Photocarcinogenesis Promotion Studies With Benzoyl Peroxide (BPO) and Croton Oil

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Previous studies demonstrated that BPO can promote chemically initiated tumor formation in SENCAR mice. In addition, a number of chemicals have been shown to promote and/or enhance UVR induced carcinogenesis. This study examined the effect of BPO on UVR initiated tumor formation.

One hundred and forty-eight Uscd mice received 270 mJ/cm² of UVB radiation to the posterior halves of their backs 3 times a week for 8 weeks. Four weeks later the mice were divided into 4 groups. Group I received croton oil in acetone applications to the back 5 times a week for the duration of the study. Group II received acetone, Group III received the BPO diluent, and Group IV received the BPO in an aqueous diluent applications as in Group I.

One mouse in Group II (acetone) and one in Group IV (BPO) developed tumors in unirradiated skin. In the UVR initiated skin 38% of the survivors developed tumors in Group I (croton oil), whereas 5% did in Group II (acetone), 8% in Group III (BPO base), and 8% group IV (BPO).

Thus under the circumstances of this study croton oil did promote UV initiated tumor formation but BPO did not. These results are consistent with those recently reported by Iversen. J Invest Dermatol 91:114–116, 1988

Benzoyl peroxide (dibenzoyl peroxide) (BPO) is an active oxidizing agent that is used as a prescription and over-the-counter drug in the treatment of acne [1,2] and it has been approved by the U.S. Food and Drug Administration for use as a direct and indirect food additive, such as a bleaching agent for flour [3].

In 1981 Slaga et al [4] first reported that BPO could promote skin tumors in SENCAR mice previously initiated with the potent chemical carcinogen 7,12-dimethylbenz(a)anthracene (DMBA). The drug, when applied without prior chemical initiation, did not act as a complete carcinogen or initiating agent. Iversen [5] recently reported that BPO in the form of the commercial product Panoxyl, containing 5% BPO, did not enhance skin tumorigenesis in hairless mice (hr/hr, Oslo) when the drug was given prior to or following ultraviolet radiation (UVR) exposure.

The current experiment was done to determine the mouse-skin tumor-promoting potential of BPO using UVR as an initiator. UV was chosen as the initiating agent because it is the primary cause of nonmelanoma skin cancer in man. In addition, it produces squamous cell carcinomas regularly in our in vivo experimental mouse-skin system [6].

MATERIALS AND METHODS

Experimental animals In this study 148 random-bred, 3- to 4-month-old Uscd (Hr) stock albino hairless mice were housed in metal cages and fed unrestricted quantities of Wayne Lab Blox and water.

Radiation source A Hanovia air-cooled hot quartz contact lamp emitting 54 mJ/cm²/sec of UVB energy at a distance of 3.4 cm was used as the light source.

Chemicals Croton oil was obtained from Sigma Chemical Co. (St. Louis, MO). Benzoyl peroxide was prepared as a 5% lotion in an aqueous vehicle.

Procedure The animals received 270 mJ/cm² of UVB energy to the posterior half of the back 3 times a week for 8 weeks. Four weeks later the mice were divided into 4 groups. Treatment consisted of the following: Group I received 0.1 cc of a 0.1% solution of croton oil in acetone to the entire back 5 times a week for the duration of the study; Group II received acetone applications (0.1 cc); Group III received BPO vehicle (0.1 cc); and Group IV received BPO lotion to the back as in Group I. Tumors greater than 4 mm were recorded.

Statistics The analyses used a computerized Life Table program. The method of comparison was Fisher’s exact test and a standard Chi square analysis for a 2 × 2 table. In addition, because times to tumor were recorded, Kaplan-Meier Survival curves were produced and tests were made to determine the equality of the survival curves. The tests used were the Cox Test and the Gehan-Breslow W statistic.

RESULTS

Tumor Onset

Anterior (nonirradiated) skin sites: For the anterior half of the back, which did not receive UV irradiation, papillomas appeared on one mouse of Group II (acetone) on week 54 and one mouse of Group IV (BPO) on week 61. Papillomas did not appear on the anterior half of the back in any of the other groups of treated animals.

Posterior (irradiated) skin sites: For the posterior half of the back, which received UV irradiation for 8 weeks prior to topical treat-
ments, skin papillomas appeared in all treated groups. Tumor onset occurred on week 39 for Group I (croton oil), week 19 for Group II (acetone), week 18 for Group III (BPO vehicle), and week 45 for Group IV (BPO). For Group I (croton oil) a total of 9 tumor-bearing animals was obtained by the end of the study. The median time for tumor appearance was 54 weeks, and 4 of the 9 tumor-bearing animals were first observed to have tumors on week 61. 1 week prior to the end of the experiment.

Tumor Incidence

Anterior (nonirradiated) skin sites: Only 2 animals developed tumors on nonirradiated skin sites; one in Group II (acetone) for an overall incidence of 1/29 or 3.4%, and one in Group III (BPO) for an overall incidence of 1/38 or 2.6% (Table I). These data and those from the other treated groups show that croton oil, BPO, and the bases were not carcinogenic to the nonirradiated skin of this strain of hairless mouse.

Posterior (irradiated) skin sites: Tumor incidence rate data show that by week 45 there were 4 tumor-bearing animals in Group I (croton oil), and for this group the number of tumor-bearing animals increased over the course of the experiment to a total of 9 by week 62 (Table I). In the other treatment groups, the number of tumor-bearing animals on week 45 ranged from 1 to 2, and this number did not increase over the remainder of the experiment (Table I). Only one tumor occurred in any of the tumor-bearing animals. A statistical analysis of tumor incidence data for week 62 showed a significant difference between Group I (UV + croton oil) and other groups (Table II), but tumor incidences for Groups II, III, and IV were not significantly different from each other (Table II). Therefore, data showed that BPO and the diluents did not act as skin tumor promoters under conditions in which croton oil promoted skin tumors following initiation by UVR.

DISCUSSION

The effects of BPO on experimental cutaneous carcinogenesis have received a great deal of attention since Slaga and co-workers reported that it and, subsequently, laurel peroxide (LP) in acetone were efficient promoters of DMBA-initiated tumor formation [4,7].

Table II. Statistical analysis of tumor incidence dataa

<table>
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<th>Fisher</th>
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<th>COX</th>
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<td>IV (BPO) vs III (BP Base)</td>
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<td>II (Acetone) vs IV (BPO)</td>
<td>&lt; .7</td>
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<td>II (Acetone) vs III (BP Base)</td>
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<td>II (Acetone) vs I (CO) &amp; II (Acetone) vs I (CO) vs III (BP Base)</td>
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<td>I (CO) vs IV (BPO)</td>
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<td>I (CO) vs III (BP Base)</td>
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* Analysis done on 62 weeks data. See Table I for incidence data and the “Materials and Methods” section for description of procedures.

No initiating or full carcinogenic effects were noted for either BPO or LP, despite their use in the carcinogen sensitive SENCAR mouse strain. Though the mechanism of this promoting effect has not been established it has been suggested that because LP and BPO are not detoxified by cellular systems such as glutathione peroxidase, free radicals generated by these agents may cause membrane peroxidation which may induce epidermal hyperplasia. Also, the free radicals may cause DNA damage which might be part of the tumor promotion mechanisms [9]. Because BPO is used commonly in the treatment of acne these findings presented some concern.

However, exposure of human skin to comparable amounts of the chemical carcinogens used experimentally does not occur. In contrast, the primary environmental carcinogen in humans is ultraviolet B (UVB) radiation from the sun [8]. It should be noted that UV radiation as well as ionizing radiation, polycyclic hydrocarbons, and nitrogenous carcinogens generate free radical formation [13]. In short, a study was designed to examine the effect of a 5% concentration of BPO in an aqueous vehicle more closely simulating clinical use circumstances. The hairless mouse used strain was used as the test animals because of their responsiveness to UVB initiation and promotion by other chemicals [8, 10–12]. The results indicate that the 5% BPO solution did not promote photocarcinogenesis in this study. In contrast, croton oil did promote tumor formation in this system indicating that an adequate amount of UV irradiation was administered to initiate carcinogenesis prior to starting the promotion applications. Subsequently, we compared the effects of chronic applications of croton oil and the 5% BPO solution on epidermal premalignant semisolid DNA synthesis [14]. These studies revealed that topical applications of croton oil for 53 weeks had a stimulating effect on epidermal cell activity which was not seen in the BPO treated mice. This effect was much more notable in mice that had been initiated with UV radiation. The effect on DNA synthesis may reflect the promotion and lack of promotion noted in the present study.

Iversen, using the hr/hr Oslo strain of hairless mice, found that 5% BPO in a gel base and the gel base alone enhanced DMBA-initiated tumorigenicity [5]. However, both the gel and the BPO applied just before UV irradiation had an apparent protective effect against photocarcinogenesis. When applied after 3 weeks of UV irradiation in a promotion type of study both the BPO and the gel base had no effect. In subsequent studies Iversen found that neither the gel nor the 5% BPO applied the day before or just after the irradiation altered tumor formation. Thus the apparent inhibition noted earlier may have been due to a screening effect. (Iversen, personal communication).

Thus our results and those of Iversen support the concept that BPO does not promote or augment UV photocarcinogenesis under the circumstances of these studies. Findings in animal or in vitro experiments certainly cannot be transposed to human responses. However, because UVR is the primary cause of human skin cancer and the concentration and bases of the applied chemical were identical or similar to those used for acne therapy it would seem that
these results were more closely related to the human experience than the DMBA initiation and BPO promotion findings.

REFERENCES

The Seventh Postgraduate Course in Medical Mycology (Dermatomycology)

The Seventh Postgraduate Course and Workshop in Medical Mycology, to be held on the Campus of The University of California School of Medicine, San Francisco, October 14 – 16, 1988, is designed for dermatologists, clinical pathologists, mycologists, pediatricians, nurses, medical technologists, clinical microbiologists, and other paramedical personnel. Lectures will emphasize clinical, immunologic, pathologic, and epidemiologic aspects of the various mycoses. An AIDS update will be included. Laboratory Sessions, supervised by the faculty, will stress self-assessment and simplified approaches to office dermatology. All presentations will be illustrated and accompanied by cultural and microscopic demonstrations. Special guest faculty join the faculty at UCSF to provide this program. Much of the information presented will be geared to those preparing for specialty boards, but the material will also be useful to those desiring to broaden their concepts of infectious diseases. For further information contact: Extended Programs in Medical Education, Room 569-U, University of California, San Francisco, CA 94143. Telephone: (415) 476-4251.