Effect of Glutathione Depletion on Sunburn Cell Formation in the Hairless Mouse

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Cutaneous protection against ultraviolet B (UVB) radiation damage by endogenous glutathione (GSH) was evaluated in the epidermis of the hairless mouse by measuring the influence of GSH depletion on sunburn cell (SBC) formation. Cellular GSH exerts antioxidant effects and recent studies have suggested a role for oxygen radicals in the production of SBC. Hairless mice (Skh/h 1) received oral treatment with buthionine S,R-sulfoximine (BSO), an irreversible inhibitor of γ-glutamylcysteine synthetase, to deplete cutaneous GSH; 4 d later their ears were exposed to UVB radiation. BSO treatment significantly reduced GSH levels in the epidermis to 10–15% of control levels. Twenty-four hours after UVB exposure, SBC counts in the ears of animals with and without BSO treatment were measured, and those exposed to UVB were found to have increased. Greater numbers of SBC were found in the ears of BSO-treated mice exposed to 15 or 20 mJ/cm² UVB, than in non-BSO-treated mice exposed to the same UVB doses. At higher UVB doses, there were no statistically significant differences between the groups. The results show that endogenous GSH provides the epidermis with measurable protection against injury by low or moderate UVB doses. *J Invest Dermatol 96:838–840, 1991*

Ultraviolet B (UVB) radiation is responsible for many of the acute effects of sun exposure, such as delayed erythema, hyperpigmentation, and thickening of the skin. These changes are accompanied by histologic changes such as epidermal hyperplasia [1], vasodilatation [2], hypermelanization [3], sunburn cell (SBC) formation [4–6], reduction in number of Langerhans cells [7], and perturbations of the cytoskeleton of the keratinocytes [8]. Although the pathogenesis of SBC formation is unclear, hypotheses related to decreased DNA repair capacity [9] and to cell-cycle dependence [10] have been proposed. Quantitative analysis of SBC formation has shown both UV dose and wavelength dependence [4]. SBC formation is most marked after exposure to UVB (290–320 nm), and may be used to quantify UV damage. In in vivo and in vitro experiments [11,12], SBC formation in mouse skin was suppressed by the administration of superoxide dismutase (SOD). In vitro, the presence of other antioxidants, including catalase, xanthine, and mannitol during UVB irradiation, was also effective. These results suggest that SBC production in the epidermis may be mediated by reactive oxygen species. Whereas UV effects must be initiated by UV photochemical reactions in the skin, the equal effectiveness of antioxidants administered before or after radiation [11], suggests that radicals may be generated in the events following exposure rather than by the initial photochemistry. Glutathione (GSH), a tripeptide thiol found in virtually all mammalian tissues [13], including human and mouse skin [14], is important for its role in cellular protection by conjugation with toxic compounds or by quenching reactive oxygen species. It is also a cofactor for the enzyme GSH peroxidase. It has been proposed that GSH functions in the skin as a photoprotective agent [14,17]. Buthionine S,R-sulfoximine (BSO), a selective and potent inhibitor of γ-glutamylcysteine synthetase [18], and therefore of GSH synthesis, has been used for experimental depletion of GSH in intact animals [18,19]. Mice and rats treated with BSO show a remarkable decline of GSH levels in many organs, especially kidney, pancreas, and skeletal muscle, and in plasma [19]. Human skin fibroblasts and keratinocytes treated with BSO showed increased sensitivity to UV radiation [15].

Previously, we have demonstrated that protection against SBC formation may be conferred by pre-treatment of animals with cadmium [20], which induces the synthesis of metallothionein, a protein that is rich in -SH groups, and which might consequently be expected to exhibit antioxidant and photoprotective properties.

In the present study, the photoprotective role of cutaneous GSH was studied in vivo in the hairless mouse. After oral administration of BSO, UVB-induced SBC production was measured and correlated with changes in skin GSH levels.

MATERIALS AND METHODS

Animals Sixty-nine female hairless mice (Skh/h 1), aged 6–8 weeks were obtained from Charles River Laboratories, Inc., Boston, MA, and randomized into 14 groups of 4–5 mice. Access to food and water was restricted following the diet plan described below.

Chemicals DL-buthionine-[S,R]-sulfoximine was purchased from Sigma Chemical Co., St. Louis, MO.

UVB Radiation Source UVB radiation was obtained from a bank of 12, closely set, FS36 fluorescent sunlamps (Elder Pharmaceuticals, Bryan, OH), mounted in a reflector housing (Dimco-Gray Co., Dayton, OH). UVB dose was measured by an IL-1700 radiometer (International Light Inc., Newburyport, MA) fitted

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Abbreviations:
BSO: buthionine S,R-sulfoximine
GSH: glutathione
SBC: sunburn cell
SOD: superoxide dismutase

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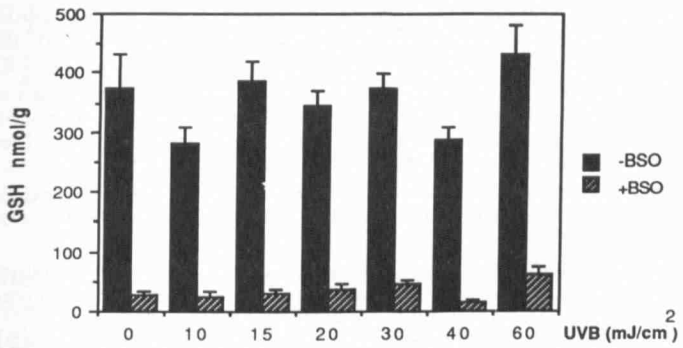

Figure 1. Effect of BSO administration on cutaneous GSH. BSO treatment depressed cutaneous GSH in each group of mice ($p < 0.001$). UVB dose corresponds to those shown in Table I. Values expressed as mean ± SE. Student t test for paired data was used to calculate significance.

with a UVB detector (peak sensitivity at 296 nm). The lamp-to-skin distance was 45 cm.

SBC Counting Paraffin sections (5 μm) of ears were prepared by routine histologic processing and stained with hematoxylin-eosin. The number of SBC/cm was counted using a light microscope fitted with an eyepiece grid. The mean count of SBC in five sections from each sample was expressed as SBC per linear centimeter of epidermis. The criteria for a SBC were cytoplasmic eosinophilia and vacuolization of the cytoplasm and a pyknotic nucleus.

GSH Analysis Skin GSH was measured spectrophotometrically using the GSH reductase catalyzed recycling assay of Griffith [21] as described [19]. Skin samples from BSO-treated and control mice were heat-treated to facilitate separation of the epidermis and dermis. After blotting and weighing, the epidermis was homogenized in 10 vol. 10% trichloroacetic acid with an Ultra-Turrax (Janke & Kunkel) homogenizer and the homogenates were centrifuged. The supernatant was assayed for GSH.

Experimental Design and UV Irradiation Four days prior to irradiation, seven groups of hairless mice were administered drinking water containing 4 mg BSO/ml, to deplete cutaneous GSH as described [19]. The seven control groups received normal drinking water. All mice were fasted for 18 h prior to irradiation (because we observed that this resulted in considerably more GSH depletion). On the fourth day, the mice were anesthetized using chloral hydrate (2.9 g/kg body weight) by intraperitoneal injection and immobilized on an animal board. The ears were fixed into a horizontal position, exposing the upper (medial) surfaces. Ears of control and BSO-treated animals were irradiated simultaneously using doses of 0, 10, 15, 20, 30, 40 and 60 mJ/cm². The board with mice attached was turned around halfway through irradiation to increase uniformity of exposure. Twenty-four hours after UVB exposure, the mice were sacrificed by cervical dislocation. A 6-mm punch biopsy was taken from each ear. The ear samples were processed for routine histology for sunburn cell counting.

Dorsal skin samples were taken from all mice, with or without BSO, to measure GSH content. Skin samples were stored at −70°C until analysis.

RESULTS

Effect of BSO on Epidermal Glutathione GSH levels in each group of mice, with and without BSO treatment, are shown in Fig 1. BSO treatment caused depletion of cutaneous GSH when compared with controls. Mean GSH levels in the skin obtained from control and BSO-treated groups of mice were 357.2 ± 19.8 and 35.8 ± 4.3 nmol/g tissue (mean ± SE), respectively, giving a reduction in GSH of 90.2%. Levels in the epidermis and dermis of controls without BSO were comparable to previous studies [14,16]. The reduction was consistent throughout the groups of animals receiving each UVB dose and reached the same level of significance in each group ($p < 0.001$).

SBC Formation The result of the UVB-dose response study is shown in Table 1, which gives the number of SBC (mean ± SE) resulting from each UVB dose. UVB dose-dependent SBC formation occurred in groups both with and without BSO. After low or moderate UVB exposures, SBC counts in the BSO-administered groups exceeded those in the controls. The difference was significant after 15 and 20 mJ/cm² ($p < 0.01$). The difference at 10 mJ/cm² UVB was not significant, but the counts were very low. After higher UVB doses (30–60 mJ/cm²), similar numbers of SBC were found in the groups with and without BSO.

DISCUSSION

SBC results from a form of individual cell necrosis after ultraviolet exposure of skin, in particular after UVB or UVC radiation. SBC formation is UV-dose related and lends itself for use as an endpoint in quantitative studies of UV effects [4,10]. Several pieces of evidence support the hypothesis that SBC formation results at least in part from free-radical mediated reactions. For example, biopsy samples of UV-irradiated skin incubated with quenchers of oxygen intermediates showed reduced SBC formation [11]. In vivo, intravenous treatment of irradiated mice with SOD was effective in reducing UVB-induced SBC [12]. Mouse ears rendered hypoxic prior to or immediately after UVB exposure also showed reduced SBC formation [22]. Because quenchers were effective after as well as before exposure, radicals generated in the events occurring after exposure may be important, rather than any that may be directly photochemically generated during UV exposure.

In this study we have evaluated the relationship between cutaneous GSH levels and susceptibility to SBC formation by UVB to clarify further the relationship between SBC formation and oxidative injury. Our aim was to evaluate changes in UV-sensitivity with depletion of cutaneous GSH, using SBC formation as a quantitative marker of UVB injury. We observed that BSO caused a marked decrease in cutaneous GSH in each group of hairless mice and that pre-treatment with BSO significantly potentiated SBC formation by UVB doses comparable with moderate environmental exposures. BSO treatment itself did not cause SBC formation. At the lowest UV doses tested, SBC formation occurred at a low level and differences associated with BSO treatment were not observed. After higher UVB exposures, SBC formation was similar in both BSO-treated and control mice, suggesting that the BSO effect was overwhelmed. Because the minimal erythemal dose for the albino hairless mouse in our laboratory is about 30 mJ/cm², the exposure doses of UVB at which effects were seen were in the 0.5 × minimal erythemal dose region. The role of other -SH rich molecules in

Table I. Effect of BSO on SBC Production in Mouse Ears by UVB Irradiation[a]

UVB Dose (mJ/cm²)	−BSO	+BSO	p
0	0.66 ± 0.15 (10)	0.99 ± 0.30 (9)	
10	0.76 ± 0.25 (10)	1.57 ± 0.46 (10)	
15	2.40 ± 0.40 (15)	4.84 ± 0.84 (14)	<0.01
20	5.13 ± 0.72 (10)	9.07 ± 0.72 (10)	<0.01
30	16.7 ± 1.5 (15)	15.5 ± 2.0 (15)	
40	20.6 ± 3.0 (5)	24.0 ± 5.1 (5)	
60	52.2 ± 4.2 (5)	61.7 ± 12.0 (5)	

[a] Numbers in parentheses represent number of ear skin in each experiment. Values are expressed as mean number of SBC/linear cm of epidermis ± standard error. Student t test for paired data was used to calculate significance.

protecting against higher UVB influences is currently being investigated [23].

Other studies have examined the relationship between GSH levels and UV-injury [14,17]. For example [14], UVB, UVA, and PUVA treatments each caused a reduction in cutaneous GSH levels, both in whole skin and in cultured keratinocytes. After UVB, reduction of GSH in the epidermis was maximal at 6 h. These results are consistent with consumption of GSH by reactions resulting in the reduction of oxidative injury. The effect of GSH depletion by BSO in cell culture has also been examined [15,17]. Human neonatal fibroblasts and epidermal keratinocytes showed increased sensitivity to UVB radiation (313 nm) after incubation in BSO to cause GSH depletion. Fibroblasts from adult skin were also sensitized to UVA radiation at 365 nm. These results emphasized the importance of GSH protection against cytotoxic UVB and UVA damage. It is of interest that keratinocytes were less sensitized to UV radiation by BSO treatment than were fibroblasts. This may reflect the higher residual levels of cellular GSH that was observed in keratinocytes (after BSO treatment) [15,17]. Administration of cysteamine, an exogenous thiol with powerful radical scavenging activity, counteracted the effects of GSH depletion on 302-nm–induced damage, but did not protect against the longer wavelengths. These results suggest that the effect of GSH depletion on damage by longer wavelengths resulted specifically from reduced efficiency of GSH peroxidase, whereas a direct free radical scavenging effect of GSH is involved in protection against 302 nm.

Skin is protected against UV injury by several mechanisms. Absorption or scattering of UV by stratum corneum components such as keratin, nuclear protein, melanin, and urocanic acid reduces transmission. The results of this study support the concept that endogenous cellular antioxidants play a significant role in photoprotection, and that oxidative damage is involved in the injury of the epidermis by UVB.

With a better understanding of the mechanisms of UV injury, strategies may be developed in the future to induce photoprotection at the cellular level.

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