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SHORT REPORTS

UVB Irradiation Induces Lipid Peroxidation and Reduces Antioxidant Enzyme Activities in Human Keratinocytes *In Vitro*

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Exposure of human keratinocytes to UVB irradiation resulted in formation of conjugated double bonds and thiobarbituric acid reactive material. The activities of superoxide dismutase and catalase, enzymes which protect cells against oxidative damage, were concomitantly reduced. The present study suggests that in keratinocytes, exposure to UVB irradiation leads to a chain of events resulting in lipid peroxidation reactions accompanied by an impairment of the cellular defence system against reactive oxygen species. These phenomena may act synergistically in UVB-induced cutaneous pathological processes, such as carcinogenesis. ***Key words: Ultraviolet irradiation; Skin; Superoxide dismutase; Catalase.***

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Exposure of skin to UVB irradiation provokes an inflammatory response, mediated by oxygenated derivatives of fatty acids, eicosanoids (1,2,3). The inflammatory reaction caused by UVB irradiation is dependent on the presence of oxygen, and scavengers of oxygen radicals have been shown to reduce the inflammatory reaction, suggesting that reactive oxygen species might be involved in the inflammatory reaction (4,5). In general, the formation of reactive oxygen species elicits peroxidation reactions in cell membrane lipids (6,7,8). The lipid peroxidation reactions and large amounts of polyunsaturated fat have been suggested to be associated with an increased risk of malignancies, and high fat intake has been shown to increase tumour yield also in mice following repeated exposure to UVB irradiation (9–12). In the present paper we describe lipid peroxidation reactions and activities of antioxidant enzymes in human keratinocytes following ultraviolet B irradiation.

MATERIAL AND METHODS

Human keratinocytes (NCTC 2544, Flow Laboratories) were cultured in 90% of MEM Eagle's medium without phenol red (Sigma, USA) containing 2 mM L-glutamine and 10% fetal bovine serum (Gibco). Before UV irradiation, the cells were first washed twice with 10 ml of Hanks' buffered salt solution (pH 7.4, 5 mM glucose; HBSS). Then 10 ml of HBSS was added and the cells attached to Petri dishes were exposed to UVB irradiation. The control dishes were treated identically except for the UV exposure.

A Dr. Honle Blue-light 2001 irradiation device served as an UV light source, emitting in the UVB range (280–315 nm) a total of 1832 $\mu W/cm^2$ (maximum at 315 nm, measured using a spectroradiometer Ag&G 580/585 and an Airam UVM-8 meter with a UV-sensitive solid-state detector). The irradiance of 1832 $\mu W/cm^2$ corresponds to 133 $\mu W/cm^2$ of erythemally effective (EE) UVB, weighed at 297 nm (13). The Erythema Units were measured with a sunburn unit meter (Model 2D) of Solar Light Co., USA, Philadelphia. After the irradiation procedure, cell viability was measured by trypan blue exclusion assay (3). As measured at 1 h post irradiation, cell viability was found not to be affected either by the dose of 330 mJ/cm^2 or by 1090 mJ/cm^2 of UVB.

At 0.5 h post-irradiation cells were disrupted and homogenized with a Potter-Elvehjem homogenizer in 0.25 M sucrose – 50 mM Tris-HCl buffer, pH 7.4 and the protein content was measured using the Biuret method (14). The amount of thiobarbituric acid reactive material was measured using 0.5 ml of the cell homogenate. 0.5 ml of 30% trichloracetic acid and 50 µl of 2% (w/v) butylated hydroxytoluene in ethanol were added to the homogenate. To start the reaction, 0.5 ml of fresh thiobarbituric acid solution (375 mg/50 mlH_2O) was added and the tubes were heated in a boiling water bath for 15 min. Then the tubes were cooled and absorbance was measured at 535 nm (14). The amount of diene conjugation was analysed from 0.2 ml of the homogenate as has been described earlier (14). For the analysis of the fluorescent products, 0.2 ml of the cell suspension was mixed with 5 ml of chloroform methanol (2 : 1) and, after mixing, 2 ml distilled water was added. Then the tubes were centrifuged and the aqueous phase was discarded. The organic phase was redissolved in 2 ml of chloroform : methanol (10 : 1, v/v) and the fluorescence was measured using a Perkin-Elmer LS-5 luminescence spectrometer. Catalase activity was determined by measuring the rate of disappearance of 15 mM hydrogen peroxide at 240 nm (15). Superoxide dismutase was assayed spectro-

Table I. *Effects of UVB irradiation on lipid peroxidation reactions in human keratinocytes in culture*

The results are expressed as percentage (Mean ± SEM) of the control value ($n = 5$–6) and the control values correspond to 4.1 nmol/mg protein and to 0.4 ΔAbs/mg protein in the analysis of thiobarbituric acid reactive material (TBARM) and conjugated dienes, respectively. The fluoresence values are expressed as arbitrary fluoresence units/mg protein. The statistical significance values have been calculated using Student's *t*-test: $^{*}p < 0.05$, $^{**}p < 0.01$.

	UVB dose		
	20 mJ/cm² (0.05 EU)	330 mJ/cm² (1 EU)	1090 mJ/cm² (3 EU)
Controls	100	100	100
TBARM	116±11 $n = 5$	153±12.6** $n = 7$	187±16** $n = 4$
Conjugated dienes	104±12 $n = 5$	138±16* $n = 8$	119±6* $n = 5$
Fluorescent products	95±5 $n = 6$	95±5 $n = 6$	91±8 $n = 5$

photometrically by inhibition of epinephrine autoxidation (15). Standard curves for the enzyme activities of catalase and superoxide dismutase were drawn by using purified enzyme preparations and enzyme activities have been expressed as μg of the purified enzyme preparation/mg protein in cell homogenate. In the analysis of catalase activity, 1 μg of the enzyme preparation (bovine liver catalase; E.C. 1.11.1.6; Sigma) corresponds to 2.5 U and 1 μg of superoxide dismutase preparation (bovine erythrocytes; E.C. 1.15.1.13; Sigma) corresponds to 3.6 U.

RESULTS

The amount of thiobarbituric acid reactive material was increased in keratinocytes in a dose-dependent manner as measured at 0.5 h after an exposure to UVB irradiation (Table I). An increase could be measured also in the amount of conjugated dienes, whereas the amount of fluorescent products, a non-specific parameter of lipid peroxidation (7,8), was essentially unchanged (Table I). After the small dose of 20 mJ/cm² of UVB, the changes in the amount of thiobarbituric acid reactive material or in that of the fluorescent products were not statistically significant, however, already the dose of 330 mJ/cm² of UVB, corresponding to one erythemal unit, induced a statistically significant response. The doses of UVB used had no effect on cell viability.

The activities of catalase and superoxide dismutase were measured following the doses of 330 mJ/cm² and 1090 mJ/cm², corresponding to approximately one and three erythema units, respectively (Table II). Following the dose of 330 mJ/cm² the activity of catalase decreased from 4.3 ± 0.3 μg/mg to 2.8 ± 0.4 μg/mg protein and that of superoxide dismutase from 2.1 ± 0.2 μg/mg to 1.3 ± 0.1 μg/mg protein. Only a slight increase in the inhibition was obtained after increasing the irradiation to 1090 mJ/cm².

DISCUSSION

Ultraviolet irradiation causes an inflammatory reaction which is accompanied by changes in membrane lipid metabolism (2,3,17), and extensive polyunsaturated fat and lipid peroxidation reactions have been assumed to be related to carcinogenesis (8). In the present study the most remarkable changes were detected in the amounts of thiobarbituric acid reactive material. Malondialdehyde, which is the major thiobarbituric acid reactive substance, arises predominantly from the oxidation of polyunsaturated fatty acids with three or more double bonds (7). It is formed in vivo both as a product of lipid peroxidation and also enzymatically as a byproduct of the cyclo-oxygenase pathway in the biosynthesis of eicosanoids (7,18). It is apparent that following UVB irradiation the malondialdehyde formed via the cyclo-oxygenase pathway also contributes to the total of malondialdehyde and, furthermore, also prostaglandins may react with thiobarbituric acid (7). The significance of the cyclo-oxygenase pathway as a

Table II. *Effects of UVB irradiation on activities of catalase and superoxide dismutase*

The activities of catalase and superoxide dismutase were measured at 0.5 h after the keratinocytes (NCTC 2544) had been exposed to UVB irradiation. The calculation of the results was based on standard curves constructed using purified enzyme preparations, and the enzyme activities are expressed as μg of the standard enzyme preparation per mg of protein in the cell homogenate (Mean ± SEM, $n = 3$–4). Statistical significance was calculated using Student's *t*-test: $^{*}p < 0.05$, $^{**}p < 0.01$. SOD = superoxide dismutase.

	Catalase	SOD
Controls	4.3±0.3	2.1±0.2
UVB 330 mJ/cm²	2.8±0.4*	1.4±0.1*
UVB 1090 mJ/cm²	2.7±0.2**	1.3±0.1**

source of malondialdehyde is indicated by the fact that inhibitors of arachidonic acid metabolism can suppress formation of thiobarbituric acid reactive material and inhibit skin tumour promotion (19,20).

UVB irradiation was found to increase the numbers of conjugated double bonds in human keratinocytes in culture. As the first reaction of lipid peroxidation, conjugated double bonds arise from abstraction of hydrogen from the methylene bridges of unsaturated fatty acids, an event which may be caused e.g. by reactive oxygen species. Ultraviolet irradiation is known to be capable of inducing the formation of reactive oxygen species, which are probably involved also in the inflammatory reaction caused by UVB irradiation (4,6,7). Thus, in the present study not only increased formation of thiobarnbituric acid reactive material was observed, but also the amount of conjugated double bonds increased, confirming the finding that in keratinocytes, an in vitro exposure to UVB irradiation leads to lipid peroxidation-type cellular processes.

Superoxide dismutase and catalase act by trapping superoxide and hydrogen peroxide, respectively (5,8). Thereby they shield cells against peroxidation reactions. The activities of the enzymes decreased immediately following UVB irradiation of 1090 mJ/cm^2 to approximately 60% of the control values. The destruction of catalase and superoxide dismutase is of particular interest, as the depletion of these enzymes might lead to potentiation of the lipid peroxidation reactions following repeated exposure to UVB irradiation.

Studies on the cutaneous effects of chemicals have shown that in epidermal cells, lipid peroxidation reactions can be induced by chemical carcinogens and tumour promoters (19,20). The present study has demonstrated that in human keratinocytes an exposure to a physical irritant, UV irradiation, results in immediate lipid-peroxidation reactions accompanied by destruction of antioxidant enzymes, catalase and superoxide dismutase. this may be of relevance in the induction of UV-related cutaneous neoplasms. Nevertheless, one must bear in mind that the present study was carried out by applying cell culture techniques and it remains to be established whether the in vivo effects of UVB irradiation on human epidermis are of a similar nature.

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