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Effects of cadmium and zinc on solar-simulated light-irradiated cells: potential role of zinc-metallothionein in zinc-induced genoprotection

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Abstract

Zinc is an essential oligoelement for cell growth and cell survival and has been demonstrated to protect cells from oxidative stress induced by UVA or from genotoxic stress due to UVB. In a recent work we demonstrated that the antioxidant role of zinc could be related to its ability to induce metallothioneins (MTs). In this study we identified the mechanism of zinc protection against solar-simulated light (SSL) injury. Cultured human keratinocytes (HaCaT) were used to examine MTs expression and localization in response to solar-simulated radiation. We found translocation to the nucleus, with overexpression of MTs in irradiated cells, a novel observation. The genoprotective effect of zinc was dependent on time and protein synthesis. DNA damage was significantly decreased after 48 h of $ZnCl_2$ (100 μM) treatment and is inhibited by actinomycin D. $ZnCl_2$ treatment (100 μM) led to an intense induction, redistribution, and accumulation of MT in the nucleus of irradiated cells. MT expression correlated with the time period of $ZnCl_2$ treatment. $CdCl_2$, a potent MT inducer, did not show any genoprotection, although the MTs were expressed in the nucleus. Overall our findings demonstrate that MTs could be a good candidate for explaining the genoprotection mediated by zinc on irradiated cells.

Keywords: Zinc; Cadmium; Metallothionein; DNA damage; Solar irradiation

Zinc is an essential oligoelement for cell growth [1] and cell survival. Zinc controls the activity of more than 300 zinc-metalloenzymes, which participate in cell metabolism, and plays an essential structural function in zinc-requiring proteins that influence gene expression at different stages of cell proliferation [2–4] and cell death [5]. Zinc has been demonstrated to protect cells from oxidative stress, whereas zinc deficiency causes oxidative damage to various molecules [6,7] and can lead to the accumulation of iron, a prooxidant metal [8]. We previously reported that increased intracellular levels of zinc can protect human cutaneous cells from the deleterious effects of UV[1] [9,10], particularly DNA damage [11] and lipid peroxidation [12,13]. These protective effects of zinc against UV-induced damage are still poorly understood.

In a recent work we demonstrated that the antioxidant role of zinc could be related to its ability to induce metallothioneins (MTs) [14]. MTs are an abundant ubiquitous family of low-molecular-weight (6000–7000 Da) metal-binding proteins, containing 25–30% cysteine residues. They are rapidly induced in cells in response to a variety of stimuli [15]. High levels of MT protein and mRNA are found in organisms and tissues exposed to high levels of zinc or cadmium [16]. Because of their high affinity to bind to, and be induced by, essential (zinc, copper) and harmful metals (cadmium, mercury), MTs

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[1] *Abbreviations used:* ActD, actinomycin D; BSA, bovine serum albumin; Cd, cadmium; Da, Dalton; DNA, deoxyribonucleic acid; MRE, metal responsive element; MTs, metallothioneins; mRNA, messenger ribonucleic acid; ROS, reactive oxygen species; SSL, solar-simulated light; TPEN, *NNN′N′*-tetrakis(2-pyridylmethyl)ethylenediamine; UV, ultraviolet; Zn, zinc; Zn(−)/Zn(+), cells cultured in basal conditions (−) or in Zn added medium (+).

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are believed to play an important role in the homeostatic function of essential metals and in the detoxification against toxic metals [17]. They may serve to buffer intracellular zinc levels [18]. Thus, MTs regulate the transport and the compartmentation of zinc, maintain adequate and appropriate intracellular zinc levels, and mediate its redistribution.

MTs have been suspected not only to control metal homeostasis but also to maintain cell survival because of their free radical scavenging activities [19–22]. MTs are quickly induced in response to some stimuli such as glucocorticoid hormones, lipopolysaccharides, interleukin-1 and -6, tumor necrosis factor, and oxidative stress, suggesting that they belong to the family of stress proteins and may be directly involved in antioxidant defense mechanisms.

MTs are expressed in mouse and human skin [23–25]. UV irradiation induces MT production in human skin, suggesting a photoprotective role [26]. Different studies have delineated the protection afforded by MTs against the deleterious effects of UV in vivo and in vitro [27–29]. In mice, subcutaneous injection of cadmium has been reported to induce MTs and reduce UV toxicity [30]. Conversely, MT-null mice exhibit reduced tolerance to UVB injury in skin [27]. Few studies have reported that MT could penetrate the nucleus [31–33], to directly protect genomic DNA. The ability of MTs to redistribute zinc [34,35] can also explain their role in cell proliferation and cell survival after UV irradiation by maintaining the activities of some Zn enzymes such as DNA polymerase and DNA repair enzymes or by restoring the functions of some transcription factors [4,25,36].

We have previously demonstrated in vitro that cells exposed to zinc deprivation entered apoptosis [11] with lower basal MTs expression [37], which could be one of the mechanisms by which zinc could influence cell viability.

We demonstrate here that both $ZnCl_2$ and $CdCl_2$ treatment leads to an intense induction, redistribution, and accumulation of MTs into the nucleus of irradiated cells. In addition, zinc treatment protected against SSL-induced DNA damage, and $CdCl_2$ treatment induced a genotoxicity. These results suggest that the nature of the metal bound to MTs is essential for genoprotection.

Materials and methods

Cell culture and chemical treatment

Cells. A spontaneously immortalized human keratinocyte cell line, HaCaT was maintained in RPMI 1640 culture medium supplemented with 10% fetal calf serum (FCS) (ATGC Biotechnologie, Noisy-le-grand, France). Cells were grown at 37 °C under a 5% CO_2 atmosphere. Culture medium was replaced once a week, and the cell monolayer was split 1/6 at 90% confluence. To avoid bacterial and fungal contamination, the medium was supplemented with 5000 UI/L penicillin (Prolabo, Paul Block and Cie, Strasbourg, France) and 50 mg/L streptomycin (Polylabo, Strasbourg, France). Cells were tested for mycoplasma using an immunostaining technique (Mycoplasma Detection Kit, Boehringer–Mannheim, Indianapolis, IN).

Chemical treatment. A sterilized stock solution of zinc chloride ($ZnCl_2$, 0.1 M) was prepared in deionized water (Merck, Darmstadt, Germany). $ZnCl_2$ (100 μM) was added in HaCaT culture medium for 24, 48, or 72 h, in order to induce MTs. This final concentration was chosen according to the laboratory results previously described [11].

A sterilized stock solution of cadmium chloride ($CdCl_2$, 0.1 M) was prepared as $ZnCl_2$ solution and was added in HaCaT culture medium for 24 h at a final concentration of 3 μM. Actinomycin D (Sigma Chemical, St. Louis, MO), an inhibitor of transcription, was used at 5 μM for 48 h, to inhibit protein synthesis.

Irradiation procedure. The light source used was a Dermolum UM-W (Müller GmbH Elektronik-optik, Moosinning, Germany) equipped with a 1 kW xenon lamp and a water filter. The UV spectrum is obtained by passing light through a 1-mm WG305 filter (Müller GmbH Elektronik-optik, Germany). This filtered xenon source provides a simulated solar light that is essentially identical to mountain sunlight (for spectrum, see Fig. 1). The irradiation effectively received by the cells (17.5 mW/cm^2) was measured using a dosimeter (Müller GmbH Elektronik-optik, Germany) with a spectral sensitivity from 270 nm to 4 μm. Cells were cultured in 9 cm^2 petri dishes and irradiated from the top at three different physiological SSL doses (0.3, 0.75, or 1.5 J/cm^2). Just prior to irradiation, the culture medium was removed and held in reserve. Cells were rinsed twice with phosphate buffer without calcium and magnesium (PBS, Gibco BRL, Life Technology, Paisley, Scotland) and maintained in 2 ml PBS for irradiation at a controlled temperature. Control cells were similarly treated and left in the dark while irradiation was carried out. After the stress, cells were returned to their initial medium and placed in the incubator for 24 h of recovery.

Single-cell gel electrophoresis [9,38]. DNA damage was evaluated using the Comet assay. Cells were detached from their culture dishes and incorporated into low-melting-point agarose. Embedded cells were immediately irradiated as previously described [39,40]. All slides were immersed overnight in a cell lysis buffer in the dark at 4 °C, in order to prevent nonspecific DNA damage and the repair process from occurring. Next, slides were placed in a horizontal electrophoresis unit containing freshly prepared electrophoresis buffer (1 mM Na_2EDTA, 300 mM NaOH). The DNA was

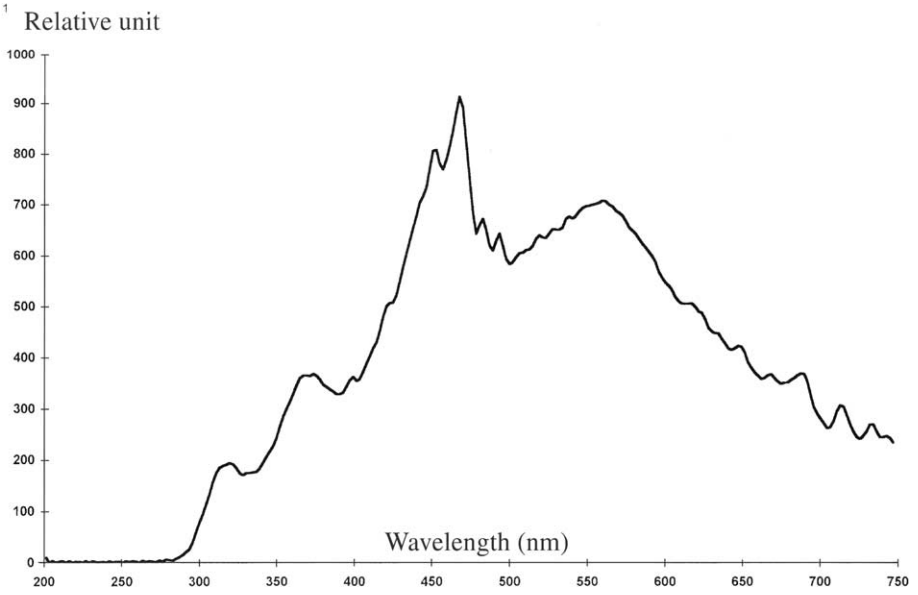

Fig. 1. Total emission spectrum of Dermolum UM-W lamp with WG-305 filter.

allowed to unwind for 40 min before electrophoresis was performed at 25 V, 300 mA for 30 min. After neutralization (0.4 M Tris–HCl, pH 7.4) the DNA was stained with 50 μl of 0.5 mg/ml ethidium bromide pipetted onto the slides. The slides were then placed in a humidified air-tight container to prevent drying of the gel, before the analysis. DNA damage was quantified using measurement of the tail moment (TM), the product of the tail distance (i.e., the distance between the center position of the head and the center of gravity of the tail), and the percentage of DNA in the tail (relative to the total amount of DNA in the entire comet [head + tail]). For each condition, the average tail moment was determined using three different slides, prepared as previously described [39]. Sixty-five cells were read per slide, and means were calculated from three independent experiments.

Immunocytochemical staining of MT. This was achieved using a mouse monoclonal antibody (clone E9, Dako, Glostrup, Denmark) produced against a mixture of horse MT-I and MT-II isoforms used as an immunogen. It has been shown immunocytochemically to be reactive against a conserved N-terminal epitope shared by MT-I and MT-II isoforms of several mammalian species. Cells were seeded on 4-well plastic Lab-Tek chamber slides (Nunc, Naperville, IL) (5000 cells/well) and cultured for 2 days. $ZnCl_2$ (100 μM) was then added for 72 h. After irradiation at the dose of 0.3 or 0.75 J/cm^2, cells were put back in their respective medium for 24 h. After washing twice with PBS, cells were fixed by 4% (w/v) paraformaldehyde in PBS and then placed for 10 min in ice-cold methanol. They were washed three times with PBS, pH 7.4, and blocked for nonspecific antibody binding by incubating for 30 min in a milk buffer (2% dried milk powder, 0.1% Tween 20 in PBS, pH 7.4). Cells were then incubated with the primary antibody diluted 1:100 (final concentration 1 μg/ml), for 2 h at room temperature. Cells were washed and then incubated with 1 μg/ml of mouse anti-human MT in PBS, pH 7.4, containing 0.1% Tween 20, 1% bovine serum albumin (BSA), and 0.1% sodium azide. After washing with PBS, pH 8.6, and saturating for 5 min with the milk buffer, cells were submitted to 2.3 μg/ml of biotinylated donkey $F(ab')_2$ fragment anti-mouse IgG (Jackson Immunoresearch Laboratories, Westgrove, PA) in PBS containing 0.1% Tween 20, 1% BSA, and 0.1% thymerosal. After two washes with PBS at pH 8.6, cells were incubated with the avidin-horseradish peroxidase complex (StreptABC Complex/HRP, Dako) for 1 h, washed three times in 50 mM Tris–HCl buffer (pH 7.6), and incubated with 3, 3′-diaminobenzidine (Sigma FAST DAB peroxidase substrate tablets) for 3 min according to the manufacturer. After washing in tap water, cells were counterstained with Harris' hematoxylin and slides were mounted with Aquamount. Blanks were treated with either 1 μg/ml nonimmune mouse immunoglobulin, or 1% (w/v) BSA in PBS. Cells under our experimental conditions did not exhibit any endogenous peroxidase activity. Positive control cells were treated with a monoclonal antibody IgG reactive against a 46 kDa cytokeratin isolated from keratinocyte (KL1, Immunotech, Marseille, France).

Western blot of MT. We developed a technique in order to detect MT without addition of radiolabeled cadmium. Cells were washed three times in PBS. The cell pellet was solubilized in a lysis buffer (10 mM Tris–HCl, 1 mM Perfabloc, 2 mM dithiothreitol (Sigma Chemical) and centrifuged at 4000 rpm for 15 min at 4 °C. Cyto-

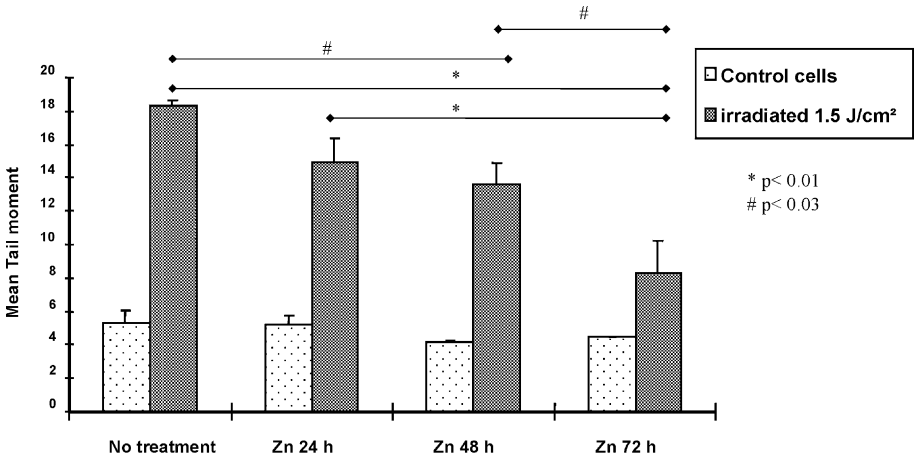

Fig. 2. DNA damage induced by a single solar irradiation (1.5 J/cm^2) on HaCaT cells treated or untreated with $ZnCl_2$ (100 μM) for 24, 48, or 72 h was evaluated using the Comet assay and compared to control cells. The mean tail moment was used to evaluate DNA lesions. Data represent the means of at least three independent assays; bars, SD.

plasmic and nuclear proteins were extracted and quantified using the Pierce technique (Micro BC Assay, Uptima Interchim, Montluçon, France). Proteins extracts (50 μg/lane) were subjected to 12% SDS–polyacrylamide in a buffer containing mercaptoethanol (2.5%) for one night at 20 mA. Proteins were transferred to a nitrocellulose membrane (Protan, Schleicher and Schuell GmbH, Germany) by electrotransfer at 60 mA for 3 h at 4 °C. The membrane was saturated with a milk buffer (5% dried milk powder, 0.5% Nonidet P-40 (Boehringer–Mannheim GmbH, Germany) in PBS, pH 7.4, for 2 h) and incubated with the monoclonal mouse anti-human MT antibody diluted 1:4600 (clone E9, Dako) in PBS for one night at 4 °C. The membrane was washed twice with PBS and incubated at room temperature for 1 h with the second antibody, peroxidase-conjugated anti-mouse IgG diluted at 1:5000. Bound antibodies were visualized by chemiluminescence using an ECL Western immunoblotting kit (Amersham Pharmacia Biotech AB, Piscataway, NJ) as specified by the manufacturer. Pure horse MT-I and MT-II solution (Sigma) was electrophoresed (6.25 μg/lane) in an independent lane to control MT migration.

Statistics. Each experiment was repeated three times. All data, expressed as means ± 1 SD, were processed statistically using one-way analysis of variance (ANOVA) and a Newman–Keuls test. Differences were considered significant at $P < 0.05$.

Results

Protein synthesis is required for protection by zinc against DNA damage induced by solar-simulated light (SSL)

To examine the zinc-dependent genoprotection, a comet assay was performed at different time points of $ZnCl_2$ treatment (Fig. 2). DNA damage was measured immediately after SSL irradiation (1.5 J/cm^2). A significant increase of the tail moment was shown in irradiated cells (18.2 + 2) compared to the tail moment measured under basal conditions (5 + 1). These results confirmed that physiological doses of SSL induced serious DNA damage. $ZnCl_2$ treatment (100 μM for 72 h) significantly decreased the tail moment (9 + 2). Zinc protection appeared within 24 h of treatment and DNA damage was significantly decreased in cells cultured with $ZnCl_2$ after 48 h, suggesting that the genoprotective action of zinc is time dependent (Fig. 2).

In order to verify the hypothesis that zinc-dependent proteins were implied, we analyzed the genoprotection afforded by zinc in cells treated by actinomycin D ($ActD^+$). Four different conditions were tested: control cells (Zn(−) $ActD^-$), cells treated with actinomycin D alone (Zn(−) $ActD^+$), cells treated with both actinomycin D and $ZnCl_2$ (Zn(+) $ActD^+$) and cells treated only by $ZnCl_2$ (Zn(+) $ActD^-$) (Table 1). The tail moment was higher in ActD-treated cells than in control

Table 1
Evaluation of zinc genoprotection in cells treated with ActD as inhibitor of protein synthesis

	Zn(−) $ActD^-$	Zn(+) $ActD^-$	Zn(−) $ActD^+$	Zn(+) $ActD^+$
Nonirradiated (mean tail moment)	5.4 ± 0.3	4 ± 0.1	35 ± 7	32 ± 2
Irradiated 1.5 J/cm^2 (mean tail moment)	18.1 ± 0.2	13.6 ± 0.6	72 ± 1.5	65 ± 3

Four conditions were tested: control cells {Zn(−) $ActD^-$}; cells treated with ActD alone {Zn(−) $ActD^+$}; cells treated with $ZnCl_2$ alone {Zn(+) $ActD^-$}; cells treated with both ActD and $ZnCl_2$ {Zn(+) $ActD^+$}. Data represent the means of at least three independent assays; bars, SD.

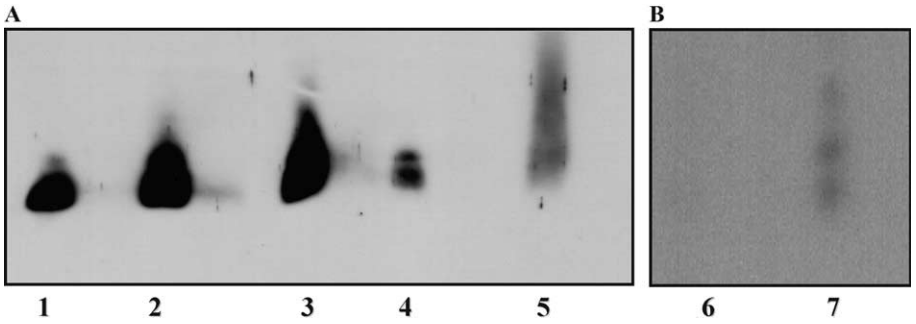

Fig. 3. Evaluation of MTs content at different time points in HaCaT keratinocyte cell line exposed to $ZnCl_2$ treatment (100 μM) (A) and in normal human skin fibroblasts after 72 h of $ZnCl_2$ treatment (100 μM) (B). MTs were visualized by Western blotting with anti-MT immunoglobulin as described in the text. Cells were treated with $ZnCl_2$ for 24 h (lane 1), 48 h (lane 2), or 72 h (lanes 3 and 7). Control cells (HaCaT cells, lane 4; and fibroblasts, lane 6) were cultured in their basal medium containing 3.4 μM $ZnCl_2$. Commercial MT-II and MT-I (6.25 μg) were loaded on lane 5 and used as positive controls.

cells, confirming that ActD was a DNA-damaging agent. The ratios of the tail moments measured in irradiated to nonirradiated cells were 2.03 in Zn(+) $ActD^+$-treated cells and 2.05 in Zn(−) $ActD^+$ cells. Zinc had no significant protective effect on ActD-treated cells. The photoprotective mechanism afforded by zinc against DNA lesions induced by SSL seemed dependent on neosynthesis of proteins.

Evaluation of MT content at different time points in $ZnCl_2$-treated epidermal cells

Intracellular levels of zinc and other transition metals are known to induce MTs. To determine if Zn-afforded protection is related to MT accumulation, we studied the kinetic of MT induction upon $ZnCl_2$ treatment and compared it with the genoprotective effect of zinc (Fig. 3). MTs were expressed in HaCaT cells (Fig. 3A, lane 4) whereas these proteins were not detectable in normal human fibroblasts (Fig. 3B, lane 6). The level of Zn-induced MTs was time dependent (compare lanes 1, 2, and 3, corresponding to 24, 48, and 72 h, respectively. This suggests that zinc genoprotection may be related to the kinetics of MT accumulation.

Effect of cadmium on DNA damage induced by solar light

To determine if cadmium and zinc afforded the same genoprotective effect, we determined the DNA damage in $ZnCl_2$ (100 μM, 24 h) and $CdCl_2$-treated cells (3 μM, 24 h) (Fig. 4). $CdCl_2$ treatment induced DNA damage per se without cell death. Moreover, when cells were treated with $CdCl_2$ (3 μM, 24 h) and then irradiated (1.5 J/cm^2), the genotoxicity of solar light was potentiated. Under our experimental conditions, the low dose of cadmium was genotoxic and the tail moment was 2.3 higher in $CdCl_2$-treated than in $ZnCl_2$-treated cells. These results clearly indicated a specific role of zinc in the protection afforded by MT.

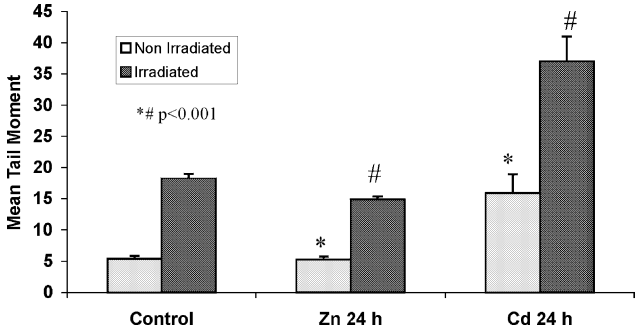

Fig. 4. DNA damage in HaCaT cells treated with $ZnCl_2$ (100 μM, 24 h) or $CdCl_2$ (3 μM, 24 h) was evaluated with the Comet assay after a single solar irradiation (1.5 J/cm^2).

Effects of zinc and cadmium on cellular localization of MT in SSL-irradiated cells

Immunostaining (Fig. 5) was used to investigate the localization of MT in both irradiated and $ZnCl_2$-treated cells. The presence of constitutive MT observed by Western blotting in HaCaT keratinocytes was confirmed (Fig. 5A). Under basal culture conditions, a very low level of staining was visualized, only localized to the cytoplasm. The immunostain control (Fig. 5B) indicated a high specificity of the two antibodies used. Single solar irradiation at the dose of 0.75 J/cm^2 (Fig. 5C) induced the synthesis of MT and a complete redistribution of the protein in the nucleus. Interestingly, the cytoplasm of HaCaT cells was not stained. In contrast, $ZnCl_2$-treated cells (100 μM for 72 h) showed a very intense staining in both cytoplasm and nucleus, indicating a new distribution of the protein (Fig. 5D).

Figs. 5E and F represented $ZnCl_2$-treated cells irradiated at either 0.75 or 0.3 J/cm^2. Under these conditions, MT was strongly induced and we observed that MT accumulated both in the nucleus and in the cytoplasm. These data suggest a synergic effect of UV irradiation and $ZnCl_2$ treatment on MT synthesis.

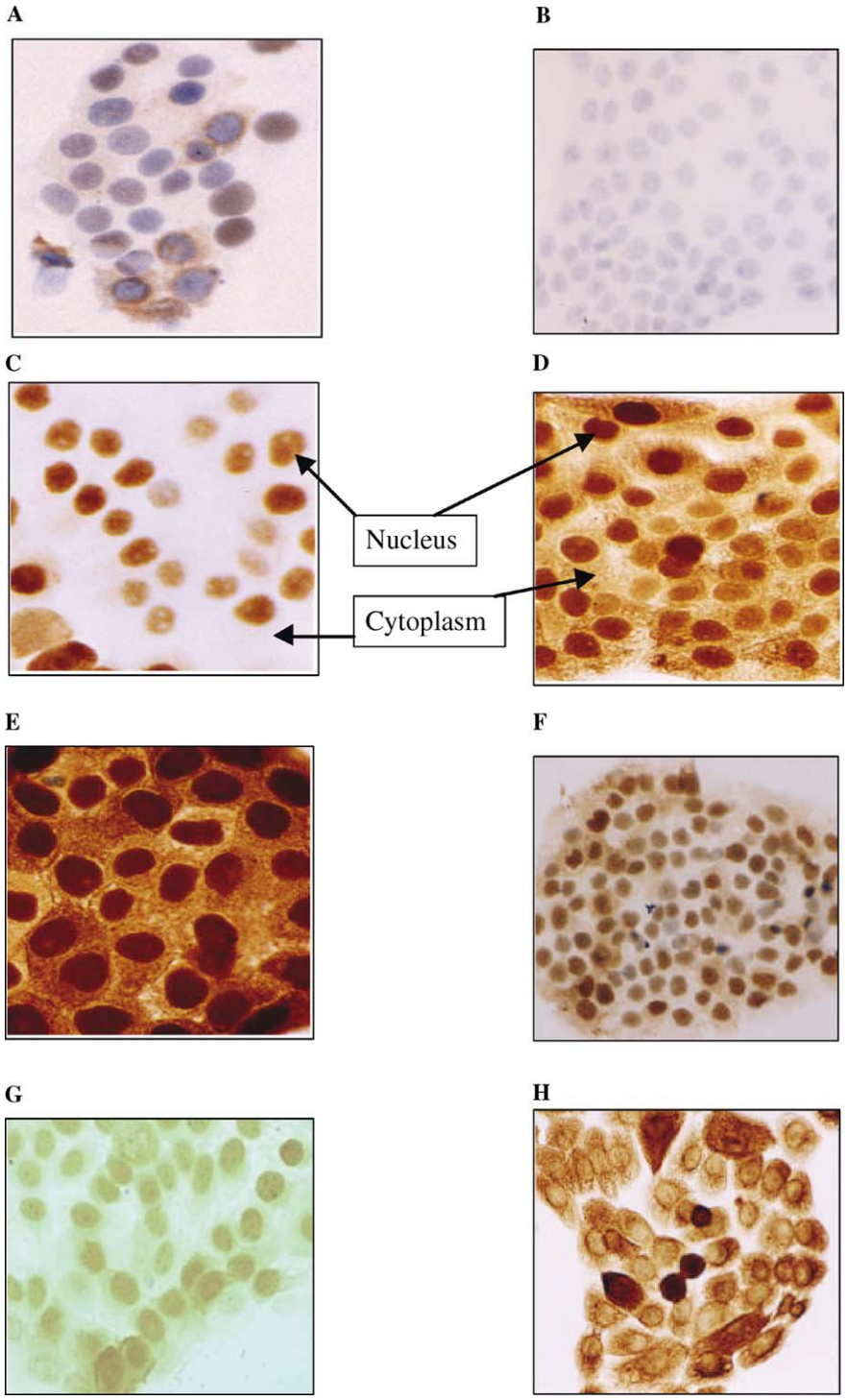

Fig. 5. Intracellular localization of MTs (MT-I + MT-II) in the HaCaT keratinocyte cell line, exposed to $ZnCl_2$ (100 μM). MTs were visualized by immunohistochemistry using light microscopy (40×) (10× for E and F) as described in the text. Representative results are shown. (A) Control cells grown in their basal medium; (B) cells labeled only with secondary antibody; (C) solar irradiated with cells (0.75 J/cm^2); (D) cells treated with $ZnCl_2$ for 72 h; E and F represented cells both $ZnCl_2$ treated and irradiated, respectively, with 0.75 or 0.30 J/cm^2; (G) $CdCl_2$-treated cells (3 μM, 24 h); (H) cells labeled with KL1 antibody as a control of DAB-peroxidase staining.

A dose-dependent intensity of MT staining was observed (compare Figs. 5E and F). These data confirm the induction of MT by SSL and clearly demonstrate the nuclear localization of the neosynthetized protein.

Treatment of $CdCl_2$ (3 μM) for 24 h induced MTs (Fig. 5G) which were localized in both cytoplasm and nucleus similar to that observed in $ZnCl_2$-treated cells (Fig. 5D), suggesting a similar mechanism of MT induction.

In Fig. 5H, cells were labeled with KL1 antibody as a positive control of DAB-peroxidase staining.

Discussion

Zinc is considered as one of the more important trace elements in humans and animals, as more than 300 zinc enzymes are dependent of this metal for their activity or structure. Although the preventive role of zinc in photoprotection is well known, the mechanism is unclear. Thus we focalized on the relation between zinc and MT, a cysteine-rich protein, widely distributed in a broad range of eukaryotic species. Previously MT was considered only a stress protein induced by heavy metals or other stimuli. Recent studies have pointed out the constitutive expression of this protein in many human cell types, especially in epidermal keratinocytes in vivo [25,26] or in vitro [37]. But their precise role in such tissues has not been elucidated. Moreover an increasing number of studies point out the overexpression of MT in a great variety of cancers [41–43], indicating an important role for cell survival and more precisely in the control of cell proliferation [32].

The present study, which extends our previous findings about cellular protection afforded by zinc, demonstrates that Zn-MTs prevent DNA genomic lesions due to a solar irradiation at physiological doses, and confirms the role of zinc in photoprotection. We again demonstrate that zinc genoprotection is an inducible mechanism, correlated with both overexpression and nuclear translocation of MTs.

The genoprotection of $ZnCl_2$ treatment is time dependent. Under our conditions, the best genoprotection is obtained after 72 h of $ZnCl_2$ treatment compared to shorter time intervals. Zinc is known to induce MT transcription. In this study, we demonstrate that zinc genoprotection is correlated with the MT content of HaCaT keratinocytes. Accumulation of MT in HaCaT cells is clearly dependent on the time period of $ZnCl_2$ treatment. Genotoxic effects of SSL decrease and the minimal DNA lesions obtained at 72 h of $ZnCl_2$ treatment corresponded to the maximum level of MT expression. Zn genoprotection is inhibited by ActD treatment, suggesting that the genoprotective mechanism implied is not only dependent on zinc per se but requires protein synthesis.

To test the hypothesis that zinc genoprotective effect is triggered through a MT-dependent pathway, we analyzed the intracellular localization of MT following $ZnCl_2$ treatment and/or irradiation. We show here that MTs are expressed constitutively in HaCaT cells, as described in normal human skin keratinocytes in vivo [25,26], or in vitro [37] indicating that HaCaT cells are a good model for studying the relation between MT expression and zinc genoprotection. The overexpression of MT induced by $ZnCl_2$ treatment is visualized in both cytoplasm and nucleus. An intense nuclear MT staining is visualized in HaCaT cells 24 h after a single dose of solar radiation. Complete redistribution of MTs into the nucleus in solar-irradiated cells is a novel observation. MT induction can be related to the free radical generation as previously described [44]. Finally, we demonstrate an additive effect of UV irradiation on Zn-MTs overexpression. Interestingly, under these conditions, the pool of MT accumulates in the nucleus. This nuclear translocation is an important finding as DNA is very sensitive to oxidative stress induced by UV irradiation, whereas the nucleus is lacking in antioxidant enzymes such as catalase and superoxide dismutase (SOD).

Nuclear translocation of MT must be quite different, by comparison with other proteins [45]. The mechanism could be an active translocation in which both MT mRNA and the cytoskeleton are needed [46].

Analysis of the specificity of the metal in the genoprotection afforded by MT revealed that $CdCl_2$ treatment induces MTs in HaCaT cells, and that the newly synthesized protein is localized to both cytoplasm and nucleus. The mechanism of MT induction by heavy metals requires a protein called MTF-1 (MRE-binding transcription factor). This protein is activated by zinc and subsequently can interact with the multiple copies of MRE located on the MT promoter. A recent study points out the fact that the activity of the *h*MTF-1 is only modulated by zinc in vitro and in vivo [47], the mechanism being different for cadmium-induced MT (for review see [48]).

The genoprotective effects of Cd-MTs are different from those obtained with Zn-MTs. $CdCl_2$ treatment acts as a genotoxic agent per se. Moreover, a potentiation of SSL genotoxic effect is observed in $CdCl_2$-treated cells. These data show that nuclear MTs are not sufficient to protect DNA. The nature of the binding metal is important. Under our conditions, the genoprotection was only afforded by Zn-MTs, which could redistribute their zinc atoms to other nuclear proteins such as transcription factors [4,36] or DNA repair enzymes to maintain genomic stability.

The induction of Zn-MTs can be considered an important adaptive mechanism for the irradiated cells. These proteins prevent DNA lesions due to UV. Zn-MT could maintain the nuclear redox potential and facilitate DNA repair mechanisms by exchanging zinc atoms. In contrast the redistribution of cadmium, a genotoxic metal, has no protective effects. Some studies have reported a protective effect of cadmium on stress-induced apoptosis [30] while others demonstrated that it was able to induce apoptosis [49,50]. Our results using the sensitive comet assay confirmed that cadmium exerts deleterious effects on DNA.

The accumulation of MTs in the nucleus of $ZnCl_2$-treated cells following irradiation suggests a genopro-

tective role for these proteins. We propose Zn-MTs as effectors of zinc genoprotective effects from SSL-induced DNA damage.

Acknowledgments

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References

[1] J.E. Coleman, Annu. Rev. Biochem. 61 (1992) 897–946.
[2] B.L. Vallee, D.S. Auld, Matrix Suppl. 1 (1992) 5–19.
[3] B.L. Vallee, D.S. Auld, Faraday Discuss. (1992) 47–65.
[4] J. Zeng, B.L. Vallee, J.H. Kagi, Proc. Natl. Acad. Sci. USA 88 (1991) 9984–9988.
[5] C. Meplan, M.J. Richard, P. Hainaut, Oncogene 19 (2000) 5227–5236.
[6] J.S. Lazo, B.R. Pitt, Annu. Rev. Pharmacol. Toxicol. 35 (1995) 635–653.
[7] M. Ebadi, M.P. Leuschen, H. el Refaey, F.M. Hamada, P. Rojas, Neurochem. Int. 29 (1996) 159–166.
[8] J.M. Rogers, B. Lonnerdal, L.S. Hurley, C.L. Keen, J. Nutr. 117 (1987) 1875–1882.
[9] N. Emonet-Piccardi, M.J. Richard, J.L. Ravanat, N. Signorini, J. Cadet, J.C. Beani, Free Radic. Res. 29 (1998) 307–313.
[10] M.T. Leccia, M.J. Richard, A. Favier, J.C. Beani, Biol. Trace Elem. Res. 69 (1999) 177–190.
[11] M.O. Parat, M.J. Richard, S. Pollet, C. Hadjur, A. Favier, J.C. Beani, J. Photochem. Photobiol. B 37 (1997) 101–106.
[12] D.J. Tate Jr., M.V. Miceli, D.A. Newsome, Free Radic. Biol. Med. 26 (1999) 704–713.
[13] A. Markant, J. Pallauf, J. Trace Elem. Med. Biol. 10 (1996) 88–95.
[14] F. Chimienti, E. Jourdan, A. Favier, M. Seve, Free Radic. Biol. Med. 31 (2001) 1179–1190.
[15] L.A. Borghesi, M.A. Lynes, Cell Stress Chaperones 1 (1996) 99–108.
[16] D.H. Hamer, Annu. Rev. Biochem. 55 (1986) 913–951.
[17] M. Nordberg, G.F. Nordberg, Cell. Mol. Biol. (Noisy-le-grand) 46 (2000) 451–463.
[18] S.R. Davis, R.J. Cousins, J. Nutr. 130 (2000) 1085–1088.
[19] J. Abel, N. de Ruiter, Toxicol. Lett. 47 (1989) 191–196.
[20] L.S. Chubatsu, R. Meneghini, Biochem. J. 291 (1993) 193–198.
[21] T.G. Rossman, E.I. Goncharova, Mutat. Res. 402 (1998) 103–110.
[22] M.A. Schwarz, J.S. Lazo, J.C. Yalowich, W.P. Allen, M. Whitmore, H.A. Bergonia, E. Tzeng, T.R. Billiar, P.D. Robbins, J.R Lancaster Jr., et al., Proc. Natl. Acad. Sci. USA 92 (1995) 4452–4456.
[23] M. Iwata, T. Takebayashi, H. Ohta, R.E. Alcalde, Y. Itano, T. Matsumura, Histochem. Cell Biol. 112 (1999) 283–290.
[24] M. Karasawa, N. Nishimura, H. Nishimura, C. Tohyama, H. Hashiba, T. Kuroki, J. Invest. Dermatol. 97 (1991) 97–100.
[25] J.J. van den Oord, M. De Ley, Arch. Dermatol. Res. 286 (1994) 62–68.
[26] A. Anstey, R. Marks, C. Long, H. Navabi, A. Pearse, D. Wynford-Thomas, B. Jasani, J. Pathol. 178 (1996) 84–88.
[27] K. Hanada, D. Sawamura, K. Tamai, T. Baba, I. Hashimoto, T. Muramatsu, N. Miura, A. Naganuma, J. Invest. Dermatol. 111 (1998) 582–585.
[28] K. Hanada, D. Sawamura, H. Nakano, I. Hashimoto, J. Dermatol. Sci. 9 (1995) 203–208.
[29] S. Kobayashi, Y. Hirota, J. Sayato-Suzuki, M. Takehana, H. Nishimura, N. Nishimura, C. Tohyama, Photochem. Photobiol. 59 (1994) 650–656.
[30] K. Hanada, R.W. Gange, E. Siebert, T. Hasan, Photodermatol. Photoimmunol. Photomed. 8 (1991) 111–115.
[31] K. Hanada, K. Tamai, D. Sawamura, I. Hashimoto, T. Muramatsu, J. Invest. Dermatol. 110 (1998) 98–100.
[32] C. Schmidt, D. Beyersmann, Arch. Biochem. Biophys. 364 (1999) 91–98.
[33] W.W. Nagel, B.L. Vallee, Proc. Natl. Acad. Sci. USA 92 (1995) 579–583.
[34] C. Jacob, W. Maret, B.L. Vallee, Proc. Natl. Acad. Sci. USA 95 (1998) 3489–3494.
[35] D.F. Cano-Gauci, B. Sarkar, FEBS Lett. 386 (1996) 1–4.
[36] J. Zeng, R. Heuchel, W. Schaffner, J.H. Kagi, FEBS Lett. 279 (1991) 310–312.
[37] M.O. Parat, M.J. Richard, C. Meplan, A. Favier, J.C. Beani, Biol. Trace Elem. Res. 70 (1999) 51–68.
[38] C. Alapetite, T. Wachter, E. Sage, E. Moustacchi, Int. J. Radiat. Biol. 69 (1996) 359–369.
[39] C. Didier, J.P. Pouget, J. Cadet, A. Favier, J.C. Beani, M.J. Richard, Free Radic. Biol. Med. 30 (2001) 537–546.
[40] S.R.M. Sauvaigo, in: C. Press (Ed.), Imaging of Nucleic Acids and Quantification in Photonic Microscopy, 2001.
[41] Y. Hishikawa, T. Koji, D.K. Dhar, S. Kinugasa, M. Yamaguchi, N. Nagasue, Br. J. Cancer 81 (1999) 712–720.
[42] Y. Tan, R. Sinniah, B.H. Bay, G. Singh, Int. J. Gynecol. Pathol. 18 (1999) 344–350.
[43] E.S. Woo, Y. Kondo, S.C. Watkins, D.G. Hoyt, J.S. Lazo, Exp. Cell. Res. 224 (1996) 365–371.
[44] T.P. Dalton, Q. Li, D. Bittel, L. Liang, G.K. Andrews, J. Biol. Chem. 271 (1996) 26233–26241.
[45] T. Nagano, N. Itoh, C. Ebisutani, T. Takatani, T. Miyoshi, T. Nakanishi, K. Tanaka, J. Cell. Physiol. 185 (2000) 440–446.
[46] M. Levadoux, C. Mahon, J.H. Beattie, H.M. Wallace, J.E. Hesketh, J. Biol. Chem. 274 (1999) 34961–34966.
[47] D. Bittel, T. Dalton, S.L. Samson, L. Gedamu, G.K. Andrews, J. Biol. Chem. 273 (1998) 7127–7133.
[48] G.K. Andrews, Biochem. Pharmacol. 59 (2000) 95–104.
[49] M. Biagioli, W. Watjen, D. Beyersmann, R. Zoncu, C. Cappellini, M. Ragghianti, F. Cremisi, S. Bucci, Arch. Toxicol. 75 (2001) 313–320.
[50] M.S. Kim, B.J. Kim, H.N. Woo, K.W. Kim, K.B. Kim, I.K. Kim, Y.K. Jung, Toxicology 145 (2000) 27–37.