Inhibition of 7-Bromomethylbenz[a]anthracene-promoted Mouse Skin Tumor Formation by Retinoic Acid and Dexamethasone

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ABSTRACT

Retinoic acid, a potent inhibitor of mouse skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate, fails to inhibit tumor formation by the complete carcinogen, 7,12-dimethylbenz[a]anthracene (DMBA). To obtain further clues about the nature of the mechanism of the carcinogenic process as well as the mechanism of the effect of retinoic acid on tumor promotion, the effect of retinoic acid and two other modifiers (dexamethasone and 7,8-benzoflavone) of tumor formation on tumor promotion by 7-bromomethylbenz[a]anthracene (BrMBA) was determined. BrMBA, a structural analogue of DMBA, is a weak mouse skin tumor-initiating agent but is a good skin tumor promoter.

Application of 10, 100, and 200 nmol of BrMBA twice weekly to DMBA-initiated skin resulted in 0, 1.6, and 2.5 papillomas per mouse, and 0, 44, and 60% of mice had papillomas at the 25th week of promotion treatment, respectively. Application of 17 nmol of retinoic acid or 76 nmol of dexamethasone 30 min prior to each twice weekly application of 100 nmol of BrMBA to DMBA-initiated skin inhibited the formation of skin papillomas by 73 and 100%, respectively. 7,8-Benzoflavone, at a 367-nmol dose, did not inhibit tumor promotion by BrMBA. Application of 200 nmol of BrMBA to mouse skin induced epidermal ornithine decarboxylase activity; a peak activity was observed between 8 and 18 hr following BrMBA treatment. Application of 17 nmol of retinoic acid or 76 nmol of dexamethasone inhibited the induction of ornithine decarboxylase activity by BrMBA. 7,8-Benzoflavone did not inhibit the induction of ornithine decarboxylase activity by BrMBA. Retinoic acid and dexamethasone, which inhibit tumor promotion by 12-O-tetradecanoylphorbol-13-acetate, also inhibited tumor promotion by BrMBA, but the nature of the mechanism of tumor promotion by BrMBA is unclear; BrMBA did not inhibit specific binding of 12-O-[3H]tetradecanoylphorbol-13-acetate to the cellular membrane fraction of mouse epidermis.

INTRODUCTION

Retinoic acid and certain of its analogues have been shown to prevent tumor formation in a number of experimental models (19, 21). Recently, we have presented evidence that the effect of retinoic acid on skin carcinogenesis is not universal (25, 27). Thus, the application of retinoic acid in conjunction with the tumor promoter TPA3 to DMBA-initiated skin inhibits tumor formation, but when applied in conjunction with a single large dose (3.2 µmol) or weekly smaller doses (1 to 200 nmol) of DMBA (no TPA treatment), it fails to inhibit but rather under some experimental conditions potentiates tumor formation by DMBA. Retinoic acid treatment did not inhibit ODC induction by DMBA (25, 27). This indicates that the nature and the mechanism of tumor promotion by TPA are different from those of the presumed promoting component of carcinogenesis by the complete carcinogen DMBA. To obtain further clues about the nature of the carcinogenic process as well as of the mechanisms of the effect of retinoic acid on tumor promotion, we determined the effect of retinoic acid and 2 other modifiers (dexamethasone and 7,8-benzoflavone) (5, 16, 27) of carcinogenesis on CD-1 mouse skin tumor promotion by BrMBA, a structural analogue of DMBA (6, 7).

BrMBA is a weak mouse skin tumor-initiating agent as well as a weak complete carcinogen (6, 18) and mutagen (4). It reacts with DNA both in vitro and in vivo (13, 14). Scribner and Scribner (18) made an interesting observation that BrMBA is a good tumor-promoting agent when applied to DMBA-initiated mouse skin. In this paper, we summarize the results that the doses of retinoic acid and dexamethasone, which inhibit skin tumor promotion by TPA (16, 22, 24, 29), also inhibit tumor promotion by BrMBA. Application of BrMBA to mouse skin induces epidermal ODC activity which was inhibited by retinoic acid or dexamethasone pretreatment; 7,8-benzoflavone inhibited neither ODC induction nor tumor promotion by BrMBA.

MATERIALS AND METHODS

Materials. Female Charles River CD-1 mice were purchased from Charles River Breeding Laboratory, Wilmington, Mass., and were used for experimentation at 8 weeks of age. TPA was purchased from LC Services Corporation, Woburn, Mass. DMBA was purchased from Eastman Organic Chemicals, Rochester, N. Y. Retinoic acid (all-frans) and dexamethasone were obtained from Sigma Chemical Co., St. Louis, Mo. α-Naphthoflavone (7,8-benzoflavone) was purchased from Aldrich Chemical Co., Milwaukee, Wis. BrMBA was a generous gift from Dr. A. Dipple (Frederick Cancer Research Facility, Frederick, Md.) and was also prepared as described by Dipple and Slade (6). α-[1-14C]Ornithine hydrochloride (specific activity, 49.9 mCi/mmol) and [20-3H]TPA (specific activity, 6.5 Ci/mmol) were purchased from New England Nuclear, Boston, Mass.

Treatment of Mice. All mice were housed in screen-bottomed stainless steel cages in light- and temperature-controlled rooms; food and water were available ad libitum. The dorsal skin of the mice was shaved 3 to 4 days before treatment, and only those mice in the resting phase of the hair cycle were used for experimentation. The solutions of TPA, DMBA, and BrMBA were prepared in acetone and delivered to the shaved backs of individual mice in a volume of 0.2 ml. Control mice were treated with the same volume of acetone.

Assay of ODC Activity. ODC activity in the soluble epidermal extracts was assayed as described (28). Protein content of soluble epidermal extracts was determined by the procedure of Lowry et al. (10).
shaved backs of mice at 8 weeks of age; 2 weeks later, BrMBA (10 to 200 nmol) or TPA (5 nmol) was applied in 0.2 ml of acetone to the shaved backs of mice twice weekly (on Days 1 and 4) for the duration of the experiment. There were at least 30 mice in each treatment group. Mice were weighed every other week. The tumor incidence was determined weekly, and the total number of papillomas appearing on each mouse was counted every other week of promotion treatment. The life history of individual papillomas was not recorded; hence, no data are available to reveal the rate of regression. The data reveal only the balance between the appearance and disappearance of papillomas in terms of the total number present at each counting.

Statistical Analysis of Tumor Data. The significance of the difference in the tumor multiplicity data was determined with the Wilcoxon rank sum test (9).

TPA Binding Assay. The binding of [3H]TPA to mouse epidermal cell membrane preparations was carried out in dichlorodimethylsilane-treated glass tubes using the cold-acetone (−78°C) filter assay as described by Ashendel and Boutwell (1), except that 2-mercaptoethanol was omitted from the buffers during epidermal cell membrane preparation and assay of [3H]TPA binding.

RESULTS

Tumor Promotion by BrMBA

In accord with previous findings, BrMBA was found to be a very weak complete carcinogen but a good tumor promoter (18). Thus, twice weekly applications of 100 nmol of BrMBA in 0.2 ml of acetone to the shaved backs of female CD-1 mice elicited only 0.12 papillomas per mouse in 11% of the mice at the 27th week of BrMBA treatment. In contrast, twice weekly applications of 100 nmol of BrMBA to DMBA-initiated skin resulted in 1.79 papillomas/mouse, and 57% of the mice had papillomas. In a separate experiment shown in Chart 1, twice weekly applications of 10, 100, or 200 nmol of BrMBA to DMBA-initiated skin resulted in 0, 1.6, and 2.5 papillomas/mouse, respectively, and 0, 44, and 60% of mice had papillomas at the 25th week of promotion treatment, respectively.

Comparison of Mouse Skin Tumor-promoting Ability of TPA and BrMBA

In this experiment (Chart 2), 0.2 μmol of DMBA was applied to mouse skin; 2 weeks later, either 5 nmol of TPA or 100 nmol of BrMBA were applied twice weekly as a promotion treatment. Tumor induction time for the appearance of first papilloma with promotion treatment with TPA was shorter than promotion treatment with BrMBA (9 weeks versus 13 weeks time to the first tumor). Furthermore, TPA treatment elicited more papillomas than BrMBA in spite of the fact that a 20 times larger dose of BrMBA than TPA was used; with TPA and BrMBA treatment, papillomas per mouse were 7.22 and 2.55, respectively.

Similarly, Scribner and Scribner (18) compared the mouse skin tumor-promoting ability of TPA and BrMBA. They used SENCAR mice in their experiments. They found that twice weekly applications of 90 nmol of BrMBA or 3.2 nmol (2 μg) of TPA to DMBA-initiated skin resulted in about 5 and 19 papillomas per mouse, respectively, at 5 and 9 weeks of promotion treatment. The available data present, by no means, a complete comparison of tumor-promoting ability of TPA and BrMBA. More recently (15), it has been shown that BrMBA, applied either once weekly or biweekly, does not affect the number of papillomas per mouse. Undoubtedly, more detailed dose and time effects of these 2 agents are mandatory for a complete comparison of their tumor-promoting ability.
Effect of Retinoic Acid, Dexamethasone, and 7,8-Benzoflavone on Tumor Promotion by BrMBA

Inhibition by Retinoic Acid and Dexamethasone of Formation of Skin Papillomas Promoted by BrMBA. As shown in Chart 3, application of either 17 nmol of retinoic acid or 76 nmol of dexamethasone 30 min before each twice-weekly application of 100 nmol of BrMBA to DMBA-initiated mouse skin inhibited skin papillomas per mouse by 73 and 100%, respectively, at the 27th week of BrMBA treatment. The number of papillomas per mouse in acetone- and retinoic acid-pretreated groups at the 27th week of BrMBA treatment was 1.8 ± 0.5 (S.E.) and 0.48 ± 0.2 (S.E.), respectively; the values are significantly (p < 0.016) different. In a separate, repeat experiment (data not shown), retinoic acid or dexamethasone pretreatment inhibited the formation of skin papillomas to a similar degree as in the experiment shown in Chart 3.

Effect of 7,8-Benzoflavone on Skin Tumor Promotion by BrMBA. As shown in Table 1, application of 367 nmol of 7,8-benzoflavone 30 min before each twice weekly application of 100 nmol of BrMBA did not inhibit tumor formation. In the same experiments, retinoic acid and dexamethasone pretreatments inhibited formation of skin papillomas promoted with BrMBA (Table 1). 7,8-Benzoflavone appears to enhance tumor formation promoted with BrMBA, but the difference between the acetone control and the 7,8-benzoflavone-treated group was not statistically significant (p > 0.1). Similarly, in a repeat experiment, 7,8-benzoflavone did not potentiate significantly tumor promotion by BrMBA (data not shown).

Induction of ODC by BrMBA

Since one of the properties ascribed to tumor promoters is the ability to induce ODC activity, we determined the effect of BrMBA applied to mouse skin on epidermal ODC activity. The results are shown in Chart 4. Application of 100 nmol of BrMBA to mouse skin led to a dramatic increase in epidermal ODC activity (about 21-fold above the acetone control level) between 8 and 18 hr, and the enzyme activity remained elevated even 36 hr following BrMBA treatment. Peak ODC activity was observed about 12 hr after BrMBA application, but for the sake of convenience, in the subsequent experiments, ODC induction was determined 5 or 6 hr after application of BrMBA.

As shown in Chart 4, inset, the induction of ODC activity was dependent on the dose of BrMBA. About a 7-fold increase in epidermal ODC activity was observed with a single application of 20 nmol of BrMBA, and application of 50, 100, 200, and 400 nmol of BrMBA resulted in 11-, 16-, 14-, and 17-fold increases in ODC activity, respectively, 6 hr following BrMBA treatment.

Effects of Retinoic Acid, Dexamethasone, and 7,8-Benzoflavone on ODC Induction by BrMBA

As shown in Table 2, application of retinoic acid (1.7 or 17 nmol) or dexamethasone (76 nmol) 1 hr prior to application of
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Table 2
Effect of retinoic acid, dexamethasone, and 7,8-benzoflavone on the induction of ODC activity by BrMBA

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose (nmol)</th>
<th>ODC activity (nmol CO2/60 min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td></td>
<td>0.45 ± 0.09*</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>1.7</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>17.0</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>0.64 ± 0.19</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>17.0</td>
<td>0.15 ± 0.04</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>76.0</td>
<td>0.05 ± 0.00</td>
</tr>
<tr>
<td>Acetone</td>
<td>367</td>
<td>0.49 ± 0.17</td>
</tr>
</tbody>
</table>

* Mean ± S.E. of determinations carried out on preparations made from 3 groups of 3 mice each.

Table 4
BrMBA failure to inhibit [3H]TPA specific binding to mouse epidermal cell membranes

<table>
<thead>
<tr>
<th>Addition</th>
<th>Concentration (w)</th>
<th>[3H]TPA bound (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>15,287 ± 570*</td>
</tr>
<tr>
<td>BrMBA</td>
<td>10-8</td>
<td>14,988 ± 304</td>
</tr>
<tr>
<td>BrMBA</td>
<td>10-7</td>
<td>15,982 ± 495</td>
</tr>
<tr>
<td>BrMBA</td>
<td>10-6</td>
<td>16,462 ± 473</td>
</tr>
<tr>
<td>BrMBA</td>
<td>10-5</td>
<td>15,723 ± 969</td>
</tr>
<tr>
<td>TPA</td>
<td>10-6</td>
<td>512 ± 33</td>
</tr>
<tr>
<td>TPA</td>
<td>10-7</td>
<td>524 ± 27</td>
</tr>
<tr>
<td>TPA</td>
<td>10-8</td>
<td>1043 ± 48</td>
</tr>
<tr>
<td>TPA</td>
<td>10-9</td>
<td>4,243 ± 140</td>
</tr>
<tr>
<td>TPA</td>
<td>10-10</td>
<td>12,837 ± 565</td>
</tr>
</tbody>
</table>

* Mean ± S.E. of determinations carried out from quadruplicate assays.

Treatment of Mouse Skin with BrMBA Did Not Inhibit ODC Induction by TPA

In this experiment, groups of mice were treated with 100 nmol of BrMBA or 2 nmol of TPA or simultaneously with 100 nmol of BrMBA and 2 nmol of TPA; ODC activity was determined 6 hr after treatment. As shown in Table 3, concurrent application of BrMBA with TPA did not inhibit the induction of ODC activity by TPA but potentiated significantly (p < 0.01) TPA-induced ODC activity.

BrMBA Does Not Compete for the Binding of [3H]TPA to Mouse Epidermal Particulate Fraction. The inhibition of [3H]TPA binding to the preparations of mouse epidermal cell membranes by various concentrations of TPA and BrMBA is shown in Table 4. As small a TPA concentration as 10 nm was sufficient to reduce the binding below 50%. In contrast, 10 μM BrMBA did not inhibit the binding of [3H]TPA.

DISCUSSION

Mouse skin provides a useful system to study the mechanism of carcinogenesis (2, 3, 17), and by the use of modifiers of carcinogenesis as well as the use of different agents for the induction of tumors, clues about the mechanism of the carcinogenic process are revealed (16, 20, 27, 29). Thus, we report that retinoic acid, at doses which failed to inhibit ODC induction and tumor formation by DMBA (25, 27), inhibited both ODC induction and tumor promotion by BrMBA (a structural analogue of DMBA). Dexamethasone inhibited ODC induction and tumor promotion by BrMBA as well. 7,8-Benzoflavone, which inhibits ODC induction and tumor formation by DMBA (25, 27), failed to inhibit ODC induction and tumor promotion by BrMBA.

Results reported earlier (18) and now presented here indicate that the mechanisms of tumor promotion by TPA, DMBA, and BrMBA are complex and different. The lack of inhibition by 7,8-benzoflavone of ODC induction and tumor promotion by BrMBA indicates that metabolic activation of BrMBA may not be required for these effects. The differential effects of retinoic acid on ODC induction and tumor formation by BrMBA and DMBA suggest that the pathways that lead to tumor formation by these polycyclic aromatic hydrocarbon congeners are not identical.

Retinoic acid inhibited both ODC induction and tumor promotion by TPA (22, 29) as well as by BrMBA (Chart 3). This suggests that there may be a common pathway for TPA and BrMBA for tumor induction and tumor promotion. However, the inability of BrMBA to compete for the specific binding of [3H]TPA to the epidermal cell membranes may argue against a completely common pathway for the promotion of tumor formation.

The mechanisms of tumor promotion by BrMBA and TPA remain speculative. It has been shown that TPA binds specifically and with high affinity to the plasma membrane (1, 8) and to a lesser extent to other cellular components, such as the nucleus (12). It is assumed that the signal(s) for gene expression that triggers tumor promotion originates from the interaction of TPA with plasma membrane receptors. In the case of BrMBA, it is known that BrMBA strongly binds to nuclear DNA, although this binding is not correlated to its weak tumor-initiating ability. Whether the binding of BrMBA to DNA-associated proteins (which may influence gene expression) is critical for its tumor-promoting ability remains to be determined. Since ODC induction by both of these tumor-promoting agents, TPA and BrMBA, was inhibited by retinoic acid pretreatment, it is likely that retinoic acid may inhibit ODC induction by direct influence on the induction of gene expression by these tumor promoters.

ODC induction is one of the properties of tumor promoters (11, 20, 29). The results presented herein strengthen the concept that the mechanism by which retinoic acid inhibits tumor promotion is by its ability to inhibit ODC induction. Thus, retinoic acid inhibits ODC induction by TPA and BrMBA and also inhibits...
tumor promotion; retinoic acid does not inhibit ODC induction by DMBA and does not inhibit tumor formation (25, 27).

It is concluded that there may not be a common biochemical pathway that leads to tumor formation by TPA, BrMBA, and DMBA. The data show that it may not be correct to expect a general inhibitory effect of a modifier of carcinogenesis on tumor formation by diverse carcinogens. The use of combinations of drugs may be a better approach in the chemoprevention of cancer (26, 30). Combinations of inhibitors may show enhanced inhibition at levels below the threshold for undesirable side effects of each when used singly.

REFERENCES


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