Inhibition of Two-Stage Carcinogenesis in Mouse Skin with Bis(2-chloroethyl)sulfide

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SUMMARY

ICR/Ha Swiss mice, 30 females/group, were given a single application of 20 μg 7,12-dimethylbenz[a]anthracene, followed 2 weeks later by three times weekly applications of phorbol myristate acetate (PMA), 2.5 μg/application. In the accompanying inhibition experiments, sulfur mustard [SM; bis(2-chloroethyl)sulfide] was applied at a dose of 20 μg/application during the promotion phase, either on the same day as PMA, i.e., three times weekly, or on alternate days, i.e., twice weekly. The experiments were continued for 385 days. The control group, i.e., without SM, resulted in 27 of 30 mice with a total of 281 papillomas. Sixteen of these mice also bore squamous cell carcinomas. In the two groups also receiving SM, two or three times weekly, 2 of 30 and 1 of 30 mice, respectively, bore papillomas. Carcinomas were not observed in these groups. The inflammatory response of mouse skin to the combined treatments of SM and PMA was no greater than with PMA alone.

INTRODUCTION

Several reports have appeared describing the partial inhibition of 2-stage carcinogenesis on mouse skin using a variety of chemical agents including actinomycin D, acridine orange, diepoxybutane, and aflatoxin B, (5, 8). These studies have been reviewed (7).

Berenblum (1–3) reported on the inhibitory effect of SM on coal tar-induced mouse skin carcinogenesis. In view of the almost complete inhibition observed in the latter experiment, the efficacy of a low dose of SM in the inhibition of 2-stage carcinogenesis on mouse skin was examined in the present study.

MATERIALS AND METHODS

Animals. Female ICR/Ha Swiss mice (ARS/Sprague-Dawley, Madison, Wis.) were vaccinated against ectromelia, and treatments were begun when they were 6 to 8 weeks old. The mice were housed on sterile hardwood chips (Iso-Dry; Fisher & Son, Bound Brook, N. J.) in stainless steel cages, fed Purina laboratory chow and water ad libitum, and weighed monthly. The animal rooms were maintained at 22–24°C. There were 30 mice to a group. All treatments were continued for 385 days when all surviving animals were killed. Animals were weighed and examined regularly and scored, and the findings were charted once monthly. Animals in poor health or with large tumor masses were killed. Except for the cranial region, animals were completely autopsied at the end of the experiment or at death. Necropsies were performed on all animals, and samples of all abnormal-appearing tissues and organs were excised for histopathological diagnosis. All tissue sections were fixed in 10% formalin, processed, blocked in paraffin, and stained with hematoxylin and eosin for histopathological examination. Included in the protocols were groups given vehicle only and groups receiving no treatment.

Bioassay Methods. The dorsal skin of the mice was shaved initially and when necessary throughout the test. All compounds were applied in 100 μl acetone in the interscapular region with a micropipet (Biopette; Carworth, Inc., New York, N. Y.). Skin lesions were diagnosed as papillomas when they reached 1 mm and persisted for 30 days or more. Dosages and complete protocols are given under "Results."

Chemicals. 7,12-Dimethylbenz[a]anthracene was recrystallized from acetone immediately before use. PMA was isolated from croton oil and purified as described before (9). Chemically pure SM was used (K & K Laboratories, Plainview, N. Y.).

RESULTS

Effect of SM and PMA on Mouse Skin. The response of mouse skin to PMA application over a 35-day period has been described (9). In the present experiments, tissue samples were taken from mice treated with SM and/or PMA over a 7-day period. The histological results were essentially identical to those reported earlier for PMA alone (9). Thus treatment of mouse skin with SM and PMA in combination neither diminished nor enhanced the inflammatory response of mouse skin to PMA or SM alone.

Long-Term Bioassays. The results of the long-term experiments are presented in Table 1. In the group that received 7,12-dimethylbenz[a]anthracene and PMA only, 27 of 30 animals developed papillomas and 16 of these animals also bore squamous carcinomas of the skin. The 2 groups that also received SM treatment resulted in 1 of 30 and 2 of 30 mice each with 1 papilloma and no carcinomas. The control groups that received no treatment showed the same results as that received only the single agents showed low incidences of papillomas and no carcinomas. Thus SM, at the low dose used, caused essentially complete inhibition of tumor induction.

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2 The abbreviations used are: SM, sulfur mustard [bis(2-chloroethyl)sulfide]; PMA, phorbol myristate acetate.

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**Table 1**

Mouse skin tumor inhibition by SM

<table>
<thead>
<tr>
<th>Primary treatment initiator</th>
<th>Secondary treatment</th>
<th>Days to 1st tumor from secondary treatment</th>
<th>No. of mice with tumors/total no. of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMBA</td>
<td>PMA</td>
<td>90</td>
<td>1/1</td>
</tr>
<tr>
<td>DMBA</td>
<td>SM*</td>
<td>209</td>
<td>2/2</td>
</tr>
<tr>
<td>None</td>
<td>PMA</td>
<td>40</td>
<td>27/281*</td>
</tr>
<tr>
<td>DMBA</td>
<td>None</td>
<td>218</td>
<td>4/7</td>
</tr>
<tr>
<td>None</td>
<td>SM*</td>
<td>385</td>
<td>1/1</td>
</tr>
<tr>
<td>DMBA</td>
<td>None</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>323</td>
<td>1/1</td>
</tr>
<tr>
<td>DMBA</td>
<td>Acetone</td>
<td>219</td>
<td>1/1</td>
</tr>
<tr>
<td>None</td>
<td>Acetone</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>No treatment</td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

* DMBA, 20 μg in 100 μl of acetone, only.
* PMA, 2.5 μg in 100 μl of acetone, 3 times/week (Monday, Wednesday, Friday) beginning 14 days after treatment with DMBA.
* Squamous cell papillomas.
* DMBA, 7, 12-Dimethylnaphtanthracene.
* SM, 20 μg in 100 μl of acetone, 2 times/week (Tuesday, Thursday) beginning 14 days after treatment with DMBA.
* SM, 20 μg in 100 μl of acetone, 3 times/week (Monday, Wednesday, Friday) following PMA application by 0.5 hr, beginning 14 days after treatment with DMBA.
* Sixteen mice with squamous cell carcinomas. The median survival time in this group was 358 days from secondary treatment.
* SM, 20 μg in 100 μl of acetone, 2 times/week (Tuesday, Thursday) beginning 14 days after treatment with DMBA.
* One mouse with a spindle cell sarcoma in the treatment area.
* SM, 20 μg in 100 μl of acetone, 3 times/week (Monday, Wednesday, Friday) beginning 14 days after treatment with DMBA.

DISCUSSION

SM is known to have a variety of biological effects and these have been reviewed (10). Two of these effects are possibly relevant to the present findings; one is the cytotoxicity of SM and the other is its ability to cause intrastrand and possibly also interstrand cross-links in DNA (10).

In this work, a low dose of SM was used on mouse skin in order to minimize cytotoxicity as revealed by light microscopy. However, cytotoxic effects cannot be eliminated as a possible explanation for the observed inhibition of 2-stage carcinogenesis. Berenblum (3) has pointed out that several compounds related to SM, e.g., the corresponding sulfone, is a moderate irritant which does not inhibit coal tar carcinogenesis on mouse skin at a concentration of 0.1%. Other inhibitory agents, however, e.g., actinomycin D, usually exhibit some degree of cytotoxicity; this property is, of course, also observed for chemical carcinogens.

The inflammatory response of mouse skin to combined application of SM and PMA was found to be essentially identical to the application of PMA by itself. Thus, if SM were causing essentially complete inhibition of 2-stage carcinogenesis in mouse skin due to a toxic effect on initiated cells, a very selective and subtle type of cytotoxicity must be involved.

Other explanations for the inhibitory activity of SM include its ability to cross-link DNA (10) and to alter the permeability and physical characteristics of cell membranes (4). It has been suggested that PMA exerts its promoting activity by interaction with the cell membrane (6), and experimental evidence for this type of interaction has been obtained (11). It is therefore possible that SM interferes with this binding of PMA to cell membranes.

ACKNOWLEDGMENTS

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