

Photoreactivation of UV-induced pyrimidine dimers and erythema in the marsupial *Monodelphis domestica*

(photodermatology/DNA damage/opossums/minimal erythema dose)

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ABSTRACT Post-UV treatment of the gray, short-tailed opossum *Monodelphis domestica* with photoreactivating light (320–400 nm) suppressed the appearance of UV-induced erythema as evidenced by an increase in the dose of UV required to elicit an erythematous response. The average erythema dose for animals held in the dark following UV exposure was 620 ± 40 J/m², whereas 2460 ± 110 J/m² were required for erythema induction with animals exposed to 90 min of photoreactivating light post-UV. Pre-UV exposure to photoreactivating light had no effect on the UV induction of erythema. The dose–response for the photoreversal of pyrimidine dimers in epidermal DNA of *M. domestica* was similar to that for the photoreactivation of erythema induction. These data not only support the notion that DNA is the primary chromophore involved in the induction of erythema but also identify pyrimidine dimers as the major DNA change responsible for its induction. These results also identify *M. domestica* as a useful whole-animal system with which to determine the role of pyrimidine dimers in other photobiological responses of mammalian skin.

UV-induced cyclobutane-type dimers between adjacent pyrimidines on the same DNA strand can be split *in situ* by a light-dependent repair process called photoreactivation (PR) (1). The process requires the presence of photoreactivating enzyme (PRE) and photoreactivating light (PRL) in the range of 300–500 nm (2). PR has been shown to occur in prokaryotes and certain eukaryotes (3), including marsupials (4, 5), in cells from some placental mammals (6), and in human skin *in vivo* (7–9). Although the PR repair process appears to be efficient in humans (7–9) and marsupials (4, 5), PR is much less evident in mice (10, 11). The specificity of this repair process for pyrimidine dimers has been used to identify this lesion as a major cause of lethal (12), tumorigenic (13), and transformation (14) events induced by UV. It would be of interest to exploit the specificity of this repair process to determine the role of pyrimidine dimers in photobiological responses of mammalian skin including erythema induction.

Exposure of mammalian skin to UV results in erythema (redness) due to vasodilatation (15, 16) and the resulting increase in blood content of the skin. It has been proposed that UV-induced vasodilatation may result from direct vascular damage (17) or from indirect effects of chemical mediators (18). For either case, the chromophore involved in the initial absorption of UV has not been identified, although nucleic acids, proteins, and lipoproteins have been discussed as possible target molecules (19). An action spectrum generated with human skin was consistent with DNA as being the primary chromophore for erythema induction when corrections for the optical effects of the stratum corneum were taken into consideration (20). The ability to photoreverse pyrimidine dimers in the skin of marsupials (4) provides a useful

whole-animal model with which to study the role of pyrimidine dimers in the induction of erythema. Although photoreversal of dimers has been reported to occur in human skin (7–9), the effects of post-UV exposure to long-wavelength radiation on photobiological responses of human skin are generally controversial (21–24).

MATERIALS AND METHODS

Experimental Animals. Five- to 6-month-old opossums (*Monodelphis domestica*), raised in this laboratory, were used for these studies. Animals were raised as described elsewhere (25) and anesthetized prior to irradiation as described (4).

UV Exposure Conditions. Hair was removed from the dorsal epidermis with small animal clippers (model no. A2, Oster, Milwaukee, WI), and aluminum foil masks, into which six holes (1 cm in diameter) had been punched, were taped to the shaved dorsal areas. These restricted areas of skin were exposed to various doses of UV from a Westinghouse FS-40 sunlamp. (Relative emissions were 0.04, 0.27, 0.69, 1.0, and 0.09 at 280, 290, 300, 313, and 360 nm, respectively, with >90% of the energy emitted between 280 and 400 nm.) The dose rate from the sunlamps was 2.0 W/m² as determined with a calibrated Optronic model 742 spectroradiometer (Optronic Laboratories, Orlando, FL).

Minimal Erythema Dose (MED) Determinations. Erythema of the skin was judged by two observers at 28–32 hr post-UV and the data were pooled. Maximal redness of the skin was observed at this time, and a MED was defined as the dose that induced a barely perceptible erythema at 28–32 hr post-UV. In the first series of experiments, MEDs were determined for three groups of animals exposed to the FS-40 sunlamp. Group 1 was held in the dark following exposure, group 2 was exposed to PRL for 90 min prior to sunlamp exposures, and group 3 was exposed to PRL for 90 min following sunlamp exposures. In a second series of experiments, MEDs were determined for animals exposed to the FS-40 sunlamp followed by a 30-, 45-, 60-, or 90-min exposure to PRL. These were the same exposure times used in the study of photoreversal of pyrimidine dimers described below. PRL was obtained from a Westinghouse BLB fluorescent lamp filtered through 3 mm of window glass (>90% of the transmitted energy was between 320 and 400 nm). The dose rate at the surface of the skin was 10 W/m² as determined with the spectroradiometer.

Pyrimidine Dimer Measurements. Pyrimidine dimers in the epidermal DNA of *M. domestica* were measured with damage-specific endonucleases from *Micrococcus luteus* as described (4). Prior to dimer determinations, animals were exposed to 1000 J/m² from the FS-40 sunlamp followed by 0,

30, 45, 60, or 90 min of PRL. Immediately following each treatment, DNA was extracted from the epidermis and analyzed for the presence of pyrimidine dimers.

RESULTS AND DISCUSSION

Series 1 Experiment. The MED observed for each animal under the three treatment conditions described above is presented in Table 1. Numerical averages and standard errors of the mean were calculated for the three groups. The average MED observed with animals exposed to PRL following exposure to the FS-40 sunlamps was considerably higher than with those animals that received no PRL treatment or received PRL prior to UV exposure. On the average, 2460 ± 110 J of UV per m^2 were required to elicit an erythema response in those animals exposed to PRL post-UV. This is significantly ($P < 0.01$) higher than the 620 ± 40 J/ m^2 or 550 ± 70 J/ m^2 required for animals that were not exposed to PRL or were exposed to PRL prior to sunlamp irradiations, respectively. The average MEDs of the latter two groups are not significantly different. The photoreactivable fraction (PRF, the fraction of the total effect that was photoreactivated) can be calculated as $PRF = 1 - [(MED \text{ without PRL}) / (MED \text{ with PRL})]$. Thus, for UV-induced erythema, the PRF equals $1 - (620/2460)$, or 0.75. Under the PR conditions used, 75% of the potential of UV to induce erythema in *M. domestica* has been reversed. This is in good agreement with the 80% photoreversal of pyrimidine dimers in epidermal DNA of *M. domestica* achieved in 90 min under similar PR conditions (4).

Series 2 Experiments. In these experiments the dose-responses for the PR of erythema induction and the photoreversal of pyrimidine dimers in epidermal DNA were determined in groups of animals exposed to PRL under similar conditions. The results of this study are presented in Fig. 1 as the PR of erythema induction as a function of photoreversal of pyrimidine dimers. The slope of the line fit to these points by linear regression analysis was 0.85, which represents a good correlation between the kinetics of photoreversal of dimers and of erythema induction. It may be concluded from these data that the primary chromophore for UV-induced erythema, in this animal at least, is DNA and the major lesion involved is the cyclobutane-type pyrimidine dimer. Confirmation of photoenzymatic action in the photoreversal of dimers and erythema induction observed in *M. domestica* will require detection of the PRE in skin. *In vitro* photoreversal of pyrimidine dimers in DNA with extracts of

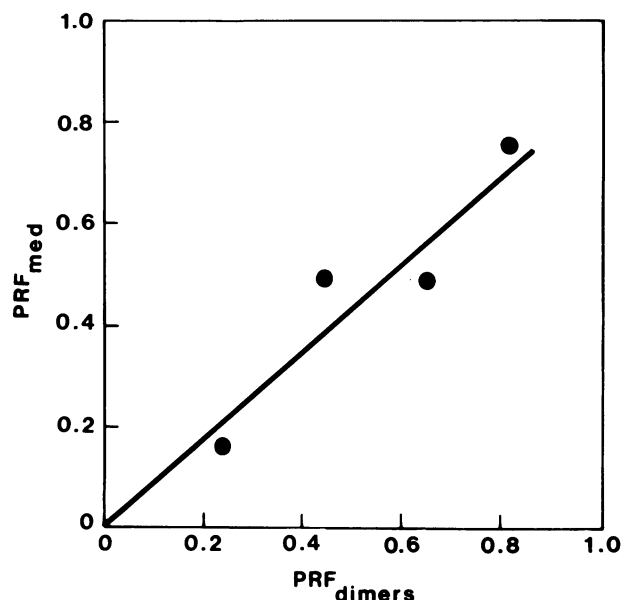


FIG. 1. PRF for MED (PRF_{med}) plotted as a function of the PRF for pyrimidine dimers (PRF_{dimers}). The MED was determined with animals exposed to 30, 45, 60, or 90 min of PRL. PRF_{med} was calculated for each PR exposure as $1 - [(MED \text{ without PRL exposure}) / (MED \text{ with PRL exposure})]$. PRF_{dimers} was calculated as the fraction of dimers reversed by 30-, 45-, 60-, and 90-min exposures to PRL after an exposure of 1000 J of UV per m^2 from the FS-40 sunlamp.

liver from *M. domestica* has been measured (data not shown).

The results reported herein identify *M. domestica* as a useful laboratory animal with which to study the role of pyrimidine dimers in various photobiological responses of mammalian skin. Previous studies on the effect of long-wavelength radiation given simultaneously either with or following UV irradiation of mice are difficult to interpret. Griffin *et al.* (26) reported that simultaneous irradiation with visible and UV light resulted in a small reduction in the incidence of tumors, whereas visible light following UV increased the incidence. In addition, Kelner and Taft (27) observed a small, statistically nonsignificant, photoreversal of UV carcinogenesis in albino mice and stated that the photoreversibility of carcinogenesis was only a tentative conclusion. Post-UV exposure of albino mice to "daylight fluorescent" lamps was reported to reduce ear damage and death rate (28). A role for the photoreversal of pyrimidine dimers in photorecovery from UV irradiation of mice is not supported by reports on the absence of PR of dimers in mouse epidermis (10, 11) and the presence of PR only in the dermis of newborn, but not adult, mice (11).

Although Van der Leun and Stoop (29) reported slight reductions in UV-induced erythema when the initial exposure was followed by several hours of glass-filtered sunlight, the considerable literature on mixed-wavelength responses in human skin is generally controversial (21-24). Furthermore, the relationship between UV and tumor formation in humans can only be inferred from epidemiological data. The studies reported herein not only show a direct relationship between a specific DNA lesion (the pyrimidine dimer) and a measurable photobiologic response (erythema) in animal skin but also demonstrate the currently unique potential of this mammalian, whole-animal model for probing the role of the pyrimidine dimer in other photobiological responses possibly including cancer induction.

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Table 1. MED determinations with *M. domestica* under various conditions of PR treatment

	MED, J/ m^2		
	Group 1 (no PRL)*	Group 2 (pre-PRL)†	Group 3 (post-PRL)‡
	550	400	2800
	700	500	2400
	600	450	2500
	600	600	2000
	800	800	2200
	450		2500
	650		2800
Mean \pm SEM	620 ± 40	550 ± 70	2460 ± 110

*Animals were held in the dark following exposure to the FS-40 sunlamps.

†Animals were exposed to PRL for 90 min prior to sunlamp exposure.

‡Animals were exposed to PRL for 90 min following sunlamp exposure.

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