

Catalase in the Stratum Corneum of Patients with Polymorphic Light Eruption

M. GUARRERA, P. FERRARI and A. REBORA

Department of Dermatology, University of Genoa, Italy

UV radiation generates reactive oxygen species, which may be involved in polymorphic light eruption. The endogenous enzymatic defense system includes catalase in the epidermis. Thirteen patients with a history of polymorphic light eruption, but free from lesions, and 13 controls were investigated from November to March. Catalase was analysed in the upper horny layer according to Colin et al.'s spectrophotometric technique. In polymorphic light eruption, catalase values were about 30% lower than in control subjects. Such deficiency was observed in patients free from the disease and not recently sun-exposed. The diminished skin catalase in irradiated polymorphic light eruption makes it possible that a longer restoration time of catalase is involved in the pathogenesis.

(Accepted March 23, 1998.)

Acta Derm Venereol (Stockh) 1998; 78: 335–336.

A. Rebora, Department of Dermatology, University of Genoa, Viale Benedetto XV7, IT-16132 Genoa, Italy.

Exposure of skin to UV radiation generates reactive oxygen species with cytotoxic effects which are involved in photoaging, skin cancer, DNA-repair deficient disorders and, possibly, idiopathic photodermatoses. The endogenous defense system against free radicals in humans includes antioxidant enzymes, among which catalase (CAT) is present in epidermis (1). In the horny layer, it is diminished after UVA irradiation (2). We suggest that CAT deficiency may occur in the most common of idiopathic photodermatoses, polymorphic light eruption (PLE).

SUBJECTS AND METHODS

Thirteen patients (3 males and 10 females), skin types II–IV, were studied. Their age ranged between 18–79 years, averaging 42.8 years. They all had a history of papular PLE for 10 years on average, but were free from lesions at the time of the study. Thirteen healthy subjects (4 males and 9 females) served as controls. They had the same skin type and were aged between 23–61 years, averaging 43.3 years. The study was performed from November to March to avoid undue sun-exposure. CAT was evaluated in the upper horny layer according to the non-invasive technique of Colin et al. (2). Briefly, the horny layer was sampled by stripping it using a 2.5-cm wide 3M Blendederm tape. In each subject, 4 consecutive tape strips were taken from the volar aspects of the forearm and arm, two commonly sun-protected areas.

All stripped tapes were transferred to a purpose-made teflon plate with 96 open-bottom wells (2). Each tape lay upon 4 adjacent well openings and was kept tightly adherent to them by an aluminium plate. A 2.5 × 8 cm tape was employed for the standard measurement.

All incubation steps were performed in the teflon wells. To determine CAT amount, we used a colorimetric method (3) as modified by Colin et al. (2). This technique exploits the CAT capacity to produce alde-

hydes in the presence of hydrogen peroxide and alcohol as hydrogen donor.

In each well, we added methanol (5.9 M), hydrogen peroxide (4.2 mM) and phosphate buffer (25 mM, pH 7.0). Phosphate buffer (25 mM, pH 7.0) served as blank. Purified bovine liver CAT (SIGMA, St. Louis, MO, USA) in phosphate buffer (25 mM, pH 7.0) at different concentrations (1.5, 2.5, 5, 10, 20, 40 and 60 µg/ml) served for standard curve. The reaction was stopped by adding Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4 triazole) (SIGMA, St. Louis, MO, USA) (22.8 mM in 2N potassium hydroxide) and potassium periodate (65.5 mM in 0.5N potassium hydroxide). The reaction produces formaldehyde, which reacts with Purpald, yielding a compound that is coloured purple by potassium periodate oxidation.

The absorbance of the adduct was determined at 550 nm by spectrophotometer (Spectra Reader, OEM version). A mean of 4 determinations from the 4 adjacent wells was calculated for each stripped tape. Data were expressed as µg CAT per cm² of horn layer and analysed by factorial ANOVA.

RESULTS

CAT values increased linearly with the depth of the sample. This was observed in both skin regions and in both PLE patients and control subjects ($p < 0.01$) (Table I). There was no difference between arm and forearm. CAT values in PLE patients were lower by 30.4% than in control subjects (Table I). This difference was statistically highly significant ($p < 0.01$).

DISCUSSION

CAT (EC 1.11.1.6) is a tetramer of about 240 kD consisting of 4 identical subunits, each having a single heme group. In aerobic organisms, CAT exerts a dual function in the decomposition of H₂O₂ to H₂O and O₂ and in the oxidation of hydrogen donors. CAT contains tightly bound NADPH which provides its protection against inactivation by H₂O₂ (4). In mammals, CAT is located in the erythrocytes, liver, kidney, lung and skin. In the human epidermis, its activity is 720% higher than in the dermis (1). In the epidermis, CAT undergoes a sunlight-induced breakdown which consists in the irreversible oxidation of the tetrapyrrole rings of the heme prosthetic group.

Two types of cutaneous CAT deficiency are known. It may develop after UV light irradiation, or it may be primary. After UV irradiation, CAT activity has been shown to diminish after solar simulator radiation in murine skin (5), with a single UVB dose in hairless mice (6, 7), in a dose-dependent manner with a single acute UVA exposure in the human stratum corneum (2) and, with a combination of UVB and UVA irradiation, in cultured human keratinocytes (8).

CAT primary deficiency is a heterogeneous disorder. The various types are caused both by the synthesis of abnormally unstable enzyme forms and by enzymes which have low specific activity. In human skin, it has been shown in xeroderma pig-

Table I. CAT values indicate $\mu\text{g per cm}^2$ horny layer obtained by stripping the skin of arm and forearm of controls and PLE patients. For each area and strip, the mean of 13 data is given. Factorial ANOVA showed that the difference between cases and controls (altogether) was highly significant, but there was no difference between cases and controls within each strip and between arm and forearm. This allowed a regression line to be drawn from the means of each strip (bottom line)

Region		Strip			
		1st	2nd	3rd	4th
Arm	PLE	0.61 ± 0.83	1.10 ± 1.22	1.45 ± 1.29	2.04 ± 1.81
Forearm	PLE	1.14 ± 1.25	1.27 ± 1.79	1.48 ± 2.05	2.19 ± 1.95
Arm	Control	1.37 ± 1.07	2.00 ± 1.38	2.28 ± 1.47	2.38 ± 1.66
Forearm	Control	1.50 ± 1.35	2.01 ± 1.54	2.22 ± 1.29	2.45 ± 1.95
Total average		1.15 ± 1.16	1.60 ± 1.51	1.86 ± 1.56	2.27 ± 1.80

mentosum (XP) (9, 10) in vitiligo patients (11) and in human epitheliomas (12).

Both sunlight and UV light exposures aggravate primary deficiency and its deleterious effects. In XP, CAT activity of the sun-protected areas at birth is about normal, while in sun-exposed areas, it is 50% that of controls. Between the first and second years of the disease, a progressive breakdown of CAT activity occurs, and cancer develops as soon as CAT activity becomes almost undetectable (10).

We confirmed Colin et al.'s finding that CAT is contained in the stratum corneum (2). We provided evidence that the amount of CAT increases linearly with the depth of the horny layers, probably depending on sunlight-induced CAT breakdown, which diminishes with the distance from the surface. Further studies with the same technique may use only the fourth strip. In addition, we found that CAT values in the stratum corneum were significantly lower in PLE patients than in controls, irrespective of the region studied.

CAT was almost undetectable in the experimentally UV-irradiated skin ($0.033 \mu\text{g/cm}^2$ vs. 0.246) in 2 of our patients. The irradiation doses were 100 J/cm^2 of UVA and 3×0.75 MED of UVB. The finding is in accordance with the 85% decrease found in the irradiated normal stratum corneum. CAT took at least 12 days to return to its normal levels (2). It may, therefore, be speculated that during the course of the disease, CAT levels were severely affected, and that months after sun-exposure their recovery was still incomplete. A longer restoration time of catalase may be involved in the pathogenesis of polymorphic light eruption.

ACKNOWLEDGMENTS

We are grateful to scientists from L'Oréal, Chevilly-LaRue (F): Dr. L. Simonetti, Dr. D. Bernard for technical advice, Dr. P. Giacomoni for his invaluable suggestions and Dr. R. Bazin for generously donating the teflon plate. Thanks are due also to MC. Divano Biol D for technical assistance. Many thanks are due also to Dr. C. Armanino from Department of Pharmaceutical and Food Chemistry and Technology, University of Genoa, for reviewing our statistics.

REFERENCES

- Shindo Y, Witt E, Han D, Epstein W, Packer L. Enzymic and non-enzymic antioxidants in epidermis and dermis of human skin. *J Invest Dermatol* 1994; 102: 122–124.
- Colin C, Boussouira B, Bernard D, Moyal D, Nguyen QL. Non-invasive methods of evaluation of oxidative stress induced by low doses of ultraviolet in humans. *Proc 18th Congr Int Fed Soc Cosm Chem (IFSCC), Venezia, Oct 3–6, 1994: 1–23.*
- Wheeler CR, Salzman JA, Elsayed NM, Omaye ST, Korte DW. Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity. *Anal Biochem* 1990; 184: 193–199.
- Kirkman HN, Gaetani GF. Catalase: a tetrameric enzyme with four tightly bound molecules of NADPH. *Proc Natl Acad Sci* 1984; 81: 4343–4347.
- Shindo Y, Witt E, Packer L. Antioxidant defense mechanisms in murine epidermis and dermis and their responses to ultraviolet light. *J Invest Dermatol* 1993; 100: 260–265.
- Fuchs J, Huflejt M, Rothfuss L, Wilson DS, Carcamo G, Packer L. Impairment of enzymatic and non-enzymatic antioxidant in skin by UV irradiation. *J Invest Dermatol* 1989; 93: 761–773.
- Pence BC, Naylor MF. Effects of single-dose ultraviolet radiation on skin superoxide dismutase, catalase, and xanthine oxidase in hairless mice. *J Invest Dermatol* 1990; 95: 213–216.
- Punnonen K, Puntala A, Ahotupa M. Effects of ultraviolet A and B irradiation on lipid peroxidation and activity of the antioxidant enzymes in keratinocytes. *Photodermatol Photoimmunol Photomed* 1991; 8: 3–6.
- Schallreuter KU, Pittelkow MR, Wood JM. Defects in antioxidant defense and calcium transport in the epidermis of xeroderma pigmentosum patients. *Arch Dermatol Res* 1991; 283: 441–455.
- Vuillaume M, Calvayrac R, Decroix Y, Best-Belpomme M, Sarasin A. Deficiency in the catalase activity in human precancerous stages of xeroderma pigmentosum. Comparison with XP, AT and Blomn's cultured fibroblasts and SV40 transformed human cell extracts. *Bioelectrochem Bioenerg* 1987; 18: 271–282.
- Schallreuter KU, Wood JM, Berger J. Low catalase in the epidermis of patients with vitiligo. *J Invest Dermatol* 1991; 97: 1081–1095.
- Rabilloud T, Asselineau D, Miquel C, Calvayrac R, Darmon M, Vuillaume M. Deficiency in catalase activity correlates with the appearance of tumor phenotype in human keratinocytes. *Int J Cancer* 1990; 45: 952–956.