ABSTRACT

The effects of several dietary supplements of antioxidants and enzyme inducers on ultraviolet light-mediated carcinogenesis were investigated. Glutathione (reduced) was without effect, but butylated hydroxytoluene, phenobarbital, and disulfiram all significantly suppressed the initiation and development of actinic lesions and tumors. On the basis of the present study and related previous ones, tumor inhibition appears to be due not to an umbra-gous effect but rather to the induction of systemic physiological responses.

INTRODUCTION

A considerable literature is accruing that attests to the moderating effects of dietary supplements of antioxidants on carcinogenesis. Compounds with antioxidant properties and affecting carcinogenesis include the various thios and sulfhydryl-generating compounds (8, 17, 24, 25, 36, 37), tocopherol (16, 18, 23), selenium (14, 19, 21, 22, 28), various phenols and quinolines (31, 33, 34), and other aromatic compounds (4, 26, 30). These compounds have been found to be effective against a wide spectrum of chemical carcinogens. Individually, however, their action may be specific for a particular species of chemical. In 1974 a mixture of compounds with antioxidant properties was shown to suppress UV-induced carcinogenesis (2, 3). The mixture included ascorbic acid, reduced glutathione, dl-alpha-tocopherol, and BHT. Although all 4 compounds demonstrated the ability to reverse the cytotoxicity of UV to cells in culture (6) only 1 was effective in altering in vivo responses to acute UV insult (11). BHT was found to be as effective as was the complete antioxidant mixture in increasing the minimal erythema dose of UV in hairless mice. These studies were undertaken to determine whether selected individual constituents of the previous dietary mixture, as well as other known moderators of chemically induced cancer, were effective in suppressing UV carcinogenesis.

MATERIALS AND METHODS

One hundred fifty female, hairless mice (SKH: hairless-1 stock; Skin and Cancer Animal Colony, Philadelphia, Pa.) were maintained for the study. The animals were divided into 5 groups of 30 each. The control group received a regular, balanced, laboratory meal (Wayne Lab-Blox; Allied Mills, Chicago, Ill.). Group 2 received the regular meal supplemented with 0.05% (w/w) phenobarbital; Group 3 received the regular meal with disulfiram added at 2 mg/g meal; Group 4 received the regular meal with 0.5% (w/w) BHT; and Group 5 received the regular meal with 0.1% (w/w) reduced glutathione. All groups were fed ad libitum and received the respective diet 10 days before initial irradiation. The animals were housed under a 12-hr dark-light schedule at 21-23°C. Mean body weights were determined monthly. The animals that received disulfiram supplement (Group 3) experienced weight loss and appeared generally unthrifty by Week 12. The animals were transferred to regular meal at the end of Week 17, and by Week 24 they had regained their normal weight. Other groups were maintained on their respective diets for the duration of the study. Animals surviving at the end of the experimental period were 29, 29, 27, 28, and 30 for Groups 1 to 5, respectively.

Animals received daily (5 days/week) irradiation from a General Electric UA-3 mercury arc lamp (General Electric Lamp Division, Cleveland, Ohio) with principal emission lines at 254, 265, 280, 297, 302, 313, and 365 nm. An initial suberythemic dose of 0.84 J/sq cm/day was delivered. The dose was increased every 2 weeks by 0.34 J/sq cm until a daily dose of 2.18 J/sq cm was attained. This level of irradiation was maintained through the 17th week at which time irradiation was discontinued. An accumulative dose of 152 J/sq cm was administered.

Animals were examined at weekly intervals to evaluate actinic effects. Elevated lesions 1 mm in diameter were considered as the biological end point in evaluation. Biopsies were taken for histopathological examination. Actinic lesions are defined as those lesions histologically interpreted as actinic keratoses. Tumors are defined as papillomas and squamous cell carcinomas.

RESULTS

Comparisons of the various dietary groups, with regard to numbers of animals bearing actinic lesions (keratoses) and tumors, were based on $\chi^2$ statistics in contingency table analysis. All 5 dietary groups were simultaneously compared to determine differences in percentages of response among the groups. Where overall differences were significant ($p < 0.05$), pairwise comparisons were made between groups to detect the source of those differences. Significant differences among the groups first occurred at Week 15. Two general types of response occur with control and glutathione representing the groups with the highest...
percentages of lesions and tumors (Chart 1A). Phenobarbital, BHT, and disulfiram all showed significant protection against UV-mediated effects. The only consistently significant pairwise comparisons of treatment groups were those between glutathione and the phenobarbital, BHT, and disulfiram groups beginning at Week 21.

Similar statistical comparisons were made between the various dietary groups for animals bearing tumors. The overall group effects separate into 2 general responses as shown in Chart 1B. Significant overall differences began to emerge at Week 20. Significant pairwise comparisons also emerged at Week 20 between control and BHT, phenobarbital, and disulfiram and between glutathione and those 3 additives. Although the data would indicate that disulfiram offers the most protection against UV carcinogenesis, it was never significantly better than was phenobarbital or BHT.

The Kruskal-Wallis test, a nonparametric procedure, was used to evaluate overall group differences in the number of tumors per animal. Pairwise comparisons were made with the Wilcoxon rank sum test. In Table 1 the groups separate into the same 2 response types as seen previously for other parameters.

It appears from evaluation of all 3 parameters, i.e., per-

- Chart 1. Plot of percentage of incidence versus time for (A) lesions and tumors and (B) tumors. •, glutathione; O, control; x, BHT; Δ, phenobarbital; A, disulfiram.

### Table 1

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- C, control; P, phenobarbital; G, glutathione; B, BHT; D, disulfiram.
- $p < 0.01$.
- $p < 0.05$.
- $p < 0.001$.

Fig. 1. Effects of dietary antioxidants on severity of actinic tumors. Top row, animals maintained on control diet (Group 1); bottom row, animals fed disulfiram-supplemented diet (Group 3). Animals with the most severe lesions in both groups were selected. Photograph taken at 30 weeks.
percentage of animals bearing lesions and tumors, percentage of animals bearing tumors, and numbers of tumors per animal, that disulfiram provided the greatest protection in all categories. This is further demonstrated in Fig. 1 in which animals bearing the most severe lesions from Groups 1 and 3 are compared.

DISCUSSION

The present study demonstrates that phenobarbital, BHT, and disulfiram, when added to the diet, provide significant systemic protection against UV carcinogenesis. Although information regarding the mode of action of these compounds is lacking, several interesting possibilities become apparent. First, the constituents in the mixture of antioxidants used in previous studies, as well as in the present study, all absorb strongly in the UV portion of the spectrum. Accumulation of any of these constituents in the target tissue could provide an umbrageous effect by absorption of photic energy and thereby diminish the effective carcinogenic dose of UV. Such an effect, however, seems unlikely for several reasons. The levels of water-soluble antioxidants in the skin of animals that receive the special diet increase over control (regular diet) levels by about 50% after 2 weeks of feeding (7), a time comparable to initiation of UV treatment in this study, and then recede by the third or fourth week to levels only about 10% above controls. Thus water-soluble antioxidant levels in skin would be minimal during the course of the current experiment. Ascorbic acid and glutathione, the 2 water-soluble constituents of the special diet, have no effect on UV-mediated erythema and, as shown here, glutathione is without effect on UV carcinogenesis. Of the fat-soluble antioxidants previously used in mixture to suppress UV carcinogenesis, only BHT provided systemic protection against UV-mediated erythema. Whereas some controversy surrounds the relationship of erythema to carcinogenesis (13, 32) and drugs such as indomethacin have reduced the erythemal response after UV insult without inhibiting other parameters of epidermal damage (29), in this study BHT provides significant protection against UV carcinogenesis. Others have shown that this compound is rapidly metabolized in the rodent, that minimal levels accumulate in the skin, and that accumulation is not progressive upon continued ingestion (10, 20). Based upon these accumulation data and upon the molar extinction coefficient, rough calculations suggest that absorption by BHT in skin would be negligible and they argue against an umbrageous effect.

If absorptive capacity, and thus reduction of the carcinogenic dose of UV, is minimal, then the anticancer action of the 3 effective agents could result from 1 of the defense mechanisms recently reviewed by Apffel (1). Induction of microsomal enzymes would be 1 system of surveillance and containment that all 3 agents would probably affect. A number of studies have shown that prevention of chemical carcinogenesis is preceded or accompanied by the induction of microsomal mixed-function oxidase activity (9, 35, 38). Previous studies by other investigators have shown that phenobarbital, BHT, and disulfiram all demonstrate anticancer properties against certain chemical carcinogens (15, 27, 31, 33, 34, 36, 37, 39) and that phenobarbital and BHT are both known to induce mixed-function oxidases. Although disulfiram has been reported to block the binding of specific carcinogens to potential target molecules, there is also evidence of its effect upon chemical carcinogen metabolism (5, 12).

If a defense mechanism of this nature were involved for carcinogenic agents as diverse as chemicals and UV, then the aspects of common oncological mechanism(s) and/or pathways arise. This would necessitate consideration of the involvement of chemical intermediates (i.e., carcinogenic photoproducts) in the etiology of UV carcinogenesis. The systemic physiological responses evoked by agents such as phenobarbital and BHT suggest the potential of extracutaneous metabolic involvement in cutaneous carcinogenesis. Whereas these considerations must await further investigation, it is clear that certain antioxidants and enzyme inducers have a pronounced effect upon UV carcinogenesis.

ACKNOWLEDGMENTS

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REFERENCES


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Effects of Antioxidants on UV Carcinogenesis

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