Antioxidant Enzyme Activity in Human Stratum Corneum Shows Seasonal Variation with an Age-Dependent Recovery

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The stratum corneum, as the body's principal barrier to the environment, is continuously exposed to environmental sources of reactive oxygen species like ultraviolet light, ozone, and pollution. Reactive oxygen species are believed to be involved in cancer, aging, and inflammatory skin disorders. We have developed a method to measure catalase and superoxide dismutase activity on tape strippings from the human stratum corneum and demonstrated a gradient of antioxidant enzyme activity across the stratum corneum with decreasing levels towards the skin surface. Sun exposure resulted in a seasonal variation of the catalase activity in stratum corneum, with low activities in summer and higher activities in winter for the same person, whereas superoxide dismutase activity in stratum corneum did not seem to vary in those conditions. Exposure of human skin to broadband ultraviolet-A resulted in a dose-dependent deactivation of the catalase activity in stratum corneum within 24 h, whereas exposure to ultraviolet-B had no effect. Superoxide dismutase activity in stratum corneum was not affected by ultraviolet-A or ultraviolet-B irradiation within 24 h. After exposure to a dose of 15 J per cm^2 broadband ultraviolet-A, full recovery of the catalase activity occurred in 3–4 wk at an age-dependent rate. We conclude that sun exposure results in a disturbed catalase to superoxide dismutase ratio in the stratum corneum. This may lead to an increased vulnerability to oxidative damage in stratum corneum barrier components. These results therefore stress the importance of providing efficient protection for this internal defense mechanism in sun-exposed areas of the skin. ***Key words: aging/catalase/stratum corneum/superoxide dismutase/ultraviolet. J Invest Dermatol 120:434–439, 2003***

The free radical theory of aging postulates that aging is caused by accumulation of damage resulting from free radical reactions and may be associated with the environment, disease, and intrinsic aging. A variant of this theory is called the mitochondrial theory of aging, which states that (DNA) damage in mitochondria accumulates with time and is responsible for a disturbed capacity for energy production and thus partly responsible for the aging process. The antioxidant protective mechanisms therefore play a crucial role of neutralizing reactive oxygen species (ROS) in order to avoid or minimize damage to proteins, lipids, and DNA.

The skin is constantly exposed to environmental sources of ROS like ultraviolet (UV) light, ozone, and air pollution. To protect against oxidative damage the skin is equipped with a large network of enzymatic antioxidant defense systems, like catalase, superoxide dismutase (SOD), and glutathione peroxidase, and nonenzymatic antioxidants, like vitamin E, ascorbate glutathione, and uric acid, which work in synergy to counterbalance oxidative stress (Thiele *et al*, 2001). Both SOD and catalase are considered as major antioxidant enzymes in the stratum corneum (SC). SOD catalyzes the dismutation of the superoxide anion into oxygen and hydrogen peroxide. Different types of SOD can be discerned, dependent on the redox-active metal at the catalytic site. Cu/Zn SOD is localized mainly in the cytosol and nucleus whereas Mn SOD is located in the mitochondria, which may suggest a different functionality. Catalase is a tetrameric, heme-containing redox enzyme. It neutralizes hydrogen peroxide to water and oxygen and thereby prevents excessive hydrogen peroxide build-up. Catalase is located intracellularly within the mitochondria and the peroxisomes. The enzymatic activity of SOD and catalase is about 2.3- and 8-fold higher in the epidermis compared to the dermis (Shindo *et al*, 1994a).

Low catalase activity levels have been measured in the skin of patients with vitiligo (Schallreuter *et al*, 1999), polymorphic light eruption (Guarrera *et al*, 1998), physical urticaria (Briganti *et al*, 2001), and xeroderma pigmentosum (Hoffschir *et al*, 1998), and in human epitheliomas (Rabilloud *et al*, 1990). In all those skin disorders the decreased catalase activity might be considered as a very sensitive marker for the increased susceptibility of the skin to external stimuli (Briganti *et al*, 2001). *In vitro* studies emphasize the importance of maintaining the optimal ratio between the different antioxidant enzymes and its relation to cellular senescence and sensitivity to oxidative stress (Amstad *et al*, 1991; de Haan *et al*, 1996). The observation that the life span of *Caenorhabditis elegans* could be extended after supplementation with SOD/catalase mimics supports the hypothesis that an increased efficiency with which ROS can be neutralized by antioxidant enzymes may counteract the aging process (Melov *et al*, 2000).

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Abbreviations: GSH-Px, glutathione peroxidase; HSP, heat shock protein; IC_{50}, inhibitory concentration 50; SC, stratum corneum, SOD, superoxide dismutase.

Conflicting data exist about the variation of the antioxidant defense capacity with aging. For SOD no or only a small decrease in enzyme activity is observed during aging in human (Rhie *et al*, 2001b), rat (Tahara *et al*, 2001), and mouse (Lopez-Torres *et al*, 1994) skin. Concerning catalase most studies did not reveal any age-associated difference in the antioxidant enzyme level in skin of mice (Lopez-Torres *et al*, 1994) or rats (Tahara *et al*, 2001), or in human skin fibroblasts (Keogh *et al*, 1996). Some authors, however, suggested a decrease in the catalase activity levels in skin fibroblasts from older donors (Lu *et al*, 1999), whereas others observed a higher catalase activity in epidermal skin of older people (Rhie *et al*, 2001a; 2001b).

It is well documented that the catalase activity is lower after an acute dose of UVA. This has been demonstrated in mouse skin (Fuchs *et al*, 1989; Shindo *et al*, 1994b), as well as in human skin fibroblasts (Moysan *et al*, 1993; Shindo and Hashimoto, 1997) and keratinocytes (Punnonen *et al*, 1991b). Chronic UVA exposure suppressed the catalase activity in hairless mouse skin (Okada *et al*, 1994), whereas acute or chronic UVB irradiation had no effect on the catalase activity in mice (Iizawa *et al*, 1994). After an acute dose of UVA the catalase activity in mouse skin recovered slowly, with activities still low after 5 d (Shindo *et al*, 1994b). Only few publications report on the catalase activity in human skin *in vivo*, however, based on the analysis of skin biopsies (Rhie *et al*, 2001a; 2001b), epidermis obtained using the suction blister technique (Punnonen *et al*, 1991a), or pathologic skin (Guarrera *et al*, 1998). Remark that although many studies have investigated whole epidermal antioxidant levels, few to no data have been published so far on the catalase and SOD activities in the human SC.

Here we used a noninvasive method to determine SOD and catalase activity *in vivo* in human SC by measuring the enzyme activity directly on tape strippings. Catalase data from a large number of panelists were used to investigate the age-associated enzyme activity. This method was also used to study the *in vivo* enzymatic activity of SOD and catalase in SC upon acute and chronic sun exposure. The recovery of the catalase activity after an acute irradiation was studied.

MATERIALS AND METHODS

Subjects Healthy volunteers, 18–80 y of age, with Fitzpatrick skin type II or III, who were in good general health, free of any dermatologic disorders, and who gave their informed, written consent, were allowed to enter the studies. In each study 20 successive D-squame® tape strippings (CuDerm, Dallas, TX) were collected on the inner upper arm and the dorsal forearm, and stored at –80°C until further analysis. On a subset of panelists ($n = 20$ for catalase activity determinations and $n = 10$ for SOD activity determinations) seasonal variations were evaluated by sampling D-squame® tape strippings from the same person in winter and in summer. Other subsets of panelists were exposed to a fixed dose of UVA ($n = 8$) or UVB ($n = 4$) for evaluating enzyme deactivation, whereas recovery was evaluated in a separate experiment on 26 subjects by sampling SC tape strippings at various time points after the irradiation. For the experiments on catalase recovery, the subjects were not allowed to expose their skin to the sun or a sun bed in the month preceding and the month following the UV exposure.

Irradiations The UVA source consisted of a high flux broadband UVA lamp with an output of 17.6 mW per cm^2 UVA (Psorisan® 900 with H1 filter and additional glass plate to remove UVB, Dr. Hönle Medizintechnik, Munich, Germany). The UVB source was a 312 nm tube (VL-115 M, Bioblock Scientific, Lille, France) with an output of 1.0 mW per cm^2. The UV dosage was measured with a radiometer (VLX-3 W, Bioblock Scientific, Lille, France) at 365 nm for UVA and 312 nm for UVB exposures.

Detection of catalase activity on tape strippings from human SC All reagents used were from Acros Organics (Geel, Belgium) unless mentioned. The method is based on the unique ability of catalase to use a lower alcohol like methanol as a hydrogen donor, resulting in the formation of formaldehyde as described by Johansson and Borg (1988). The detection of catalase activity on tape strippings from SC was modified from Guarrera *et al* (1998) as described in Giacomoni *et al* (2000). Briefly, in a 24-well plate (Corning, Costar, Acton, MA) the D-squame® tape strippings were fixed on top of a well containing a solution of 5.9 M methanol and 4.2 mM hydrogen peroxide in 68.2 mM phosphate buffer pH 7.0. The tape strippings were firmly fixed by a cover and the well plate was turned upside down and incubated for 60 min with continuous shaking. After the incubation the tape strippings were removed and stored in a tube for protein analysis, whereas the remaining solution in the wells was used for quantification of the formaldehyde concentration. Formaldehyde was quantified spectrophotometrically at 550 nm after reaction with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald, Sigma-Aldrich, St. Louis, MO), followed by complete oxidation with potassium periodate at high pH. One unit (U) of catalase is defined as the amount of enzyme that causes the formation of 200 μM formaldehyde in the test conditions specified above. Specific catalase activity was obtained after normalizing to the total protein content on the stripping. Catalase inhibition experiments were performed with 3-amino-1,2,4-triazole (Sigma-Aldrich).

Detection of SOD activity on tape strippings from human SC All reagents were from Sigma-Aldrich. Stock solutions of all reagents were prepared in a 100 mM phosphate buffer at pH 7.8. Superoxide was generated *in situ* by the hypoxanthine/xanthine oxidase system according to McCord *et al* (1977). The detection of the superoxide radical is based on luminol-dependent chemiluminescence. The assay was adapted to a well plate method. A D-squame® tape stripping was perforated with a punch and the small piece of stripping ($Ø = 5$ mm) was put on the bottom of a well of an opaque 96-well plate (Corning, Costar, Acton, MA). Xanthine oxidase, luminol, and hypoxanthine were added and the chemiluminescence intensity was recorded immediately for 30 s with a luminometer (Luminoskan, Ascent, Labsystems, Helsinki, Finland). The end concentrations of the reagents in the wells were 20 mU per ml, 50 μM, and 367 μM for xanthine oxidase, luminol, and hypoxanthine, respectively. As an external standard we used SOD from bovine erythrocytes, in which case a blank strip was put in the well. The integrated area under each curve was calculated from exactly 10 to 30 s by the Ascent software™. One unit of SOD is defined as the amount of enzyme that results in a 50% reduction of the integrated area compared to the experiment without SOD. After the measurement the tape strippings were removed and stored in a tube for protein analysis. Specific SOD activity was obtained after normalizing to the total protein content on the stripping.

Protein analysis on tape strippings The total protein amount on the D-squame® tape stripping was quantified as the total amount of amino acids after acid hydrolysis at elevated temperature. The D-squame® tape strippings were incubated in a tube containing 1.5 ml HCl 6 N at 120°C for 20 h for complete hydrolysis. After centrifugation the supernatant was diluted (1:20) in 1 M borate buffer pH 9. The total amino acid content of this solution was determined by high pressure liquid chromatography with fluorescence detection after derivatization with orthophtalaldehyde (modified from Lindroth and Mopper, 1979). This sample pretreatment allowed the estimation of the total protein amount on the strip. The total protein amount was always determined on the same stripping as the enzymatic analysis (SOD or catalase) occurred. Keratin being the most abundant protein in SC, we used a keratin standard (ICN, Irvine, CA) to establish a calibration curve.

Data analysis The experiments on seasonal variation of enzymatic activity were analyzed with repeated ANOVA and further analyzed using the Tukey HSD test to determine whether differences were statistically significant among the various experimental conditions. The data set obtained after acute UV irradiation was also analyzed with repeated ANOVA and further analyzed using Dunnett's test. The SOD data as a function of age were analyzed with a Student *t* test. The linear correlation between parameters was evaluated by the Pearson product–moment correlation coefficient (*r*). Results are presented as the mean value and the error bars correspond to the standard error of means. Differences between data sets were defined as being significant (*, $0.05 > p > 0.01$), highly significant (**, $0.01 > p > 0.001$), or very highly significant (***, $p < 0.001$).

RESULTS

3-Amino-1,2,4-triazole is known to be a specific inhibitor of catalase (Darr and Fridovich, 1986). The addition of 3-amino-1,2,4-triazole at several concentrations to the catalase assay reaction mixture resulted in a dose-dependent inactivation of the catalase activity on the tape stripping with an IC_{50} of 20.6 mM. Addition

of the same inhibitor to a standard of catalase from bovine erythrocytes showed a very similar behavior with an IC_{50} of 17.1 mM. This confirms the presence of enzymatically active catalase on the tape stripping, detectable with this assay.

On a series of 20 successive D-squame® tape strippings, specific catalase and SOD activities were determined. Because it was not possible to analyze both specific catalase and SOD activities on the same stripping, we used alternately the odd strippings for the specific catalase activity and the even strippings for the specific SOD activity. In human SC a gradient of specific catalase and SOD activity was found across the first 10 layers, with lower values towards the skin surface (**Fig 1**). From layer 10 on both the specific SOD and catalase activities reached a plateau. Therefore the specific enzymatic activity was determined on layer 14 or deeper for all further experiments. Remark that all enzymatic activity data on tape strippings were normalized to the total protein amount, which was measured on the same tape strippings.

The specific SOD and catalase activity was evaluated on tape stripping samples collected from the same person in summer and in winter at two distinct areas of the arm. The inner upper arm was considered as a sun-protected site, and the dorsal forearm was considered to be a sun-exposed site. The SOD activity in human SC was not affected by chronic sun exposure and no difference in SOD activity was found between samples taken from the same person in winter and in summer at an exposed and a nonexposed site (**Fig 2**). On the other hand the specific catalase activity on tape strippings from the dorsal forearm was significantly lower in summer compared to winter ($p = 0.0002$) (**Fig 2**). Furthermore in summer the specific catalase activity was significantly lower on a sun-exposed site compared to a sun-protected site ($p = 0.0002$). Even for samples taken at a sun-protected site, there was a statistically significant decrease in catalase activity in summer compared to winter ($p = 0.006$). Note that all panelists are living in a mild maritime climate with a daily maximum temperature of about 22°C with 200 h of sunshine in the summer and 6°C with 56 h of sunshine in the winter.

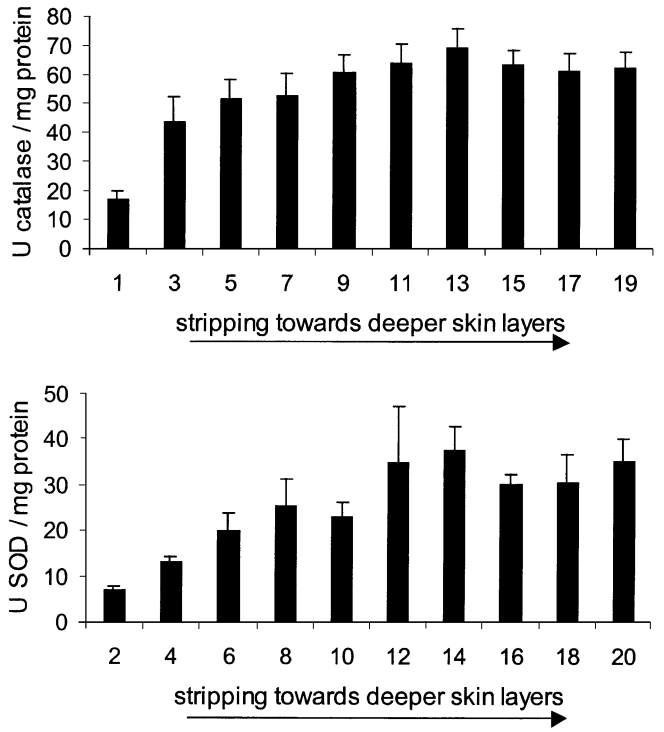

Figure 1. The specific activities of both catalase and SOD show a gradient across the first 10 layers of the human SC, with lower activity towards the skin surface. Tape stripping samples were taken on the inner upper arm of the panelists. The specific enzyme activity was measured directly on the tape strippings, by using alternating strippings for specific catalase and SOD measurements, as described in *Materials and Methods*. Results are expressed as mean values for the specific enzyme activity ± SEM. The upper frame shows the results for catalase ($n = 10$). The lower frame shows the results for SOD ($n = 4$).

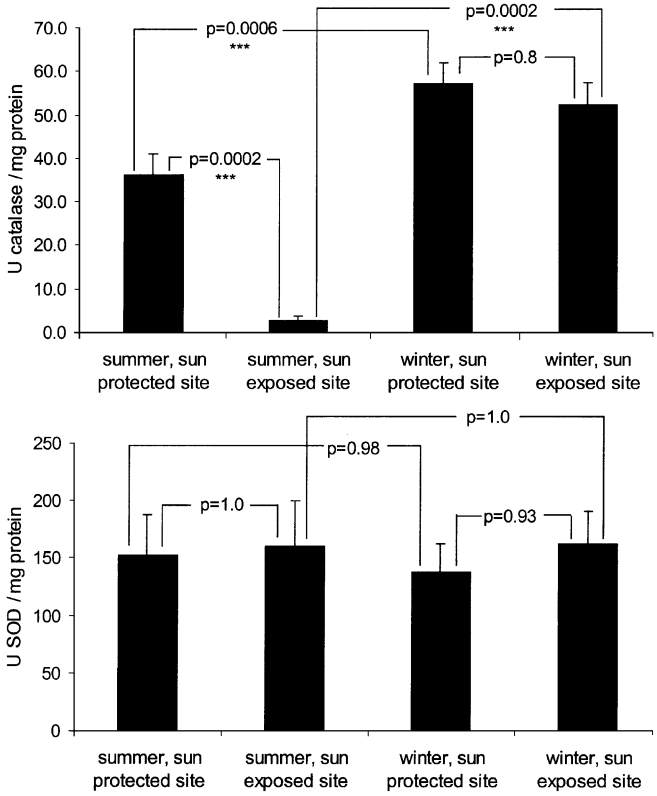

Figure 2. Seasonal variation is only observed for the specific catalase activity in human SC and not for the SOD activity. Tape stripping samples were collected on the inner upper and lower outer arm in summer and in winter on the same panelists. Results are expressed as mean values for the specific enzyme activity ± SEM. The upper frame shows the results for catalase ($n = 20$). The lower frame shows the results for SOD ($n = 10$).

Evaluation of 107 tape strippings taken in winter on the inner upper arm of people who never used a sun bed for tanning revealed that there seemed to be no correlation between the specific catalase activity in SC and the age of the panelist ($p = 0.32$). Data of specific SOD activity on a limited subset of panelists also showed no statistically significant difference ($p = 0.14$) in SOD activity between a group of young and old panelists [91 ± 7.3 and 77 ± 6.3 U SOD per mg protein for the young panelists ($n = 4$, aged 26–37 y) and old panelists ($n = 4$, aged 62–78 y), respectively].

We evaluated the effect of controlled UV exposures on SOD and catalase activity in SC. In line with the chronic sun exposure experiments the specific SOD activity, evaluated 24 h after the exposure, was not affected by 15 J per cm^2 of broadband UVA or 0.15 J per cm^2 [0.75 minimal erythema dose (MED)] of UVB. As shown in **Fig 3** the specific catalase activity decreased in a statistically significant and dose-dependent way after exposure to broadband UVA (dose ranging from 0 to 15 J per cm^2). Irradiation with a single dose of UVB (0.15 J per cm^2) did not affect the specific catalase activity in SC within 24 h following the exposure.

When a dose of 6 J per cm^2 UVA was repeatedly given on three consecutive days, a cumulative deactivation of SC catalase was observed (results not shown). The decrease was proportional to the total dose of UVA that was administered and there did not seem to be a substantial recovery in catalase activity during the 24 h interval between two exposures.

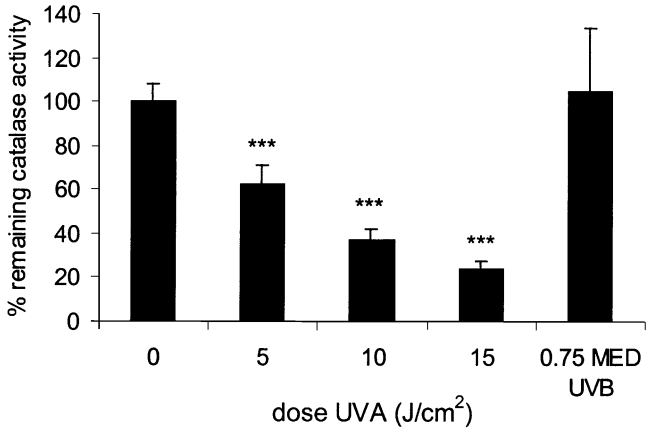

Figure 3. UVA but not UVB irradiation results in a dose-dependent decrease of the specific catalase activity in human SC. Tape stripping samples were collected on the lower arm of the panelists after exposure to various doses of UVA or to 0.15 J per cm^2 (0.75 MED) UVB. A nonirradiated site was used as the control to express the specific catalase activity as a percentage. Results are expressed as mean values ± SEM and significance is calculated compared to the nonirradiated site. Eight and four panelists were included for the UVA and UVB experiment, respectively.

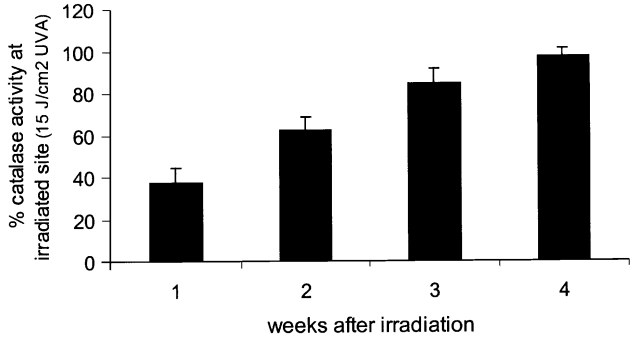

Figure 4. The specific catalase activity is completely recovered 4 wk after UVA irradiation (15 J per cm^2). Tape stripping samples were collected on the lower arm of the panelists at different time points after exposure to 15 J per cm^2 UVA. A nonirradiated site at each time point was used as the control to express the specific catalase activity as a percentage. Results are expressed as mean values ± SEM. Twenty-six panelists were included in this study.

The recovery of the specific catalase activity in SC after a single exposure to 15 J per cm^2 broadband UVA was monitored over a period of 4 wk ($n = 26$, mean age 47 y). **Figure 4** shows the residual specific catalase activity expressed as a percentage. Within the first week after the exposure the catalase activity in the SC did not recover at all. A gradual recovery of the specific catalase activity in SC was observed after the first week and it took about 4 wk for complete recovery. For samples taken 3 wk after the exposure there was a clear correlation between the residual catalase activity and the age of the panelist, as shown in **Fig 5**. At this time point the specific catalase activity in SC of younger subjects was already completely recovered whereas older subjects needed at least 4 wk to reach a full recovery.

DISCUSSION

Our skin is a major target for UV light, ozone, and pollution but is also constantly exposed to oxygen. For that reason efficient defense mechanisms of the skin to protect against oxidative damage

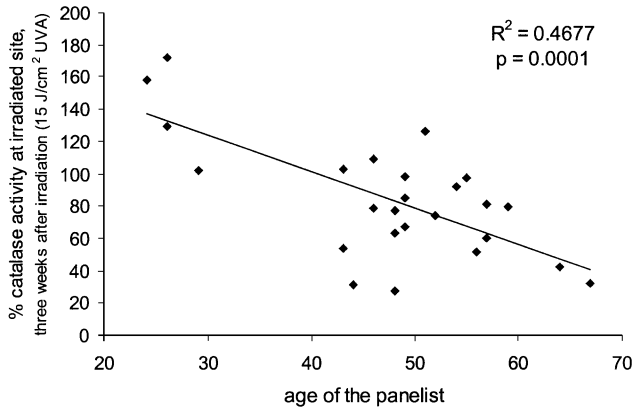

Figure 5. The recovery of the catalase activity in human SC following UVA irradiation is age dependent. Tape stripping samples were collected on the lower arm of the panelists 3 wk after exposure to 15 J per cm^2 UVA. A nonirradiated site was used as the control to express the specific catalase activity as a percentage. Results are expressed as mean value ± SEM. Twenty-six panelists were included in this study.

are essential. Two foremost antioxidant defense enzymes in the SC are SOD and catalase. The dismutation of superoxide by SOD results in the production of hydrogen peroxide, which is subsequently converted to water and oxygen through a reaction that is catalyzed by catalase. An imbalance in the ratio of antioxidant enzymes may thus contribute to an excessive accumulation of ROS, increasing oxidative stress and damage.

In this study we presented a noninvasive method for the *in vivo* determination of SOD and catalase activity in human SC, by measuring the enzymatic activity directly on D-squame® tape strippings. All enzymatic activity data on tape strippings were normalized to the total protein amount that was present on the same tape strippings. It has been reported that the amount of SC removed by tape stripping decreases towards deeper layers. This can be explained by an increased cohesion between cells at deeper layers. We adopted an acid hydrolysis step at elevated temperatures to quantify the combination of insoluble and soluble proteins on the tape stripping. As such it is presumed that normalization to the total protein amount on tape strippings resulted in an efficient correction for differences in the amount of sample collected on the tape strippings. This should decrease strip-to-strip and person-to-person variation of the data and should enable comparison of results.

Guarrera *et al* (1998) measured the catalase activity in SC with a similar method and demonstrated a linear increase of the catalase activity in human SC from the surface down to layer 4. We evaluated a larger cross-section of the SC down to layer 20, and showed a decrease in both specific SOD and catalase activity towards the skin surface (**Fig 1**). A constant value is measured approximately from layer 10 onward. Recent results on pooled tape strippings of the human SC also showed increasing activities with depth over the first 10 layers for the enzymes β-glucocerebrosidase, acid phosphatase, and phospholipase A2 (Redoules *et al*, 1999). Analogous observations were published with respect to several nonenzymatic antioxidants, showing the existence of a redox gradient across the SC with decreasing vitamin E (Thiele *et al*, 1998), vitamin C, uric acid, and glutathione concentrations (Weber *et al*, 1999) towards the skin surface. Several reasons for the existence of such a gradient can be put forward. (i) The outer layers of the skin are exposed to high loads of different kinds of environmental sources of ROS, like UV light, air pollution, and ozone in the presence of relatively high concentrations of oxygen. It can therefore be expected that the oxidative damage to proteins and other biomolecules is higher towards the outer layers of the SC. This was already demonstrated for tape strippings where an increase in keratin oxidation was observed towards the outer

layers of the SC (Thiele *et al*, 1999). (ii) In the case of catalase, its activity can most probably also be lost by a mechanism of direct photodestruction (Shindo *et al*, 1994a, and our observation), which will also contribute to the gradient. (iii) Interestingly, a reversed activity gradient is observed for the trypsin-like or chymotrypsin-like activities over the first 10 layers of the SC with higher activity towards the surface (Redoules *et al*, 1999). These proteases are supposed to be involved in the desquamation process and are presumably quite resistant to the oxidative stress that leads to the activation of the other enzymes. Here we hypothesize that a gradual enzymatic proteolysis of the antioxidant enzymes by these proteases may also partly contribute to their deactivation towards the SC surface.

Some confusing data are found in the literature concerning the age-related changes of the catalase activity in the skin. We reported earlier that there seemed to be no correlation between the specific catalase activity in SC on the inner upper arm and the age of the panelist for a test group of 176 volunteers (age range 18–77 y) (Giacomoni *et al*, 2000). Here we further expanded the total number of samples and evaluation of these data revealed that the specific catalase activity of people who regularly used sun beds for tanning was significantly lower ($p = 0.03$) compared to subjects who never used a sun bed for tanning (57 ± 5 and 70 ± 4 U catalase per mg protein for the users ($n = 83$) and nonusers ($n = 107$) of sun bed tanning, respectively), which confirms the pronounced loss of catalase activity in the SC upon exposure to UV light. It follows that the effect of intrinsic aging on the catalase activity in the human SC can only be estimated if direct deactivation of the catalase activity is avoided. We therefore excluded all results on sun bed tanners and restricted the observation to samples that were collected in winter. Analysis of the 107 tape stripping samples, selected to the criteria described above, suggested that the specific catalase activity in SC does not vary with age ($p = 0.32$).

We observed a seasonal variation in the enzymatic activity of catalase, with low activities in summer, especially at sun-exposed sites, and high activities in winter (**Fig 2**). The activity of SOD was not affected, however. Amstad *et al* (1991) reported that a decrease in the catalase to Cu, Zn SOD ratio in mouse epidermal cells may result in an *in vitro* chronic prooxidant state mediated by hydrogen peroxide, which resulted in low reduced glutathione concentrations, hypersensitivity to the formation of DNA single-strand breaks, growth retardation, and killing by an extracellular burst of superoxide. Our results imply that the ratio of specific catalase to SOD is dramatically lower in summer compared to winter in the upper layers of the human SC. It can therefore be expected that a reduction in the catalase to SOD ratio may lead to a local overproduction of hydrogen peroxide and thus an increase in oxidative stress. This is in accordance with very recently published data on human SC where it is shown by immunohistochemistry that acute and chronic UV exposure lead to a significant depletion of the antioxidant enzyme expression especially in the SC and epidermis concomitant with increased protein oxidation, mainly in the dermis (Sander *et al*, 2002). On the other hand they showed that exposure of keratinocytes and fibroblasts to hydrogen peroxide resulted in protein oxidation, and suggested that the UV-induced oxidative damage in human skin might partly occur via the formation of hydrogen peroxide. Also other authors have suggested that oxidative damage to the SC not only affects antioxidant levels in the SC, but might also induce cellular responses deeper in the cutaneous tissue. Filipe *et al* (1997) suggested this mode of action as he found a reduction of the psoralen-plus-UVA-induced inflammatory reaction in the dermis upon topical application of SOD, whereas the SOD itself was not able to penetrate into the dermis. Valacchi *et al* (2002) recently reported that ozone exposure caused a rapid upregulation of heat shock protein 27 (HSP27) and a more delayed induction of HSP70 and heme oxygenase-1 in the active deeper layers of mouse skin. This was also explained via an indirect mechanism in which second messengers activate the stress response, as it is very unlikely that ozone itself penetrates deeply in the protective SC layers.

One may speculate that for the *in vivo* situation the loss in catalase activity upon sun exposure is at least partly compensated by the activity of glutathione peroxidase (GSH-Px), which is also capable of neutralizing hydrogen peroxide. There seem to be some functional differences, however, between catalase and GSH-Px in human dermal fibroblasts (Masaki *et al*, 1998): GSH-Px mainly shields the cell from damage at high hydrogen peroxide concentrations whereas catalase protects from oxidative stress at relatively low hydrogen peroxide concentrations and acts as a primary defense mechanism. Furthermore literature data suggest that in the epidermis the enzymatic activity of GSH-Px is much lower than that of catalase (Shindo *et al*, 1994a; Rhie *et al*, 2001b). The latter could be confirmed on tape strippings from human SC. Using a method modified from Flohé and Günzler (1984) we were not able to detect any GSH-Px activity on tape strippings taken from human SC. Thus we propose that after catalase deactivation GSH-Px in the SC is not able to take over the neutralization of hydrogen peroxide without any loss in efficiency.

We showed that the deactivation of catalase could be mimicked by exposure to a source of broadband UVA, whereas UVB did not affect the catalase activity (**Fig 3**). As expected the SOD activity is not affected by the irradiation. The decrease in catalase activity after UVA irradiation has been well documented both *in vitro* and in animals (Fuchs *et al*, 1989; Punnonen *et al*, 1991a; 1991b; Moysan *et al*, 1993; Shindo *et al*, 1994a; Shindo and Hashimoto, 1997). Very recently it was shown by immunohistochemistry that protein levels of catalase are depleted in human SC upon acute UV exposure (Sander *et al*, 2002). *In vitro* experiments have revealed that the dose-dependent decline in catalase activity is mainly due to direct photodestruction (own observation and Shindo *et al*, 1994a) in which the heme group, which absorbs at 410 nm, might be involved. Our experiments further show that systemic effects on the SC catalase activity upon chronic sun exposure and controlled UVA irradiation do not occur, as the decrease in SC catalase activity is only seen at irradiated sites and not at sham irradiated sites. These observations, together with the fact that the catalase activity in mouse SC (Iizawa *et al*, 1994) and human SC (**Fig 3**) is not affected by UVB irradiation, support the hypothesis of direct photodestruction *in vivo*.

We have found that the catalase activity in human SC gradually recovered after an acute dose of UVA, with full recovery within 3–4 wk over a dose range that caused 40% (5 J per cm^2) to 60% (15 J per cm^2) deactivation ($n = 26$) (**Fig 4**). The rate of the recovery in SC catalase activity after acute UV exposure was shown to decrease significantly with increasing age (**Fig 5**). Only few publications report on the recovery of the catalase activity in skin after UV irradiation. In mice a slow recovery after UVA exposure, with activities still low after 5 d (Shindo *et al*, 1994b), has been demonstrated. A study on human skin biopsies demonstrated full recovery of catalase activity in epidermis and dermis within 72 h after exposure to 2 MED of a UV source with peak emission in the UVB range (Rhie *et al*, 2001a). Our data indicate that the time of recovery of the catalase activity corresponds to the renewal time of the SC, which is approximately 20 d for young adults and around 30 d for older people (Grove and Kligman, 1983). This confirms that the UVA-induced deactivation of catalase in SC is irreversible and suggests that functional catalase has to be replenished from the deeper living cell layers. The reduced capacity of older skin to replenish the antioxidant capacity of the SC after UV exposure may exacerbate the damage that occurs upon subsequent exposure to oxidizing conditions. It is therefore important to provide long-term protection for this internal defense mechanism.

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