[CANCER RESEARCH 48, 620–623, February 1, 1988]

# Enhancement of Chemical Carcinogenesis in Mice by Systemic Effects of Ultraviolet Irradiation[1]

**Helen L. Gensler**

*Department of Radiation Oncology and Cancer Center, University of Arizona, College of Medicine, Tucson, Arizona 85724*

## ABSTRACT

**The present study was designed to determine the systemic influence of ultraviolet (UVB) irradiation upon subsequent carcinogenesis induced by benzo(*a*)pyrene. The source of UV irradiation consisted of six Westinghouse FS-40 fluorescent sunlamps. Female BALB/c mice received five 30-min dorsal UVB radiation treatments per week for 13 wk. At the end of 13 wk, irradiated and unirradiated mice received ventral applications of 0.1 or 1.0 mg of benzo(*a*)pyrene twice weekly for 20 or 10 wk, respectively. At 18 wk after the first benzo(*a*)pyrene treatment, mice receiving 0-, 0.1-, or 1.0-mg benzo(*a*)pyrene treatments bore 0, 12, or 29 tumors per group of 18 mice, respectively. Tumor-free survival was significantly shortened in the UV-irradiated hosts as compared with unirradiated hosts, as analyzed by the Kaplan-Meier method of survival analysis. Therefore, ultraviolet irradiation induced a systemic effect which enhanced subsequent tumor induction by benzo(*a*)pyrene in a manner which was dependent on the dose of benzo(*a*)pyrene.**

## INTRODUCTION

Early studies concerning the interactions of UV radiation and chemicals in tumorigenesis involved sequential exposure of the same areas of skin to these agents (1–3). In experiments in which chemical treatment preceded UV irradiation, conflicting results were reported, presumably because UV radiation can lead to degradation of polycyclic aromatic hydrocarbons or to photosensitization, which can enhance the carcinogenic response (4). In experiments in which UV irradiation preceded each treatment with DMBA,[2] a 30% enhancement of tumor formation was reported (5). In that experiment, the UV irradiation treatments were interspersed with the DMBA applications, and both were at a carcinogenic dose, so that the study demonstrated an additive effect of DMBA and UV irradiation as complete carcinogens.

In addition to its local DNA-damaging role in carcinogenesis (6, 7), UV radiation is a systemic immunomodulating agent. After repeated UV radiation exposures, mice acquire a state of immunosuppression that renders them incapable of mounting an effective immunological rejection response to syngeneic UV-induced skin tumor cells (8, 9). Normal mice can reject transplanted UV-induced tumor cells, whereas UV-treated mice cannot. This immunosuppression can be passively transferred with lymphoid cells and is due to the presence of suppressor T-lymphocytes in UV-irradiated mice (10). Fisher and Kripke (11) demonstrated that the UV radiation-induced suppressor lymphocytes could enhance the development of primary skin cancers induced by chronic UV irradiation. The importance of this UV-induced systemic effect on cutaneous carcinogenesis from UV irradiation raised the question of whether or not there is a systemic influence of UV irradiation on chemically induced skin carcinogenesis. Roberts and Daynes (12) had reported that dorsal UVB irradiation for 3 wk before ventral applications of 50 μl of 0.2% benzo(*a*)pyrene twice weekly for 30 wk resulted in a 2- to 6-wk reduction in tumor latency. We wished to determine if there was a dose response in UV influence on benzo(*a*)pyrene-induced tumorigenesis and if ventrally applied benzo(*a*)pyrene influenced the yield of tumors induced by previous dorsal UVB irradiation.

## MATERIALS AND METHODS

**Materials.** Specific-pathogen-free BALB/c mice were purchased from SASCO, Inc. (Omaha, NE). Females at 6 wk of age were used for these experiments. Benzo(*a*)pyrene was obtained from Aldrich Chemical Co. (Milwaukee, WI).

**UVB Irradiation.** The mice were housed 5 per cage on a shelf 20 cm below a bank of 6 Westinghouse FS40 sunlamps. The dorsal fur of the mice was shaved with electrical clippers (Oster, Model 40) once each week during the UV treatment period. The cage order was systematically rotated along the shelves to compensate for differences in flux at various positions under the sunlamps.

The average UV irradiation at the position of the mice was 6.0 J/m$^2$/s over the wavelength range of 280 to 320 nm. This was determined using a cosine-correcting UVX digital radiometer (Ultraviolet Products, San Gabriel, CA). Mice received five 30-min treatments weekly, as described previously by Roberts and Daynes (12), for 13 wk. The total UV dose received by the mice during the 13-wk period, as measured under the wire cage top at the level of the mice, was approximately 7.0 $\times$ 10$^5$ J/m$^2$.

**Chemical Carcinogenesis.** Tumors were induced by a complete carcinogenesis protocol which began 1 wk after the termination of UV irradiation. Benzo(*a*)pyrene was applied to the shaved ventral surface of the mice twice weekly. Mice received 1.0 mg of BP for 10 wk, 0.1 mg of BP for 20 wk, or acetone solvent alone for 20 wk.

**Experimental Design.** The incidence of both dorsal and ventral skin cancers was determined in 6 different treatment groups, each consisting of 18 mice. The groups were treated as follows: (*a*) UV irradiation plus acetone; (*b*) UV irradiation plus 0.1 mg of BP for 20 wk; (*c*) UV irradiation plus 1.0 mg of BP for 10 wk; (*d*) acetone alone; (*e*) 0.1 mg of BP alone for 20 wk; or (*f*) 1.0 mg of BP alone for 10 wk. All animals were inspected weekly for skin cancers. The time of appearance, size, and location of tumors were recorded. At the termination of the experiment, tumors were randomly selected for histological examination.

**Statistical Analysis.** Tumor incidence data are presented in the format of tumor-free survival as a function of time. Thus, only the first tumor that developed was included in the tumor-free survival analysis which was performed by the method of Kaplan-Meier (13) and the censored rank order test derived by Breslow (14). Tumor occurrence did not follow a normal distribution, so that a nonparametric analysis of the data was required (15). Wilcoxon rank-sum tests were performed on the mean number of tumors per mouse between UV-irradiated and -unirradiated groups to test the hypothesis that there was a significant difference between the medians of the irradiated and unirradiated groups.

## RESULTS

**Enhancement by Dorsal UVB Irradiation of Subsequent Ventral Skin Tumorigenesis Induced by Benzo(*a*)pyrene.** The numbers of tumors which developed in the ventral skin of mice

Received 7/31/87; revised 10/29/87; accepted 11/4/87.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This investigation was supported by NIH Grants CA-27502 and CA-44504.

[2] The abbreviations used are: DMBA, 7,12-dimethylbenz(*a*)anthracene; BP, benzo(*a*)pyrene; UVB, electromagnetic radiation in the wavelength range of 280 to 320 nm.

treated ventrally with benzo(*a*)pyrene at a dose of 1.0 mg per treatment are depicted in Fig. 1. At 18 wk after the first BP treatment, there were 2 tumors in the group of unirradiated mice. Pretreatment of such mice with dorsal UVB radiation dramatically increased the number of BP-induced tumors to 29 in the group of irradiated mice, at the same time point. Of 20 tumors in irradiated mice examined histologically, 90% were squamous cell carcinomas, and 10% appeared to be undifferentiated sarcomas. Both tumors in the unirradiated mice were squamous cell carcinomas. Thus, the systemic effects of UV irradiation significantly enhanced skin carcinogenesis by benzo(*a*)pyrene ($P < 0.05$).

The effect of UV irradiation of dorsal skin was tested on the tumor-free survival of mice treated subsequently with benzo(*a*)pyrene. Ten % of mice receiving 1.0 mg of BP ventrally twice weekly for 10 wk developed carcinomas by 12 wk after treatment began (Fig. 2). Sixty-seven % of the mice which had been preirradiated had ventral carcinoma (Fig. 2; $P = 0.0003$, compared with unirradiated animals).

**Influence of the Dose of Benzo(*a*)pyrene on UV Modulation of Tumorigenesis.** Tumor formation in mice exposed to 0.1 mg of BP twice weekly for 20 wk is shown in Fig. 3. At 21 wk after the first BP treatment, there were 9 tumors in the 18 unirradiated mice and 25 tumors in the 18 mice which had been UV irradiated at a distant site. Thus, a 10-fold reduction in the concentration of the carcinogen resulted in a pronounced decrease in the UV enhancement of tumorigenesis at this time point. However, the overall effect of the UV pretreatment was a 3-wk delay in tumor formation, with a similar slope of the curves in both irradiated and unirradiated groups. UVB irradiation of dorsal skin did result in a significant reduction in tumor-free survival of mice treated ventrally with 0.1 mg of BP (Fig. 4; $P = 0.0011$, compared with unirradiated animals).

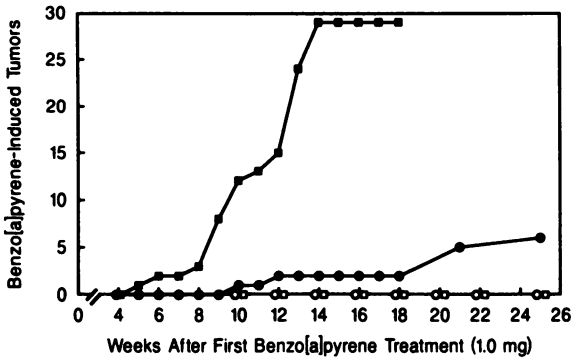


Fig. 1. Rate of tumor development in ventral skin of mice treated with 1.0 mg of benzo(*a*)pyrene, with and without dorsal UV irradiation. Groups of 18 mice each received ventral applications of 1.0 mg of benzo(*a*)pyrene twice weekly for 10 wk. ■, mice which had received UVB irradiation dorsally; ●, mice which did not receive UVB irradiation; □ and ○, mice which did not receive benzo(*a*)pyrene, with (□) or without (○) UVB irradiation.

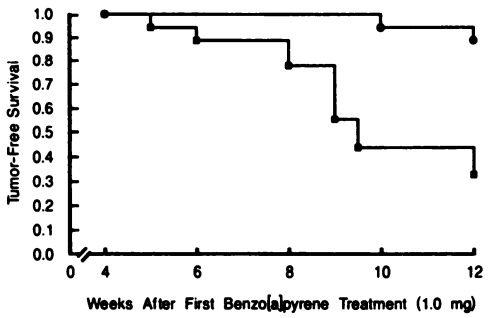


Fig. 2. Reduction of tumor-free survival in mice pretreated with UVB irradiation. Only benzo(*a*)pyrene-induced tumors were considered in these results. Mice were treated as described in the legend of Fig. 1. ■, mice which had received UVB irradiation; ●, unirradiated mice.

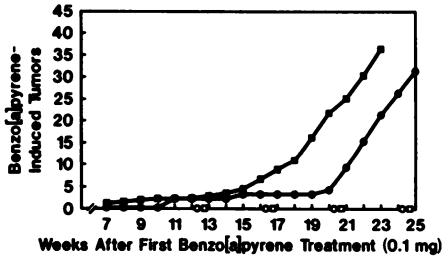


Fig. 3. Rate of tumor development in ventral skin of mice treated with 0.1 mg of benzo(*a*)pyrene, with and without dorsal UV irradiation. Treatments and symbols are similar to those described in the legend of Fig. 1.

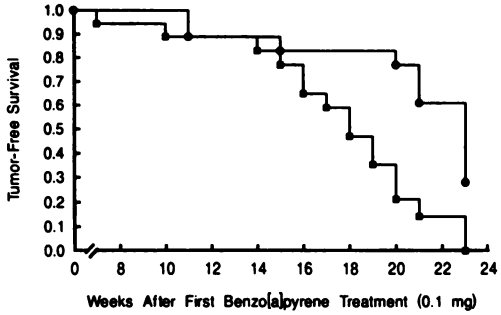


Fig. 4. Reduction of tumor-free survival in mice pretreated with UVB irradiation. Only benzo(*a*)pyrene-induced tumors were considered in these results. ■, irradiated mice; ●, unirradiated mice.

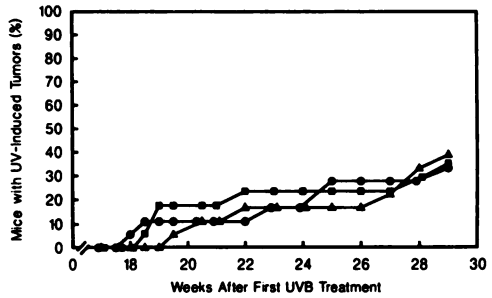


Fig. 5. Incidence of UV-induced tumors in dorsal skin of mice treated with UVB irradiation. The mice received five 30-min exposures per week for 13 wk. One wk later mice began to receive either 0.1 or 1.0 mg of benzo(*a*)pyrene ventrally, twice weekly, for 20 or 10 wk, respectively. ■, UV irradiation alone; ●, UV irradiation plus 1.0 mg of benzo(*a*)pyrene; ▲, UV irradiation plus 0.1 mg of benzo(*a*)pyrene.

**Influence of Ventrally Applied Benzo(*a*)pyrene on the Yield of Tumors Induced by Previous Dorsal UVB Irradiation.** The rate of dorsal UVB-induced tumorigenesis was similar in control mice and mice which received 1.0 or 0.1 mg of BP twice weekly for 10 or 20 wk, respectively (Fig. 5). The BP treatments began 1 wk after the termination of UVB irradiation. Fourteen of the UV-induced tumors were examined histologically. Sixty-four % were classified as squamous cell carcinomas, 21% were fibrosarcomas, 7% were anaplastic carcinomas, and 7% were keratoacanthomas. No influence of ventral benzo(*a*)pyrene treatment was evident in the histology of the UV-induced tumors.

## DISCUSSION

A systemic influence of UV radiation markedly enhanced cutaneous carcinogenesis resulting from topical application of

benzo(*a*)pyrene to unirradiated skin. The extent of the UV potentiation was dose dependent on the concentration of benzo(*a*)pyrene applied. A comparison of Figs. 1 and 3 reveals that the difference between tumor formation of UV-irradiated *versus* nonirradiated mice was maintained over time when 1.0 mg of benzo(*a*)pyrene was applied twice weekly for 10 wk, whereas the UV enhancement in mice treated with 0.1 mg of benzo(*a*)pyrene for 20 wk consisted of a 3-wk reduction in latency. Thus, the UV enhancement of chemical carcinogenesis was more profound at the higher dose of the carcinogen. Schmitt and Daynes (16) have reported a correlation between the inducing dose of a chemical carcinogen and the antigenicity of the resulting tumors. Our results are consistent with the hypothesis that UV-induced immunosuppression allowed the growth of chemically induced antigenic tumors which were rejected in the unirradiated controls. At the lower carcinogenic dose, the tumors were not rejected by the unirradiated mice, but did show accelerated growth in the UV-irradiated, immunosuppressed mice. These findings suggest that the tumors induced by the lower dose of carcinogen were weakly immunogenic and that a state of tolerance was achieved in the unirradiated mice.

Our results with 0.1-mg treatments of benzo(*a*)pyrene were similar to those reported by Roberts and Daynes (12), who had used 0.1 mg of benzo(*a*)pyrene applied ventrally twice weekly. They found that dorsal UV treatments had to precede carcinogen applications in order to influence tumorigenesis. This would be expected if UV-induced immunosuppression were responsible for the accelerated carcinogenesis. Kripke has demonstrated that mouse skin tumors induced by benzo(*a*)pyrene (0.1 mg applied twice weekly) were not immunogenic, based upon growth in unirradiated as well as in immunosuppressed recipients (17). Yet, she also reported that two of three methylcholanthrene-induced tumor cell lines grew at an increased rate in UV-treated mice (18). Roberts and Daynes (12) found that the immunogenicity of tumors induced by a single s.c. innoculum of 5 mg of methylcholanthrene was influenced by the immunosuppressive state of the host. UV pretreatment of the host resulted in a 3-fold increase over control levels in the percentage of tumors which were immunogenic. Thus, the immunogenicity of chemically induced tumors is influenced by the carcinogen dose (16) and the immunological status of the host (12).

It is noteworthy that polycyclic aromatic hydrocarbons can induce suppression of humoral and cellular immunity (19, 20). In the present study there was no increase in dorsal UV-induced tumorigenesis when benzo(*a*)pyrene was applied ventrally during the course of UV tumor development. Thus, we detected no additive effect in immunosuppression caused by the application of benzo(*a*)pyrene subsequent to UV irradiation.

The systemic effect of UVB irradiation could be immunological, as has been demonstrated in the case of UV-induced tumorigenesis (10, 11). A second possibility is that UV irradiation activates leukocytes which circulate to distant sites and release high energy oxygen species which damage DNA of adjacent target cells (21, 22). Such damage would be at a subcarcinogenic level, requiring further DNA damage for carcinogenesis to occur. The higher level of carcinogenesis at the lower dose of benzo(*a*)pyrene, rather than at the higher dose of BP, argues against the second possibility. Experiments are under way to determine if the UV enhancement of chemical carcinogenesis can be passively transferred with splenocytes. It is possible that the systemic effect of UVB irradiation upon benzo(*a*)pyrene-induced carcinogenesis is due to induction of T-suppressor cells, as has been shown for methylcholanthrene-induced tumors (12). The significance of this would be that UV-induced T-suppressor cells would recognize antigens expressed on skin tumors induced by UV irradiation, methylcholanthrene, or benzo(*a*)pyrene. Studies in Daynes' laboratory (23) suggest that UV-induced and methylcholanthrene-induced tumors display oncofetal antigens. Negative regulation of cell-mediated immunity by fetal antigens has been previously reported (24–26). Thus, UV-induced immunosuppression may allow the escape from immunological surveillance of tumors, induced by a variety of agents, all of which express a common or similar tumor-associated antigen recognized by UV-induced T-suppressor cells.

The finding that UV radiation can increase the tumorigenic potency of chemical carcinogens broadens the potential role of UV irradiation in human cancers. It is not yet known if the systemic influence of UVB irradiation upon antigenic benzo(*a*)pyrene-induced tumors applies to other organs in addition to the skin. Nor is it known if the presence of multiple carcinogens would act in an additive manner to induce antigenic tumors. Considering that carcinogens are mutagens and that the increase in antigenicity of tumors is likely due to an increase in mutations, it appears probable that multiple carcinogens could additively increase the antigenicity of tumors. Clearly, the role of UV-induced immunosuppression in allowing the development of antigenic tumors induced by chemical agents warrants further investigation.

## ACKNOWLEDGMENTS

We thank Karen Holladay for technical assistance, Jonelle Davidson for statistical analysis, and Sally Anderson and Robin Dyer for secretarial assistance.

## REFERENCES

1. Davies, R. E., and Dodge, H. A. Modification of chemical carcinogenesis by phototoxicity and photochemical decomposition of carcinogen. *In:* Proceedings of the First Annual Meeting of the American Society of Photobiology, p. 138, 1973.
2. Epstein, J. H. Ultraviolet light carcinogenesis. *In:* W. Montagna and R. Dobson (eds.), Advances in Biology of Skin, Vol. 7, pp. 215–236. New York: Pergamon, 1966.
3. Urbach, F., Forbes, P. D., Davies, R. E., and Berger, D. Cutaneous photobiology: past, present, and future. J. Invest. Dermatol., *64:* 253–257, 1975.
4. Ullrich, R. L. Interactions of radiation and chemical carcinogens. *In:* T. J. Slaga (ed.), Carcinogenesis, Vol. 5, pp. 169–184. New York: Raven Press, 1980.
5. Stenback, F. Studies on the modifying effect of ultraviolet radiation on chemical skin carcinogenesis. J. Invest. Dermatol., *64:* 253–257, 1975.
6. Haseltine, W. A. Ultraviolet light repair and mutagenesis revisited. Cell, *33:* 13–17, 1983.
7. Kraemer, K. H., Lee, M. M., and Scotto, J. DNA repair protects against cutaneous and internal neoplasia: evidence from xeroderma pigmentosum. Carcinogenesis (Lond.), *5:* 511–514, 1984.
8. Kripke, M. L., and Fisher, M. S. Immunologic parameters of ultraviolet carcinogenesis. J. Natl. Cancer Inst., *57:* 211–215, 1976.
9. Spellman, D. W., Woodward, J. G., and Daynes, R. A. Modification of immunological potential by ultraviolet radiation. Transplantation (Baltimore), *24:* 112–119, 1977.
10. Daynes, R. A., and Spellman, C. W. Evidence for the generation of suppressor cells by ultraviolet radiation. Cell Immunol., *31:* 183–187, 1977.
11. Fisher, M. S., and Kripke, M. L. Suppressor T lymphocytes control the development of primary skin cancers in UV-irradiated mice. Science (Wash, DC), *216:* 1133–1134, 1982.
12. Roberts, L. K., and Daynes, R. A. Modification of the immunogenic properties of chemically induced tumors arising in hosts treated concomitantly with ultraviolet light. J. Immunol., *125:* 438–447, 1980.
13. Kaplan, E. L., and Meier, P. Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc., *53:* 457–481, 1958.
14. Breslow, N. A generalized Kruskal-Wallis test for comparing K samples subject to unequal patterns of censorship. Biometrika, *57:* 579–594, 1970.
15. Conover, W. J. Practical Nonparametric Statistics. New York: John Wiley & Sons, 1980.
16. Schmitt, M., and Daynes, R. A. Heterogeneity of tumorigenicity phenotype in murine tumors. Transplantation (Baltimore), *33:* 387–392, 1982.

17. Kripke, M. L. Antigenicity of murine skin tumors induced by ultraviolet light. J. Natl. Cancer Inst., *53:* 1333–1336, 1974.
18. Kripke, M. L. Ultraviolet radiation and tumor immunity. J. Reticuloendothel. Soc., *22:* 217–222, 1977.
19. Blanton, R. H., Lyte, M., Myers, M. J., and Bick, P. H. Immunomodulation by polyaromatic hydrocarbons in mice and murine cells. Cancer Res., *46:* 2735–2739, 1986.
20. Wojdani, A., and Alfred, L. Alterations in cell-mediated immune functions induced in mouse splenic lymphocytes by polycyclic aromatic hydrocarbons. Cancer Res., *44:* 942–945, 1984.
21. Frenkel, K., and Chrzan, K. Hydrogen peroxide formation and DNA base modification by tumor promoter-activated polymorphonuclear leukocytes. Carcinogenesis (Lond.), *8:* 455–460, 1987.
22. Cerutti, P. A. The role of active oxygen in tumor promotion. Biochem. Mol. Epidemiol. Cancer, UCLA Symposia on Molecular and Cellular Biology, New Series, Vol. 40, pp. 167–176. New York: Alan R. Liss, Inc., 1986.
23. Beeson, J. H., Scott, J. R., and Daynes, R. A. The presence of oncofetal antigens on ultraviolet light-induced and methylcholanthrene-induced murine tumors which are common to placental and fetal tissues. Am. Reprod. Immunol., *3:* 92–96, 1983.
24. Evans, D. L., Frank, M. K., McKinnon, K. P., and Cerrone, M. C. Suppression of mitogen and tumor-cell-induced stimulation by tumor-associated fetal antigens. Cell Immunol., *57:* 155–174, 1981.
25. Zoller, M., Price, M. R., and Baldwin, R. W. Inhibition of cell-mediated cytotoxicity to chemically induced rat tumours by soluble tumor and embryo cell extracts. Br. J. Cancer, *17:* 129–137, 1976.
26. Brag, M., Burton, R. C., Smith, J. A., Luckenbach, G. A., Decker, J., and Mitchell, G. F. Effects of placenta tissue on immunologic responses. Clin. Exp. Immunol., *34:* 441–448, 1978.

# Enhancement of Chemical Carcinogenesis in Mice by Systemic Effects of Ultraviolet Irradiation

Helen L. Gensler

*Cancer Res* 1988;48:620-623.