Adaptive Antioxidant Response of Manganese-Superoxide Dismutase Following Repetitive UVA Irradiation

Arndt Poswig, Jutta Wenk, Peter Brenneisen, Meinhard Wlaschek, Christina Hommel, Gudrun Quel, Katrin Faisst, Joachim Dissemond, Karlis Briviba,* Thomas Krieg, and Karin Scharffetter-Kochanek

Department of Dermatology, University of Cologne, Germany; *Department of Physiological Chemistry, I Heinrich Heine University of Düsseldorf, Germany

In response to the attack of reactive oxygen species, the skin has developed a complex antioxidant defense system including among others the manganese-superoxide dismutase (MnSOD). MnSOD dismutates the superoxide anion (O$_2^-$) derived from the reduction of molecular oxygen to hydrogen peroxide (H$_2$O$_2$), which is detoxified by glutathione peroxidase to water and molecular oxygen. We have addressed the question whether MnSOD is inducible upon UVA irradiation and whether repetitive UV exposure, as practiced for the light-hardening during phototherapy of various photodermatoses, can even enhance the adaptive antioxidant response. Single exposure of four different strains of fibroblasts to UVA irradiation resulted in a dose- and time-dependent increase in specific MnSOD mRNA levels. Interestingly, repetitive UVA exposure at days 1, 2, and 3 at a dose rate of 200 kJ per m$^2$ resulted in a 5-fold induction of specific MnSOD mRNA levels following the third UVA exposure. Similar results were obtained for MnSOD activity. This adaptive response in terms of upregulation of the antioxidant enzyme MnSOD correlates with the protection against high UV doses, if cells were pre-exposed to sublethal UV doses. Importantly, MnSOD substantially differed between the tested individuals in both mRNA and activity levels. Taken together, we here provide evidence for the increasing induction of MnSOD upon repetitive UVA irradiation that may contribute to the effective adaptive UVA response of the skin during light hardening in phototherapy. Interindividual differences in the inducibility of MnSOD might account for differences in the susceptibility to develop photodermatologic disorders related to photosensitivity, photoaging, and skin cancer. The molecular basis for interindividual differences in the inducibility of antioxidant enzymes remains to be elucidated.

Key words: adaptation/MnSOD/skin/UV response. J Invest Dermatol 112:13–18, 1999

The skin is always in contact with oxygen and now is increasingly exposed to ultraviolet (UV) irradiation. Therefore, the risk of photodermatoses related to UV exposure species (ROS) has increased substantially (Darr and Fridovich, 1994). The term ROS collectively includes oxygen-centered radicals such as the superoxide anion (O$_2^-$) and the hydroxyl radical (HO·), but also some nonradical species, such as hydrogen peroxide (H$_2$O$_2$) and singlet oxygen ($^1$O$_2$), among others, all being produced in the skin upon UV irradiation. A complex antioxidant defense system has evolved in the skin and protects against ROS (Fridovich, 1989; Shindo et al., 1993); however, UV-generated ROS at least at high levels may substantially compromise the antioxidant defense of the skin (Witt et al., 1993; Biesalski et al., 1996), thus tilting the balance towards a prooxidant state (Sies, 1986). The resulting oxidative stress causes damage to cellular components and changes the pattern of gene expression, finally leading to skin pathologies such as skin cancer, phototoxicity, and photoaging (Oikarinen et al., 1985; Gallagher et al., 1989; Urbach, 1989; Kligman, 1992; Scharffetter-Kochanek et al., 1997). As shown earlier, UV-generated ROS are able to induce the synthesis and activity of various matrix-metalloproteinases responsible for the breakdown of dermal interstitial collagen and other connective tissue components in vitro and in vivo (Scharffetter et al., 1991; Scharffetter-Kochanek et al., 1992; Wlaschek et al., 1994, 1995; Brenneisen et al., 1998).

Whereas high UV doses and ROS levels are known to overwhelm the antioxidant enzymatic defense system, clinical studies have shown that repetitive low UV doses prevent phototoxicity and UV-induced pathologies upon subsequent higher UV doses (Rucker et al., 1991), indirectly suggesting that the skin – apart from other major protection mechanisms like inducible melanogenesis – is possibly equipped with an inducible adaptive antioxidant defense response. The individual antioxidant enzymes are located in specific subcellular sites and reveal substrate specificity. Among these, manganese-superoxide dismutase (MnSOD) has been the subject of particular interest in terms of an inducible antioxidant defense, because it is located in the mitochondria and is in the first line of defense against superoxide radicals produced as a by-product of oxidative phosphorylation, upon UV irradiation and during inflammatory processes. Interestingly, specific mRNA levels and MnSOD activity were found to be upregulated upon γ-irradiation in human lung fibroblasts (Akashi et al., 1995). The induction of MnSOD can be mediated by its substrate, the superoxide anion.
itself, and various cytokines such as IL-1 and TNF-α (Akashi et al., 1995). The presence of MnSOD appears to be involved in processes like tumor suppression and cellular differentiation (Harris et al., 1991; Church et al., 1993; St. Clair et al., 1994; Akashi et al., 1995; Sato et al., 1995). Superoxide anions are dismutated by MnSOD to hydrogen peroxide, which is subsequently detoxified by catalase located in peroxisomes or by glutathione peroxidase located in mitochondria and the cytosol. Isolated deficiencies of superoxide dismutases as seen for the copper-zinc superoxide dismutase (Cu/ZnSOD) in patients suffering from amyotrophic lateral sclerosis and in mice completely deficient for MnSOD, are associated with premature aging, neurodegeneration, and death (Li et al., 1996). Recently, we were able to show that isolated overexpression of MnSOD by stable transfection revealed specific resistance to the superoxide anion (O₂⁻) generating agent paraquat (Wenk, personal communication). A combined UVA/UVB ultravioletomter (Centra-UV-dosimeter, Osram, Munich, Germany) (Mutzhas et al., 1981). During irradiation, cells were incubated in phosphate-buffered saline and maintained at 37°C in a thermostatically controlled water bath. Following irradiation, phosphate-buffered saline was replaced by fresh medium with 10% fetal calf serum and the cells were incubated for various periods of time. Applied UVA doses were in the range between 50 and 300 kJ per m². Even the highest dose of 300 kJ per m² represents a dose easily acquired during 3 h sun exposure in June at latitude 40°N (Bruls et al., 1984; Kligman and Kligman, 1986), thus showing the physiologic relevance of the applied doses. RESULTS UVA irradiation results in a dose- and time-dependent induction of MnSOD on RNA and protein level In order to study the effect of different UVA doses on the synthesis and activity of MnSOD, we subjected total RNA isolated at different time points after UVA irradiation to northern blot analysis. In a parallel set of experiments, homogenized fibroblast monolayers were prepared for spectrophotometric determination of MnSOD activity using the nitroblue tetrazolium reduction method. We found a dose- and time-dependent increase in specific MnSOD mRNA levels. While already at an UVA dose of 50 kJ per m² both MnSOD mRNA species of 4 and 1 kb were slightly increased at 3 h post-irradiation, the most prominent increase occurred 1 h post-irradiation at an UVA dose of 300 kJ per m², with a maximal 2-fold induction at 9–12 h and a subsequent decline to almost basal levels 24 h post-irradiation. Interleukin-1β, a well-known MnSOD-inducing cytokine, served as a positive internal control in all experiments (Fig 1). We then determined whether the induction of MnSOD on RNA level is followed by an increase in the activity of MnSOD. There was no increase in MnSOD activity at any studied time point upon UVA irradiation at a dose of 50 kJ per m²; however, following UVA irradiation at doses of 150 kJ per m² or 300 kJ per m², a biphasic dose-dependent increase was observed. The first smaller peak occurred 3 h post-irradiation with an increase in MnSOD activity to 140% at a UVA dose of 150 kJ per m² and to 120% at a UVA dose of 300 kJ per m², compared with the mock treated control that was set as 100%. A second more impressive peak in MnSOD activity was observed 12 h post-irradiation with an increase to 160% at a dose of 150 kJ per m² and to 170% at a dose of 300 kJ per m² compared with the mock treated control. At 24 h post-irradiation MnSOD activity levels corresponded to constitutive basal levels (Fig 2). Repetitive low-dose UVA irradiation further increases specific MnSOD mRNA and activity In a first attempt to study a potential adaptive antioxidant response in fibroblasts, fibroblast monolayer cultures were irradiated three times at a dose of 200 kJ per m² with a 24 h incubation period between each irradiation. MnSOD mRNA levels and activity were determined after the first, second, and third irradiation using northern blot analysis and spectrophotometric determination of MnSOD activity following standard procedures. After a single UVA dose of 200 kJ per m², there was an increase in specific MnSOD mRNA levels with a maximal 1.5-fold induction. This induction was further increased by 1.8- and 4.0-fold after the second and third UVA irradiation compared with values after the first UVA irradiation, suggesting that repetitive UVA irradiation results in a further enhancement of specific MnSOD mRNA levels (Fig 3). These findings were also reflected on the level of MnSOD activity as shown in Fig 4. After the first UVA irradiation at a dose of 200 kJ per m², a 2-fold induction of MnSOD activity was observed compared with the mock treated control, whereas fibroblast monolayer cultures that had been subjected to two or three repetitive UVA irradiations revealed a 2.3- and 3.3-fold increase in MnSOD activity compared with the mock treated control (Fig 4).
Preirradiation of fibroblast monolayer cultures with low UVA doses confers protection from the cytotoxic effect of a subsequent high-dose UVA irradiation. In order to study the potential protective role of low-dose UV preirradiation prior to a cytotoxic high UVA dose, the viability of fibroblasts after three exposures to low-dose UVA irradiation (200 kJ per m²) and a final high-dose UVA irradiation (450 kJ per m²) (experimental groups 1–3) was determined and compared with the viability of fibroblasts that had been irradiated by a single high-dose UVA irradiation (experimental group 4) using the 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test. There was a significant protection from the cytotoxic action of high-dose UVA irradiation in fibroblast monolayer cultures, which had been preirradiated only once prior to exposure to the high UVA dose (Fig 5). The extent of protection from the cytotoxic effect of a single high-dose UVA irradiation could not be further increased by repetitive low-dose UVA irradiation, indirectly suggesting that the adaptive antioxidant response upon a single preirradiation at a low UVA dose of 200 kJ per m² is sufficient to prevent cytotoxicity by a subsequent high dose of UVA irradiation.

Evidence for interindividual differences in the constitutive activity and inducibility of MnSOD. In order to study potential interindividual differences in the constitutive synthesis and inducibility of MnSOD, fibroblast monolayer cultures were prepared by outgrowth from preputial biopsies from different healthy individuals, and MnSOD activity was determined in mock treated controls 12 h post-irradiation of fibroblasts at a dose of 200 kJ per m².
m², as well as 12 and 24 h after exposure of fibroblast cultures to recombinant IL-1β at a concentration of 20 U per ml (Table I). There were strong interindividual differences in the constitutive synthesis and the inducibility of MnSOD upon various stimuli. The spontaneous constitutive MnSOD activity among the studied fibroblast cultures of eight different individuals differed 1.7-fold, with the lowest activity of MnSOD of 1.96 U per mg and the highest activity of 3.36 U per mg. There are, however, most dramatic interindividual differences following UVA irradiation with a maximal 3.8-fold difference in MnSOD activity in different fibroblast strains. Similarly, IL-1β preincubation of individual fibroblast strains for 12 and 24 h resulted in differences in MnSOD activities ranging from 2.5 at 12 h post-treatment to 2.4 at 24 h post-treatment. Strong interindividual differences in the spontaneous activity and inducibility of MnSOD may, thus, confer differences in the interindividual susceptibility for the development of skin aging and cancer.

**DISCUSSION**

The incidence of nonmelanoma and melanoma skin cancers, by far the most common forms of human cancer, has increased substantially over recent decades, with annually new cases for nonmelanoma skin cancer in the U.S.A. between 600,000 (Kwa et al., 1992) and 1,200,000 (Miller and Weinstock, 1994). This tendency has been noted worldwide (for review, see Scharffetter-Kochanek and Mauch, 1994). Also, the incidence of photoaging has increased tremendously. The increasing incidence of skin malignancies and photoaging is considered to be at least in part due to UV-generated ROS. This is particularly relevant in terms of UV irradiation, which is predicted to increase on the earth’s surface upon ozone depletion (Coldiron, 1992; Slaper et al., 1996). Therefore, an understanding of the antioxidant enzymes within resident skin cells and the regulation upon exposure to UV-generated ROS is important for our understanding of UV-protective mechanisms in skin, urgently required for proper individual risk assessment and the protection of sun-exposed skin.

The overall aim of this study was therefore to define adaptive antioxidant enzymatic mechanisms that confer protection to resident cells against the oxidative attack following UVA irradiation. We found that UVA irradiation of fibroblast monolayer cultures in vitro resulted in a significant increase in MnSOD on both mRNA and protein levels. The administered UVA doses in our experiments

---

**Figure 4. Induction of MnSOD activity after repetitive UVA irradiation.** Fibroblast monolayer cultures had repetitively been subjected for three times to UVA irradiation at a dose of 200 kJ per m² with a 24 h incubation period between each irradiation. MnSOD activity was determined as described in Materials and Methods 12 h after each irradiation and expressed as percentage of the mock treated control. The experiments were performed in triplicate in three independent experiments with SD < 5%; *p < 0.005 compared with the mock treated controls that were set at 100%.

**Figure 5. Low-dose UVA preirradiation confers protection from cytotoxicity of a subsequent high dose of UVA irradiation.** Four experimental groups were defined, as indicated on the y axis. In experimental group 1, fibroblasts had been exposed once, in experimental group 2, fibroblasts had been exposed twice, and in experimental group 3, fibroblasts had been exposed three times to low doses of UVA irradiation (200 kJ per m²) prior to being exposed to a subsequent high UVA dose (450 kJ per m²). In a parallel experiment fibroblasts of experimental group 4 were directly exposed to a single high UVA dose (450 kJ per m²). Viability of low-dose preirradiated and subsequently high-dose irradiated fibroblasts (experimental groups 1, 2, and 3) and the viability of fibroblasts that had been exposed to a single high dose (experimental group 4), was determined using the 3-(4,5-di-methylthiazol-2-yl)-2–5-diphenyltetrazolium bromide assay as described in Materials and Methods. Viability was expressed as percentage of the viability of mock treated fibroblasts that was set 100%. The viability of fibroblasts of experimental groups 1, 2, and 3 was compared with that of fibroblasts of experimental group 4. The experiments were performed in triplicate in three independent experiments with SD < 8%; *p < 0.001.

**Table I. Interindividual differences in the constitutive and inducible activity of MnSOD**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Age (y)</th>
<th>Mock treated (200 kJ per m²)</th>
<th>UVA (200 kJ per m²)</th>
<th>IL-1β (20 U per ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KU</td>
<td>52</td>
<td>1.96</td>
<td>3.14</td>
<td>2.74</td>
</tr>
<tr>
<td>ER</td>
<td>5</td>
<td>3.22</td>
<td>3.7</td>
<td>6.44</td>
</tr>
<tr>
<td>PL</td>
<td>9</td>
<td>3.28</td>
<td>3.64</td>
<td>6.78</td>
</tr>
<tr>
<td>GO</td>
<td>59</td>
<td>3.6</td>
<td>5.43</td>
<td>n.d.</td>
</tr>
<tr>
<td>MB</td>
<td>5</td>
<td>2.6</td>
<td>1.95</td>
<td>3.5</td>
</tr>
<tr>
<td>ED</td>
<td>10</td>
<td>2.8</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>FK</td>
<td>6</td>
<td>3.04</td>
<td>4.0</td>
<td>n.d.</td>
</tr>
<tr>
<td>FD</td>
<td>9</td>
<td>3.36</td>
<td>7.52</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

*MnSOD activity was determined in fibroblast monolayer cultures from different healthy individuals after treatment with UVA or IL-1β at the indicated dose and concentration. MnSOD activity is expressed in U per mg. n.d., not done. The experiments were performed in three independent experiments with SD < 10%.*
are physiologically relevant and can be easily acquired (Bruls et al., 1984; Kligman and Kligman, 1986), thus underlining the relevance of our findings. Furthermore, we found that repetitive low-dose UVA irradiation could even dramatically enhance the synthesis and activity of MnSOD, and that this induction is related to a substantial protection against the cytotoxic effect of a subsequent high-dose UVA insult.

Earlier, it was reported that chronic UV exposure of hairless mice for 2 h per day with a source mainly emitting UVA including 2% UVB, resulted in a significant increase in SOD activity that, however, following continued irradiation for 24 wk, substantially decreased below the level of mock treated animals (Maeda et al., 1991), whereas glutathione peroxidase–activity remained raised. These results suggest that chronic UV exposure for months, even at suberythermal doses, may compromise the SOD-dependent antioxidant defense. It remains to be determined whether short-term repetitive UVA irradiation – as observed in our in vivo experiments – may also result in an increase in MnSOD activity in human skin in vivo and whether repetitive irradiation over months – similar to the findings in mice – will compromise SOD activity in human skin. The MnSOD is the first line of defense in the protection against the attack of the superoxide anion and reveals tumor suppressing properties (Church et al., 1993). Given that the gene of the MnSOD is deleted in some but not all melanomas, our finding of an adaptive and superinducible response of the MnSOD upon repetitive UVA irradiation is particularly interesting, and may have in vivo relevance for the protection of cellular structures and functions. In fact, loss of cellular redox homeostasis, due to a failure of the antioxidant enzymes, as occurs in severe sunburns, represents a major risk factor in the etiology of melanoma (MacKie and Aitchison, 1982; Setlow et al., 1993; Autier et al., 1994; Klein-Szanto et al., 1994; Holly et al., 1995; Donawho and Wolf, 1996).

Similar to nonmelanoma skin cancer, melanoma also undergoes a multistage development towards the fully malignant phenotype. A failure of proper detoxification of ROS by antioxidant enzymes results in an increase of the load of ROS that had been shown to be involved in all three stages of carcinogenesis (Cerutti et al., 1994), and is produced by both the UVA and the UVB components of sunlight (Masini et al., 1994). As well as causing permanent genetic changes involving protooncogenes and tumor suppressor genes, ROS activate cytoplasmic signal transduction pathways that are related to growth, differentiation, senescence, and tissue degradation (Cerutti, 1994; Brenneman et al., 1998). The adaptive induction of the MnSOD upon UVA irradiation definitely contributes to the detoxification of the superoxide anion (O_2·−), which if not detoxified could at least partly drive the Fenton reaction resulting in the generation of the highly aggressive hydroxyl radical (HO·) (Darr and Fridovich, 1994). The overall relevance of MnSOD becomes particularly clear in MnSOD deficient mice recently generated by gene targeting and homologous recombinant techniques. Complete absence of MnSOD results in lethality of newborn mice due to defects in the oxidative phosphorylation pathway resulting in a dilatative cardiomyopathy as early as 10 d after birth (Li et al., 1995).

Single UVA irradiation and to a significantly greater extent repetitive irradiation for three times, resulted in a dramatic increase in the two specific 1.0 and 4.0 kb sized MnSOD mRNA species as well as in MnSOD activity. Whereas other investigators found that the half times of the MnSOD-specific transcripts substantially differed (Melendez and Baglioni, 1993), we did not detect any difference in the steady-state mRNA levels of both species upon UVA irradiation. It is still unclear whether both transcripts are equally required for the overall synthesis and activity of MnSOD (Melendez and Baglioni, 1993). MnSOD activity occurred in two peaks upon UVA irradiation. Interestingly, this biphasic time course in the induction of MnSOD activity has earlier been described by Oberley et al. (1987) following x-irradiation of mouse heart. Based on the lack of [3H]arginine incorporation, the nature of the first peak appears to be due to a MnSOD precursor protein that becomes active upon still unidentified stimuli, whereas the second peak in MnSOD activity – as shown by the dramatic incorporation of [3H]arginine – in fact, requires new protein synthesis (Oberley et al., 1987). The biphasic response in MnSOD activity may be of physiologic relevance in that the first peak may confer protection against O_2·− early after the oxidative insult, whereas the second peak may protect cells from repetitive UVA irradiation or from a sustained ROS-generating inflammation following UVA irradiation or other ROS-generating stimuli.

As to the regulation of the induced synthesis and activity of MnSOD upon UVA irradiation, it is most likely that O_2·− and IL-1α and IL-1β, both released following UVA irradiation, qualify to mediate UVA effects (Kupper et al., 1987; Masini et al., 1994; Wlaschek et al., 1994). At least, both O_2·− and IL-1α and IL-1β are well-known stimuli of the MnSOD (Marklund, 1992; Akashi et al., 1995). Preliminary data, in fact, show that neutralizing antibodies against IL-1α and IL-1β can at least partly suppress the induction of MnSOD activity following UVA irradiation (data not shown). It is, however, unresolved whether O_2·− and IL-1 represent completely independent mechanisms or act as causally related sequential events. So far, there is some evidence that IL-1 is able to induce the generation of ROS in fibroblasts (Meier et al., 1989; Lee et al., 1996).

Low-dose preirradiation definitely conferred protection from the cytotoxic effects of a subsequent high dose of UVA irradiation. Even though we only provide correlative evidence, it is most likely that the induction of MnSOD at least in part contributes to this protection.

Our finding that strong interindividual differences exist in terms of spontaneous activity and inducibility of MnSOD upon stimulation with UVA irradiation or IL-1β, may represent a molecular determinant conferring differences in the observed interindividual susceptibility for the development of premature aging and skin cancer. Further elucidation of the adaptive superinducible antioxidant defense systems in physiologic and pathologic conditions will not only allow individual risk assessment but will also stimulate the development of novel therapeutic strategies.

We thank Irene Smith (Genetech, San Francisco, CA) for supplying us with the 3H-DNA done for human MnSOD. Karin Schaffner-Kochanek was a recipient of a Heisenberg grant from the German Research Foundation (DFG).

REFERENCES


Cerutti P, Gosh R, Oya Y, Amstad P: The role of the cellular antioxidant defense of a Heisenberg grant from the German Research Foundation (DFG).