Role of Oxygen Intermediates in UV-Induced Epidermal Cell Injury

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To investigate the role of oxygen intermediates (OIs) in sunburn cell (SC) formation and development of UV-inflammation in vivo, groups of mice were injected intravenously with OI scavengers, including bovine blood superoxide dismutase (SOD), bovine liver catalase, l-histidine, d-mannitol, and saline (controls) both before and/or after UV irradiation with sunlamp tubes (mainly 280–320 nm; 300 mJ/cm²; UV). Ear thickness was measured before and 6 and 24 h after UVIR. Ears were removed 24 h after UVR and the number of SCs per unit length of ear epidermis was counted using hematoxylin-eosin stained sections. The number of SCs was significantly decreased (p < 0.02) by a single injection of SOD (10–30 units/g body weight) given either just before or immediately after (<15 min) UVR, while SC formation was no longer suppressed by injections given more than 2 h before or after UVR. Four repeated injections of SOD (10 units/g) also reduced SC counts but did not significantly alter ear-swelling responses (ESR). Neither SC counts nor ESR were remarkably suppressed by 4 injections of any of the other active OI scavengers, inactivated SOD, or bovine serum albumin. A single injection of diethyldithiocarbamate, an SOD inactivator, significantly augmented SC formation (p < 0.05), but did not change ESR. These findings suggest that OIs generated by UVR participate in SC formation but are not apparently involved in UV-edema.

The sunburn cell (SC) is a histologic characteristic of UV-induced epidermal injury. By electron microscopy, the SC contains aggregated masses of tonofilaments with a variable admixture of other organelles, sometimes including nuclear fragments. The morphologic observations suggest that the SC represents a model of cutaneous apoptosis [1–3]. However, the pathomechanism of SC formation is still obscure, although several possible chromophores, including nuclear DNA, have been proposed [4]. It has been suggested that oxygen intermediates (OIs) generated by ultraviolet radiation (UVR) may account for cellular damage [5]. Recently, we used an in vitro system to demonstrate that SC formation was significantly suppressed by addition of scavengers of OIs such as superoxide dismutase (SOD) to the culture medium containing slices of guinea pig skin that had previously been given UVR [6]. In this study we have expanded earlier observations to investigate the role of OIs in SC formation in vivo. After intravenous administration of OI scavengers to UV-exposed mice, the number of SCs in ear epidermis and the degree of UV-inflammation as assessed by ear-swelling responses (ESR) were examined.

MATERIALS AND METHODS

Animals

ICR strain female mice (6-week-old, body weight 25–30 g) were used.

Ultraviolet Source

Five tubes of fluorescent sunlamp (Toshiba FL 20 SE) were used as a radiation source of middle-wave ultraviolet radiation (UVR). This lamp emits wavelengths mainly between 280–320 nm, peaking at 310–315 nm. The total energy output was 0.2 mJ/cm²/s at a distance of 30 cm, as measured with the aid of a UVR-meter (Toshiba Medical Supplies, Tokyo), with spectral sensitivity in the range of 280–320 nm.

Ultraviolet Radiation

UVR was administered by placing cages containing a maximum of 10 mice under the lights. The cage is 2-cm high and covered by thin wire netting so that mice can move freely without lying one over another during irradiation. The mice received a total energy dose of 300 mJ/cm². Irradiation time was approximately 25 min.

Chemicals

We purchased bovine serum albumin (BSA; Armour Pharm Co., Phoenix, Arizona), catalase from bovine liver (approximately 3000 units/mg protein, P-L Biochemicals, Milwaukee, Wisconsin), N,N'-diethyldithiocarbamate sodium trihydrate (DDC), SOD from bovine blood (approximately 2700 units/mg protein; Sigma Chemical Company, St. Louis, Missouri), L-histidine dihydrochloride, and d-mannitol (Wako Pure Chemical Company, Osaka, Japan).

All reagents were dissolved in sterile physiologic saline at final concentrations of 40–1200 µg/ml (SOD), 0.4 mg/ml (BSA), 8–80 mg/ml (catalase, histidine, and mannitol), and 24–120 mg/ml (DDC), and the pH was adjusted to 7.4. A part of SOD was inactivated in an autoclave (1 kg/cm², 120°C) for 10 min (inactivated SOD). The reagents (each 0.25 ml) were injected intravenously via tail veins without anesthesia, except for DDC, which was given intraperitoneally.

Dosage and route of administration of OI quenchers and DDC were selected on the basis of the quantitative data of the experiments by Oyanagi [7] and Heikila and Cohen [8], respectively.

Sunburn Cell Counting

Tips of ears were removed 24 h after completion of UVR; the specimens were fixed in 10% Formalin and embedded in paraffin, and 5-µm-thick sections were stained with hematoxylin-eosin. The total number of SCs in the epidermis was counted, and the length of the epidermis was measured with a scale bar under 10X ocular and 40X object lenses. SC counts per millimeter of epidermis were calculated after observing at least two different sections per ear specimen.

Ear Swelling Response

Before UVR, the baseline thickness of both ears was measured with a dial thickness gauge (Peacock, Tokyo, Japan) under anesthesia with pentobarbital sodium (50 mg/kg). Ear thickness was measured 6 h (in some experiments) and 24 h after UVR, and ear swelling was expressed as the mean increment in thickness above baseline control values.

Statistical Analysis

Student's t test was employed to determine statistical differences between the means.
Experimental Protocols

A single and repeated injections of SOD: Groups of mice were injected intravenously with SOD (10–300 μg), inactivated SOD (100 μg), or BSA (100 μg) (1) just before UVR (mice received irradiation within 15 min) or (2) immediately after completion of UVR (within 15 min). Positive controls were given saline plus UVR, and negative controls were injected with SOD without UVR. Other groups of mice received 4 repeated injections of SOD or inactivated SOD (100 μg), which were given (1) immediately, (2) 1 h, (3) 3 h, and (4) 5 h after UVR.

Injection timing: A single injection of SOD (100 μg) was given (1) 5 h before, (2) 2 h after, (3) just before, (4) immediately after, (5) 2 h after, or (6) 5 h after UVR. Positive controls were given saline just before UVR.

Injection of DDC: A single injection of DDC (6–30 mg) was given intraperitoneally 3 h before or 1 h after UVR. Positive controls were given saline plus UVR, and negative controls were given DDC only.

Injection of catalase, histidine, and mannitol: Mice received 4 injections of catalase (2–10 mg), histidine (2–10 mg), or mannitol (2–20 mg) (1) before UVR, and (2) 1 h, (3) 3 h, and (4) 5 h after UVR. Positive controls were given 4 injections of saline plus UVR.

RESULTS

A Single and Repeated Injections of SOD (Table I)

The number of SCs was significantly decreased by a single injection of SOD that was given either before or after UVR (100 μg) or after UVR (100–300 μg) and also by 4 repeated injections of SOD. Injection of 10 μg of SOD, inactivated SOD, and BSA had no inhibitory effect on SC formation. ESR was not significantly suppressed by 1–4 injections of SOD or inactivated SOD (data not shown). Injection of SOD without UVR produced no detectable SCs or ESR.

Injection Timing of SOD (Table II)

The number of SCs was significantly decreased by a single injection of SOD that was given either just before or immediately after UVR, while suppression in SC counts was no longer observed when the reagent was given more than 2 h before or after UVR.

Injection of DDC (Table III)

SC counts were significantly increased by a single injection of DDC (6–30 mg) that was given before UVR. Injection of DDC after UVR showed no such effects. ESR were not apparently affected by DDC (data not shown). Injection of DDC without UVR produced no SCs or ESR.

Injection of Catalase, Histidine, and Mannitol

Neither SC formation nor ESR were significantly suppressed by repeated injections of each one of the chemicals (data not shown).

DISCUSSION

Formation of SCs was significantly suppressed by SOD that was given either before or after UVR. This inhibitory effect may be caused by the enzymatic activity of the reagent, i.e., by quenching superoxide anion (O₂⁻) [9]. Absence of significant suppressive effects by injection of inactivated SOD and BSA supports our interpretation. These findings are in keeping with the results of in vitro studies by Miyachi et al [6] and suggest that O₂⁻ and a subsequent production of other active Ols through O₂⁻ following UVR may account for SC formation. Since SOD that was given 2 h after UVR no longer showed inhibitory effects, the toxicity of Ols generated within a short period (< 2 h) after UVR may be responsible for SC formation. In agreement with our results, Petkau et al [10,11] have demonstrated the reduced lethality in x-irradiated mice by a single intravenous injection of SOD, given either before or after irradiation, suggesting a prophylactic effect of SOD against radiation damage. Furthermore, the protective effect of endogenous cellular SOD against epidermal cell injury was suggested by our experiments using DDC that is known to cause a marked inactivation of copper-zinc SOD in situ by its copper-chelating capacity [8] (copper-zinc SOD is the major type in mammalian tissues). Alternatively, this result may also be expected if DDC photosensitizes cell damage (DDC shows a broad absorption spectrum between 210–380 nm, as demonstrated by means of a spectrophotometer).

Superoxide anion itself, however, is relatively unreactive toward biologic substrates such as nucleic acids, proteins, and membrane lipids. The active species are believed to be other Ols, including hydrogen peroxide (H₂O₂), hydroxy radical (OH⁻), and singlet oxygen (¹O₂), which are generated from O₂⁻ [12]. The observation that catalase (quenching mainly H₂O₂), histidine (OH⁻ and others), and mannitol (OH⁻) did not inhibit SC formation may be due to the rapid reaction of H₂O₂ and OH⁻ with substrates or to formation and reactions of the Ols in regions of the cell that are not accessible to these quenchers.

A single injection of SOD given just before UVR (< 15 min) remarkably inhibited SC production, while injection 2 h before UVR was no longer effective. Native SOD is known to be cleared quite rapidly from the circulation. Consequently, these results may be explained by supposing that during a limited period after injection of SOD, a sufficient concentration of the
reagent that achieves the protective effect against UV damage is maintained in skin, particularly in the epidermis, where the circulation is presumably much slower than in other organs. However, SOD levels in skin remain to be assayed.

Whether or not OIs play a primary role in SC formation is, however, obscure. Following UVR, OIs are highly generated by a variety of mechanisms, including photochemical reduction of epidermal protein molecules [13] and in the course of several enzyme/substrate reactions [14]. If production of OIs overcomes the quenching capacity of endogenous SOD and other quenchers, OIs may give a lethal alteration to epidermal keratinocytes, preferentially to a UV-sensitive population [15,16]. In SC formation, nuclear DNA has been proposed as the most plausible candidate for primary chemophores [17,18], and membrane damage, including lysosomal rupture [19], may be a secondary phenomenon [20]. Since repeated administrations of SOD and other OI scavengers to UV-irradiated mice failed to interfere with development of ESR, it is unlikely that OIs are involved in UV-edema. Our findings further suggest that UV-induced epidermal cell injury and UV-inflammation may occur via different mechanisms, respectively.

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REFERENCES