Photoreactivation of Ultraviolet Radiation-induced Skin and Eye Tumors of *Monodelphis domestica*

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**ABSTRACT**

Chronic exposure of the opossum *Monodelphis domestica* to UV radiation (UVR) leads to the formation of cutaneous and corneal tumors. Groups of shaved opossums were exposed 3 times/week to: (a) UVR alone; (b) UVR followed immediately by 1 h of photoreactivating light (PRL) (320–700 nm); (c) 1 h of PRL followed by UVR; and (d) 1 h of PRL alone. Exposures were terminated after 70 weeks of treatment. Analysis of data plotted as probability of tumor formation versus weeks from first exposure shows that post-UVR exposure to PRL significantly (*P* < 0.005) delayed the time to appearance of cutaneous tumors from a 50% probability of tumor formation at 73 weeks for those animals exposed to UVR alone to 128 weeks for those animals exposed to PRL after UVR. Pre-UVR exposure to PRL delayed the appearance of tumors by 6 weeks when compared to the UVR alone group, but the difference between the two groups was not statistically significant. The yield (number of tumors/surviving animal) of cutaneous tumors at 70 and 110 weeks following initiation of treatments also was significantly less in those animals exposed to PRL after, but not before, UVR. Based on the specificity of the PR repair pathway to act only on pyrimidine dimers, these results suggest that dimers are involved in the induction of cutaneous tumors. The results obtained with the induction of corneal tumors are more difficult to interpret. While exposure to PRL significantly delayed the appearance of corneal tumors, the magnitude of the effect was the same regardless of whether the PRL was given before or after each UVR exposure.

**INTRODUCTION**

A number of observations support the hypothesis that DNA damage is involved in the induction of cancer by UVR. Similar action spectra for the induction of pyrimidine dimers in DNA and the induction of *in vitro* neoplastic transformation suggests a causal relationship between the induction of pyrimidine dimers and UVR-induced transformation (1, 2). However, as the action spectra for the induction of dimers as well as other forms of DNA damage, e.g., the pyrimidine (6-4) pyrimidone photoproduct (6-4 photoproduct), are similar (3, 4), these nondimer photoproducts cannot be ruled out as the lesions(s) responsible for *in vitro* neoplastic transformation.

The well-studied observation that cells derived from patients with xeroderma pigmentosum lack certain DNA repair functions also supports a causal relationship between DNA damage, or unrepaired damage, and cancer induction (5, 6). The xeroderma pigmentosum population exhibits a susceptibility to skin cancer several orders of magnitude greater than for the general population (6). These skin cancers presumably are induced by the UVR portion of the solar spectrum.

PR studies not only indicate that DNA damage is involved in UVR-induced neoplastic transformation, but also that pyrimidine dimers are the specific lesions involved. Photoreactivation is a DNA repair pathway that requires the presence of a photoreactivation enzyme (photolyase) which recognizes and binds specifically to pyrimidine dimers in DNA. Illumination of the photolyase-dimer complex with wavelengths in the range of 300–500 nm (PRL) results, upon absorption of a photon, in the conversion of dimerized pyrimidines to their monomeric form (7). Photoreactivation has been reported to suppress UVR induction of *in vitro* neoplastic transformation of cultured, human fibroblasts (8), and UVR induction of neoplastic changes in cells *in vitro* appeared as thyroid neoplasia upon injection into the Amazon molly, *Poecilia formosa* (9). This latter study utilized the ability of fish cells to efficiently photoreactivate pyrimidine dimers in DNA. Post-UVR exposure of *P. formosa* cells to PRL resulted in a decrease in the number of UVR-induced thyroid tumors observed after injection of exposed cells into isogenic recipients.

In view of these findings with fish cells, it would be of interest to determine whether post-UVR exposure to PRL alters the induction of skin tumors in mammals. Results of previous photoreactivation studies with mice are difficult to interpret. Simultaneous irradiation of mice with visible light and UVR resulted in a small reduction in the incidence of skin tumors, whereas visible light following UVR increased the incidence (10). In addition, a small, statistically nonsignificant photoreversal of UVR carcinogenesis in mice was reported by others (11). These results are difficult to interpret in terms of a role for DNA damage in carcinogenesis because PR of pyrimidine dimers does not appear to occur in adult mouse epidermis and was reported to occur in the dermis only of newborn, and not adult, mice (12, 13).

The ability of the gray short-tailed opossum *Monodelphis domestica* (hereafter referred to as *Monodelphis*) to photoreactivate pyrimidine dimers in cutaneous (14) and corneal (15) DNA, in conjunction with its susceptibility to UVR-induced benign and malignant tumors of the skin and the eyes, make this animal a valuable model with which to determine whether pyrimidine dimers are involved in UVR tumorigenesis. *Monodelphis* is a small (~100 g) South American opossum that has been used in our laboratory to study acute and chronic effects of UVR exposure on mammalian skin and eyes. We have used the specificity of the PR pathway to show that dimers are involved in the induction of a number of pathologic changes in UVR-exposed *Monodelphis* including erythema (16), edema (17), sunburn cell formation (18), hyperplasia (18) of the skin, loss of ATPase-positive Langerhans cells (19), suppression of contact hypersensitivity (20), and opacification and neovascularization of the cornea (21). Herein we report on the effects of post-UVR exposure to PRL on the induction of cutaneous and corneal, nonmelanoma tumors.
MATERIALS AND METHODS

Animals. Opossums used in this study were obtained from the breeding colony of the Lovelace Medical Foundation (Albuquerque, NM). During the course of the study, animals were housed individually in cages with newspaper or paper towels provided for bedding. Water and dry fox food (Reproduction Diet; Milk Specialties Products, New Holstein, WI) were provided ad libitum (22). Animals were maintained at 26–27°C on a 12-h light/12-h dark cycle. To prevent unscheduled photoreactivation, opossums were housed under red light (Westinghouse F40R lights).

Radiation Sources. Ultraviolet radiation was obtained from a bank of Westinghouse FS40 fluorescent sunlamps that emit energy in the 280–400-nm range with a peak output at 313 nm (Fig. 1). Animals were exposed individually in cages with steel wire cage lids in place. The dose rate at the bottom of exposure cages was ~3.9 W/m². A calibrated Optronic model 742 spectroradiometer (Optronic Laboratories, Orlando, FL) was used to monitor the dose rate and emission spectrum of the source. Photoreactivation light was obtained from a bank of Westinghouse F40CW lights filtered with 3 mm of window glass. The glass-filtered light had an emission spectrum between 320 and 700 nm (Fig. 1) and a dose rate of 10 W/m² as determined with the spectroradiometer.

Exposure Conditions. Prior to initial exposure, animals were anesthetized by inhalation of methoxyflurane (Metofane) in a closed-chamber system, and dorsal hair was removed with small animal clippers followed by shaving with a Remington Microscreen shaver (Remington Products, Inc., Bridgeport, CT). Prior to each subsequent exposure, any regrowth of hair was removed with the electric shaver. Groups of 29 to 36 shaved animals were exposed 3 times/week for 70 weeks to: (a) UVR alone (250 J/m² = 0.025 J/cm²); (b) UVR followed immediately by 1 h of PRL (3.6 × 10² J/m²)(UVR/PRL); (c) 1 h of PRL preceding UVR (PRL/UVR); and (d) 1 h of PRL alone. A fifth group of 15 animals was sham-treated (shaved twice a week for 70 weeks) to determine possible deleterious effects of handling and shaving. Animals were observed at least once a week, during shaving, for the appearance of pathological changes of the skin and eyes. A thorough examination of both skin and eyes was carried out at monthly intervals during the course of exposures and following termination of treatments. Nonmelanotic, cutaneous growths of >1 mm in diameter which did not regress during a 2-month observation period were scored as nonmelanoma skin tumors. Eyes which appeared upon gross examination to have tumorous or nontumorous cell proliferation. The average time to appearance of tumors was shortest (73 weeks) in animals exposed 3 times/week to UVR alone (Fig. 2). While exposure to PRL immediately before each UVR exposure appeared to have delayed the appearance of skin tumors, the difference (6 weeks) was not statistically significant (P > 0.05). However, exposure of animals to PRL immediately following UVR significantly delayed the appearance of tumors (P < 0.005). In those animals exposed to UVR alone or to PRL/UVR, a 50% probability of skin tumor formation was reached at 73 and 79 weeks following initiation of treatment, respectively. A similar level of probability was not reached in those animals exposed to PRL immediately following UVR until 128 weeks following initiation of treatment.

In addition to delaying the time to appearance of UVR-induced skin tumors, the yield of tumors (number of tumors/surviving animal) was less in those animals exposed to PRL immediately following UVR. Tumor yields for the various treatment groups at 70 and 110 weeks from first exposure are presented in Table 1. The 70-week point is the time at which treatments were terminated, and 110 weeks marks the time when the last cutaneous tumor appeared. Clearly, post-UVR exposure to PRL has significantly reduced the yield of tumors between animals in the various treatment groups was determined by the method of Peto et al. (24). Significant differences in the yield of tumors in each group was determined by the Kruskal-Wallis test.

RESULTS

Cutaneous Tumors. Cutaneous outgrowths appeared between 30 weeks (90 exposures) and 40 weeks (120 exposures) following initiation of treatments with UVR alone, PRL/UVR, and UVR/PRL. Papilloma was the predominant tumor type observed in these groups, but keratoacanthomas, squamous cell carcinomas, and spindle cell tumors, probably fibrosarcomas, also occurred. A detailed description of UVR-induced tumors in opossums has been published elsewhere (25). In those animals exposed to PRL alone, a single tumor appeared following 65 weeks of exposure. No tumors were observed in sham-treated animals. The last skin tumor arose 40 weeks following the termination of exposures. The appearance of skin tumors in the various treatment groups is presented in Fig. 2 as the probability of skin tumor induction versus weeks from first exposure.

The average time to appearance of tumors was shortest (73 weeks) in animals exposed 3 times/week to UVR alone (Fig. 2). When the last cutaneous tumor appeared. Clearly, post-UVR exposure to PRL has significantly reduced the yield of tumors between animals in the various treatment groups was determined by the method of Peto et al. (24). Significant differences in the yield of tumors in each group was determined by the Kruskal-Wallis test.
PHOTOREACTIVATION OF UVR-INDUCED TUMORS

Table 1: Nonmelanoma tumor number and distribution at 70 and 110 weeks following initiation of exposure in the four treatment groups

<table>
<thead>
<tr>
<th>Treatment</th>
<th>UVR alone</th>
<th>UVR/PRL</th>
<th>PRL/UVR</th>
<th>PRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks</td>
<td>70 110</td>
<td>70 110</td>
<td>70 110</td>
<td>70 110</td>
</tr>
<tr>
<td>(n^a)</td>
<td>21 10</td>
<td>24 15</td>
<td>26 14</td>
<td>22 17</td>
</tr>
<tr>
<td>Low(^b)</td>
<td>0 1</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>High(^b)</td>
<td>5 6</td>
<td>5 5</td>
<td>8 7</td>
<td>7 1</td>
</tr>
<tr>
<td>Total tumors/group</td>
<td>37 22</td>
<td>4 5</td>
<td>38 28</td>
<td>1 1</td>
</tr>
</tbody>
</table>

* UVR: 250 J/m² from a bank of Westinghouse FS-40 fluorescent sunlamps. The dose rate at the bottom of the irradiation cubicles was ~3.9 W/m² between the wavelengths of 280 and 400 nm as determined with a calibrated spectroradiometer (Optronic Laboratories, Inc.); UVR/PRL: UVR followed immediately by exposure for 1 h to photoreactivating light from glass-filtered Westinghouse “cool white” fluorescent lamps. PRL/UVR: PRL exposure immediately before UVR; PRL: 1-h exposure to photoreactivating light alone. Treatments were given 3 times/week for 70 weeks.

\(^a\) Weeks from initiation of first exposure.

\(^b\) Number of surviving animals.

\(^c\) Lowest number of tumors found on an individual animal.

\(^d\) Highest number of tumors found on an individual animal.

observed at both 70 and 110 weeks following initiation of exposures.

Corneal Tumors. As with cutaneous tumors, the first tumors of the anterior eye were scored between 30 and 40 weeks following initiation of exposure. In addition, the average time to appearance of tumors was shortest in those animals exposed to UVR alone (Fig. 3). The time course for appearance of corneal tumors in the UVR alone group was very similar to that observed for skin tumors (Fig. 2), with a 50% probability of corneal tumor formation reached at 69 weeks (Fig. 3). A majority of the UVR-induced tumors of the eye of Monodelphis appear, based on light and electron microscopic examination, to be fibrosarcomas that arise in the corneal stroma.1 A similar type of tumor was reported to be induced in UV-irradiated mice (26, 27).

In contrast to what we observed with skin tumors, exposure of PRL immediately before UVR was as effective in delaying the appearance of corneal tumors as was PRL given immediately following UVR. In both cases, the delay in appearance of corneal tumors was statistically significant \((P < 0.005)\). The small difference in time to appearance of tumors observed between groups of animals given PRL before UVR or PRL after UVR was not significant \((> 0.05)\). No tumors of the eye were observed in sham-treated animals or in those animals exposed to PRL alone. The last eye tumor was scored at 72 weeks following termination of exposures.

DISCUSSION

The specificity of PR repair in acting solely on pyrimidine dimers has been used to identify the involvement of dimers in lethal (28), mutagenic (28), tumorigenic (9), and transformational (8) events. The tumorigenic potential of pyrimidine dimers was determined with UV-irradiated cells from the Amazon molly, P. formosa (9). We wished to expand on this observation made with fish cells and determine whether photoreactivation would reduce the capacity of chronic UVR exposure to induce tumors in mammalian skin. In a number of single UVR exposure studies we have shown that photoreactivation suppressed the induction of various nontumorigenic changes in the skin of Monodelphis.

The data presented in Fig. 2 and Table 1 clearly show that post-UVR exposure to PRL reduced the tumorigenic potential of chronic UVR exposure. Photoreactivation has delayed the appearance of an animal’s first tumor and reduced the average number of tumors induced per surviving animal. We have previously reported a similar result with UVR-induced melanotic lesions (29). Photoreactivation has also been shown to reduce the incidence of melanoma in UV-irradiated platyfish-swordtail hybrids (30).

These results suggest that UVR-induced pyrimidine dimers in DNA are involved in UVR tumorigenesis. The role, however, of pyrimidine dimers in tumorigenesis may be indirect. Photoreactivation may result, through reduced competition for a limited number of repair enzymes, in a more rapid excision repair of nondimer photoproducts that might be the true initiating lesions for tumor induction. Previous studies with frog cells \textit{in vitro} (31) and marsupial cells \textit{in vivo} (32) have shown that photoreactivation of dimers resulted in a more rapid removal of 6-4 photoproducts. Thus the 6-4 photoprodust could be the true initiating lesion for tumorigenesis, and pyrimidine dimers could be acting only indirectly in the tumorigenic process.

In either case, the photoreactivation results show that DNA is the primary chromophore of skin tumorigenesis.

Results from UVR-induced corneal tumors are more difficult to interpret. While exposure to PRL was observed to significantly delay the appearance of UVR-induced corneal tumors, the magnitude of the delay was similar regardless of whether the PRL was given before or after each UVR exposure. There are at least two possible explanations for this observation: (a) The delayed appearance of corneal tumors in those animals exposed to PRL resulted from a photoprotection phenomenon and not from the photoreactivation of pyrimidine dimers. With photoprotection, it has been postulated that exposure to long-wavelength radiation may induce a cell division delay which allows time for excision repair processes to occur (33). (b) The delayed appearance of corneal tumors was associated with the photoreactivation of dimers regardless of whether the PRL was given before or after each UVR exposure. This seems inconsistent with the requirement that to photoreactivate dimers, the PRL exposure must follow the UVR treatment that induces dimers. However, in the 3 times/week treatment regime used in this tumorigenesis study, an exposure to PRL before UVR on Wednesday, for example, also represents a PRL exposure 48 h after the UVR exposure Monday. If during this 48-h period incomplete excision repair of dimers occurred, residual dimers would presumably be subject to photoreactivation when

![Fig. 3. Probability of corneal tumor formation as a function of weeks from first exposure](https://cancerres.aacrjournals.org)
the animals were eventually exposed to PRL. A persistence of pyrimidine dimers that could be reversed by PR in *Xenopus* cells has been reported (34). It is interesting to note that cell lines derived from UVR-induced corneal tumors appear to be less efficient in excision repair when compared to a primary culture from skin. Twenty-four h following the induction of equal numbers of pyrimidine dimers, <10% of the dimers had been repaired in corneal tumor cell lines as compared to ~50% repaired in primary cultures from skin (35). If the excision repair kinetics of tumor cell lines reflects the repair capacities of progenitor cells in the cornea, one would predict that dimers would be present and capable of being photoreactivated for up to 48 h after induction.

In summary, exposure of UV-irradiated opossums to wavelengths of light that drive the PR repair pathway extended the time to appearance of and decreased the yield of tumors. Based on these observations it is clear that UVR-induced pyrimidine dimers are in some way involved in skin carcinogenesis. It is not clear, however, what role dimers play in that process. It is also unclear at this time whether pyrimidine dimers are involved in the induction of corneal tumors in chronically irradiated opossums.

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REFERENCES


* Unpublished data.


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