in mammary tumor inhibition. We compared the tumor inhibitory action of dibenzoylmethane with that of curcumin. Dietary curcumin (1.0%) did not inhibit DMBA-induced rat mammary carcinogenesis. This response to curcumin is similar to that reported by others (6) and is consistent with the lack of inhibition of genesis. This response to curcumin is similar to that reported with that of curcumin. Dietary curcumin prior to DMBA administration did indeed result in mammary was important to establish that its incorporation in the diet

Table II. Effect of dietary curcumin and dibenzoylmethane on DMBA-induced mammary carcinogenesis

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Final body wt (g)</th>
<th>Final tumor incidence (%)</th>
<th>Final palpable tumors/rat (mean)</th>
<th>Adenocarcinomas/rat (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>295.0 ± 12.0a</td>
<td>92.6</td>
<td>3.4 ± 0.6a</td>
<td>4.3 ± 0.7a</td>
</tr>
<tr>
<td>0.1% dibenzoylmethane</td>
<td>302 ± 10.0a</td>
<td>91.7</td>
<td>3.3 ± 0.6a</td>
<td>4.2 ± 0.7a</td>
</tr>
<tr>
<td>0.5% dibenzoylmethane</td>
<td>310 ± 8.7a</td>
<td>50.0</td>
<td>1.5 ± 0.4b</td>
<td>1.8 ± 0.4b</td>
</tr>
<tr>
<td>1.0% dibenzoylmethane</td>
<td>300 ± 12.5a</td>
<td>37.0</td>
<td>0.7 ± 0.2b</td>
<td>1.0 ± 0.3b</td>
</tr>
<tr>
<td>1.0% curcumin</td>
<td>297.0 ± 18.1a</td>
<td>96.0</td>
<td>4.1 ± 0.6a</td>
<td>5.0 ± 0.7a</td>
</tr>
</tbody>
</table>

1Values represent means ± SE. Means among treatment groups sharing unlike superscripts are statistically significant.
2For statistical comparison of tumor incidence values, see legend to Figure 3.
3Means for adenocarcinomas/rat are greater than for final palpable tumors/rat because of the presence of non-palpable tumors removed at necropsy.

Acknowledgement
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