Stimulation of Ultraviolet-induced Carcinogenesis by 1,3-Bis(2-chloroethyl)-1-nitrosourea

John H. Epstein

Department of Dermatology, University of California School of Medicine, San Francisco, California 94143

ABSTRACT

The influence of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) on ultraviolet-induced carcinogenesis was examined in hairless mouse skin in vivo. Noncarcinogenic amounts of topically applied BCNU and carcinogenic levels of UVB energy were utilized in the study. The applications of BCNU significantly accelerated the appearance and growth of the cutaneous tumors in this study. Thus, the BCNU acted as either a promoting or a cocarcinogenic agent for ultraviolet-induced cancer formation. Although the mechanism of this effect has not been established, avoidance of extensive sun exposure would probably be prudent when topical BCNU is being utilized therapeutically.

INTRODUCTION

The nitrosourea compounds are a relatively new group of alkylating agents which have shown efficacy in the treatment of certain human cancers and experimental tumors (15). Recently, certain of these chemicals have proven to be topically therapeutically effective in mycosis fungoides (17) and the benign disease psoriasis (14).

In contrast, nitrosourea compounds have also produced cancers under experimental conditions in a variety of organs ranging from the liver to the skin (8, 10, 11).

A recent study indicated that topically applied BCNU (carmustine) was effective in the treatment of mycosis fungoides (18). Since it is an effective topical antimitotic agent, it might well prove useful in the treatment of common epidermal proliferative diseases such as psoriasis. The present study was designed to evaluate the potential carcinogenic or anticarcinogenic effect of BCNU on UV energy-induced cutaneous squamous cell carcinoma formation. UV was chosen as the carcinogenic stimulus because it regularly produces cancers in our experimental system and it is primarily responsible for human skin cancers (5). In addition, UV energy is commonly used in the treatment of psoriasis.

MATERIALS AND METHODS

Experimental Animals. In this study, 145 random-bred 3- to 4-month-old female Uscd:(HR) hairless mice were housed in metal cages and fed on unrestricted quantities of Wayne Lab Blox and water. Visible light exposure was minimal except during treatment and examination procedures.

Light Source. A Hanovia air-cooled hot quartz contact lamp produced UVB energy, 0.25 × 10³ mJ/sq cm/sec (280 to 320 nm) at a distance of 3.4 cm. A Hanovia UV meter (Model AV-971) was used to measure the applied energy.

Chemical. A solution of BCNU dissolved in acetone at a concentration of 5 mg/ml was prepared freshly before application. The BCNU solution and the solvent were applied to the posterior half of the backs of the mice with a 1-ml syringe.

Procedure. The mice were divided into 4 groups. Group 1 (40 mice) received a single weekly application of 0.5 mg (0.1 ml) of the BCNU solution (Thursdays) and a thrice weekly administration of UVB energy (1.25 × 10³ mJ/sq cm Mondays, Wednesdays, and Fridays) to the posterior half of their backs for the duration of the study. Group 2 (36 mice) received acetone applications and UVB exposures as in Group 1. Group 3 (29 mice) received applications of BCNU as in Group 1 but no UVB exposures, and Group 4 (40 mice) received acetone applications as in Group 2 but no UVB exposures.

The mice were examined regularly, and mice with tumors greater than 4, 50, and 100 cu mm were tabulated. The tumors were measured in 2 diameters at right angles on the surface, and the protrusion above the surface was also measured. These sizes were chosen to allow comparison of tumor development and growth with previous reports on UV and chemical carcinogenesis (1, 2, 4, 5).

RESULTS

Tumor Onset. No tumors occurred in Groups 3 (BCNU alone) and 4 (acetone alone). The first tumors greater than 4 cu mm occurred by 2.25 months in Group 1 (BCNU plus UV) and by 2.5 months in Group 2 (acetone plus UV). Tumors reached a size greater than 50 cu mm by 3 months in both groups, and tumors larger than 100 cu mm were noted at 3 months in Group 1 and at 3.25 months in Group 2.

Tumor Incidence. One hundred % of the survivors in Group 1 and 94% of those in Group 2 developed tumors. Ninety % of the tumors reached a size of greater than 100 cu mm in each group. Between 1 and 2 tumors occurred per mouse in each group (Group 1, 1.3; Group 2, 1.4). As the tumors enlarged, they became confluent, forming one large growth if there was more than one initially.

Effect of BCNU. A life table analysis (actuarial analysis) was performed on the tumor development data for tumor sizes greater than 4, 50, and 100 cu mm for Groups 1 and 2 separately. From these analyses, distribution curves representing cumulative probabilities of developing tumors were...
The mechanism of this response is not clear. Certain UV irradiation. This effect was statistically significant. The UV energy is a potent carcinogenic stimulus. As noted in this study, over 90% of the mice receiving radiation developed tumors by the 12th month (Groups 1 and 2). The vast majority of these tumors grew larger than 100 cu mm. However, the tumors appeared earlier and grew more rapidly in the mice receiving BCNU plus UV. A Mantel-Haenszel (12, 13) 1 degree of freedom continuity-corrected x^2 was computed for each tumor size, allowing simultaneous comparison over all the contingency tables of the differences in tumor development probabilities for the 2 groups. All 3 summary x^2's were statistically significant (tumor >4 cu mm, x^2 = 17.044, p < 0.001; tumor >50 cu mm, x^2 = 10.249, p < 0.005; tumor >100 cu mm, x^2 = 9.286, p < 0.005).

Thus, we conclude that BCNU plus UV induced tumors of all 3 sizes more quickly than did acetone plus UV.

### DISCUSSION

UV energy is a potent carcinogenic stimulus. As noted in this study, over 90% of the mice receiving radiation developed tumors by the 12th month (Groups 1 and 2). The vast majority of these tumors grew larger than 100 cu mm. However, the tumors appeared earlier and grew more rapidly in the mice receiving BCNU applications in addition to the UV irradiation. This effect was statistically significant. The mechanism of this response is not clear. Certain nitrosourea compounds are potent carcinogens for rodent skin as well as other organs (8, 10, 11).

In contrast, BCNU is a weak cutaneous tumorigenic agent. In the present study, no tumors were produced with 0.5-mg weekly applications. However, the pattern of tumor development simulated that described for the repeated applications of subcancinogenic amounts of complete chemical carcinogens or repeated UV exposures following initiation with a potent polycyclic hydrocarbon such as 7,12-dimethylbenz(a)anthracene (4). Tumor appearance time was markedly shortened, regression was not noted, and the tumors were carcinomas. However, at present there is no evidence that BCNU and UV act on comparable structures or through similar mechanisms to produce tumors, although both do indeed adversely affect DNA.

A recent study by Fornace et al. (7) suggests a possible mechanism for this combined effect. These authors reported that 2-chloroethyl isocyanate, a decomposition product of BCNU, inhibits the ligase step of DNA excision repair following UV irradiation in human fibroblasts. Defects in DNA excision repair previously have been noted in the rare genetic human disease xeroderma pigmentosum (3, 6, 16). The defects have been considered to be responsible for the inordinate susceptibility to the development of sunlight-induced skin cancers in these patients. It is possible that 2-chloroethyl isocyanate inhibited the repair of UV-damaged DNA in the present study, resulting in an acceleration of cutaneous cancer formation. However, the studies of Ley et al. (9) indicate that mouse epithelial cells in vivo have little or no capacity for excision repair of pyrimidine dimers. Thus, further inhibition of this process may well have no significant effect in the skin of these animals.

Perhaps the most important aspect of these findings concerns the use of BCNU in the treatment of human cutaneous disorders. The drug has been used successfully in the topical treatment of the lymphoma mycosis fungoides and the benign epidermoproliferative disease psoriasis. Information obtained in mouse systems cannot be translated into human responses. In addition, the present studies do not define the time relationships between the UV exposures and the BCNU applications which are responsible for the experimental results. However, avoidance of extensive sun or artificial UV irradiation would appear to be of value for patients being treated with BCNU topically.

### ACKNOWLEDGMENTS

Statistical analysis was provided by Calvin Zippin and Kenneth Resser of the Cancer Research Institute of the University of California, San Francisco.

### REFERENCES


Stimulation of Ultraviolet-induced Carcinogenesis by 1,3-Bis(2-chloroethyl)-1-nitrosourea

John H. Epstein