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Zinc-Metallothionein Genoprotective Effect Is Independent of the Glutathione Depletion in HaCaT Keratinocytes After Solar Light Irradiation

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Abstract UV radiations are the major environmental factors that induce DNA damage of skin cells either by direct absorption (UVB), or after inducing an oxidative stress (UVA and UVB). Cells maintain a reducing intracellular environment to avoid genomic damage. MTs have been expected not only to control metal homeostasis but also counteract the glutathione (GSH) depletion induced by oxidative stress because of their high thiol content. Induction and redistribution of MTs in cultured human keratinocytes (HaCaT) in response to SSL, is an important cellular defense mechanism against DNA damage. Reduced glutathione (GSH) is another way of cellular protection against UV-induced oxidative stress. This study which extend our previous finding focused on the relation between intracellular GSH and Zn genoprotective effects after solar irradiation. HaCaT cells, depleted or not in GSH by a chemical treatment were used to compare MTs induction by Northern blot, expression by Western blot and localization using immunocytochemistry. Zn genoprotection experiments after SSL irradiation was carried out by the comet assay. We demonstrated that in absence of GSH, Zn-MTs could protect DNA after SSL irradiation and that GSH depletion has no effect on MTs induction and localization. Nuclear Zn-MTs could be responsible for this observed genoprotection in GSH depleted cells. So the GSH/Zn and the MT/Zn systems could be two independent but interacting mechanisms of cellular protection against SSL injury. J. Cell. Biochem. 92: 631–640, 2004.

Key words: zinc; metallothionein; glutathione; DNA damage; solar irradiation

UV radiation is the major environmental factor that affects the DNA of skin cells. UVB radiations (290–320 nm) are mainly genotoxic by direct absorption of UV radiation by DNA, but more recently UVB have also been shown to induce an oxidative stress [Kuluncsics et al., 1999]. Like UVB, UVA radiations (320–400 nm) can induce cell mutations [Perdiz et al., 2000]. The carcinogenic effectiveness of UVA seems to be related to an oxidative stress that oxidizes DNA bases and subsequently induces DNA damage. The respective and synergistic roles of both UVB and UVA in carcinogenesis are now established [Burren et al., 1998]. Cells develop numerous mechanisms for maintaining their genetic integrity. Under physiological conditions, cells maintain an intracellular environment that is reducing in the face of a highly oxidizing extracellular environment. The machinery used for the redox regulation of protein activity is due to GSH, reduced thioredoxin, glutaredoxin, and some small proteins such as MTs [Arrigo, 1998; Wei et al., 2000; Sun et al., 2001; Tanaka et al., 2001].

MTs are an abundant, ubiquitous family of low molecular weight (6,000–7,000 Da) metal

Abbreviations used: BSO, L-Buthionine-SR sulfoximine; GSH, reduced glutathione; GSSG, oxidized glutathione; MTs, metallothioneins; ROS, reactive oxygen species; SSL, solar simulated light; SSR, solar simulated radiation; TPEN, NNN'N'-tetrakis (2-pyridylmethyl) ethylene diamine; UV, ultraviolet radiation; UVB (290–320 nm): ultraviolet B radiation; UVA (320–400 nm), ultraviolet A radiation; Zn, zinc.

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binding proteins, containing 25–30 % cysteine residues. Because of their high affinity to bind to, and be induced by, essential (Zn, copper) and harmful metals (cadmium, mercury...), MTs are believed to play and important role in both the homeostatic function of essential metals and in detoxification against toxic metals [Nordberg and Nordberg, 2000].

MTs have been expected, not only to control metal homeostasis but also to maintain cell survival by their free radical scavenging activities [Abel and de Ruiter, 1989; Chubatsu and Meneghini, 1993; Schwarz et al., 1995; Rossman and Goncharova, 1998]. MTs are quickly induced in response to a variety of 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 [Borghesi and Lynes, 1996]. Indeed, because of their high thiol content, MTs could maintain the intracellular redox potential and counteract the GSH depletion induced by oxidative stress.

Zn is essential for cell growth and cell survival [Coleman, 1992]. Zn is essential for the activity of more than 300 Zn-metalloenzymes participating in cell metabolism. It plays an essential structural function in Zn-requiring proteins that influence gene expression at different stages of cell proliferation and cell death [Zeng et al., 1991b; Vallee and Auld, 1992a,b; Meplan et al., 2000]. We previously reported that increased intracellular levels of Zn can protect human cutaneous cells from the deleterious effects of UV particularly DNA damage and lipid peroxidation [Markant and Pallauf, 1996; Parat et al., 1997b; Emonet-Piccardi et al., 1998; Leccia et al., 1999; Tate et al., 1999]. In a previous study we proposed MTs as an effector of antioxidant and genoprotective effects of Zn [Chimienti et al., 2001; Jourdan et al., 2002].

These protective effects are still poorly understood. Nevertheless several hypothesis could explain the biological activities of Zn. Zn can protect the thiol groups from ROS oxidation and then maintain the GSH scavenging activity [Parat et al., 1997a]. Part of the antioxidant role of Zn could be related to its ability to induce MTs. They may act as intracellular Zn buffers [Davis and Cousins, 2000]. Thus, MTs regulate the transport and the compartmentalization of Zn, maintaining adequate and appropriate intracellular Zn levels and should be mediate its delivery to Zn proteins.

Previous studies have shown that MTs are expressed in the mouse and human skin [Karasawa et al., 1991; van den Oord and De Ley, 1994; Iwata et al., 1999]. Anstey et al. [1996] demonstrated that UV-radiation induced MTs in human skin, thereby suggesting their photoprotective role. Different studies have delineated the protection afforded by MTs against UV deleterious effects both in vivo and in vitro [Kobayashi et al., 1994; Hanada et al., 1995, 1998a]. In mice, subcutaneous injection of cadmium has been reported to induce MTs and to reduce UV toxicity [Hanada et al., 1991]. Conversely, MT-null mice exhibit reduced tolerance to UVB injury [Hanada et al., 1998a]. A few studies reported that MTs could penetrate into the nucleus and then directly protect the genomic DNA [Nagel and Vallee, 1995; Hanada et al., 1998b; Schmidt and Beyersmann, 1999]. Similar results were found with lipid peroxidation. Once more the ability of MTs to redistribute Zn can also explain their role in cell proliferation and cell survival after UV irradiation by maintaining the activities of some Zn-dependent enzymes such as DNA polymerase, DNA repair enzymes, or by restoring the functions of some transcription factors [Zeng et al., 1991a,b; van den Oord and De Ley, 1994; Cano-Gauci and Sarkar, 1996; Jacob et al., 1998].

We have previously demonstrated that cells deprived of Zn by exposure to the diffusible chelator TPEN entered apoptosis [Parat et al., 1997b]. We observed both a loss of intracellular levels of GSH and DNA fragmentation after 4 h of treatment. These effects were prevented by the addition of equimolar amount of Zn and TPEN. In the same time we have demonstrated that TPEN treatment inhibited basal MTs expression which could be one of the mechanisms by which Zn could influence cell survival [Parat et al., 1999]. These results confirmed the importance of Zn, MTs, and GSH in cell survival and DNA integrity.

In this study we demonstrate that SSR induces GSH depletion. The neosynthesis of MTs by Zn treatment led to an intense induction, redistribution, and accumulation of MTs into the nucleus of irradiated cells. After a chemical GSH depletion of HaCaT cells, no MTs induction could be observed. Moreover, these GSH depleted HaCaT cells express and redistribute MTs after Zn treatment. Induction of MTs

by Zn in GSH depleted cells could be an important antioxidant defense mechanism that could maintain cell survival after solar irradiation.

MATERIALS AND METHODS

Cell Culture and Chemical Treatments

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 5% CO_2 atmosphere. Culture medium was replaced twice a week, and cell monolayer were split 1/6 at 90% confluence. To avoid bacterial and fungus contamination, the medium was supplemented with 5,000 IU/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 kit (Mycoplasma Detection Kit, Boehringer Mannheim Corp., Indianapolis, IN.

Chemical treatments

Zn. A sterilized stock solution of Zinc chloride ($ZnCl_2$, 0.1 M) was prepared in desionized water (Merck, Darmstadt, Germany). Zn (100 μM) was added in HaCaT culture medium for 24, 48, or 72 h. This final concentration was chosen according to previous results obtained in the laboratory [Parat et al., 1997b].

BSO. BSO (Sigma Chemical Co., St Louis, MO), an inhibitor of the GSH synthesis, was added to the culture medium at the concentration of 80 μM for 18 h.

Irradiation procedure. The light source 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 UVR spectrum that nearly eliminates visible and infrared radiation [for the spectrum see Jourdan et al. [2002]]. The irradiance effectively received by the cells (17.5 mW/cm^2) was measured using a dosimeter (Müller GmbH Elektronik-optik) with a spectral sensitivity from 270 nm to 4 μm. Cells were cultured in 9 cm^2 petri dishes and irradiated from the top with different physiological UV doses (0.75 or 1.5 J/cm^2). Just prior to irradiation, the culture medium was removed and reserved. Cells were rinsed twice with phosphate buffer without calcium and magnesium (PBS, Gibco BRL, Life Technology Ltd., Paisley, Scotland) and maintained in 2 ml PBS for irradiation. Temperature was controlled. Control cells were similarly treated and left in the dark while irradiation was carried out. After the stress, cells were put back into their initial medium, and placed in the incubator for 24 h of recovery.

Single-cell gel electrophoresis. [Alapetite et al., 1996; Emonet-Piccardi et al., 1998]. DNA damages were evaluated using the comet assay. Cells were detached from their culture dishes and included in low-melt agarose. Embedded cells were immediately irradiated as previously described [Didier et al., 2001]. All slides were immersed overnight in a cell lysis buffer in the dark at 4°C, in order to prevent nonspecific DNA and also prevent repair processes 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 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) 50 μl of 0.5 mg/ml ethidium bromide was pipetted on the slides to stain the DNA. The slides were placed in a humidified air-tight container to prevent drying of the gel, until the analysis was performed. The quantification of DNA damage was performed using 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 averaged TM was determined using three different slides prepared as previously described [Didier et al., 2001].

Immunocytochemical staining. 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-wells plastic Lab-Tek® chamber slides (Nunc, Naperville, IL) (5,000 cells/well), cultured for 2 days and then $ZnCl_2$ (100 μM) was 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 non-specific 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 saturation 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 pH 8.6, cells were incubated with the avidin–biotin complex (StreptABCComplex/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 haematoxylin and slides were mounted with Aquamount. Blanks were either 1 μg/ml nonimmune mouse immunoglobulin, or 1% (w/v) BSA in PBS. In our experimental conditions cells did not exhibit any endogenous peroxidase activity. Positive control cells were treated with a monoclonal antibody IgG reactive against a 46 Kd cytokeratin isolated from keratinocyte (KL1, Immunotech, Marseille, France).

Western blotting. 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 Co.) and centrifuged at 4,000 rpm for 15 min at 4°C). Cytoplasmic and nuclear proteins were extracted and quantified (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 electro transfer at 60 mA for 3 h at 4°C. The membrane was saturated with a milk buffer (5% dried milk, 0.5% Nonidet P40 in PBS pH 7.4) for 2 h and incubated with the monoclonal mouse anti human MT antibody diluted 1:4,600 (clone E9, Dako) 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 1:5,000. Bound antibodies were visualized by chemiluminescence using an ECL Western immunoblotting Kit (Amersham Pharmacia Biotech, Piscataway, NJ) as specified by the manufacturer. Pure Horse MT-I and MT-II solution (Sigma Chemical Co.) was electrophoresed (6.25 μg/lane) in an independent lane to control MT migration.

Northern blotting. Samples were collected at 3 h after treatment and lysed directly on the culture dishes. Total RNA was prepared in accordance with the QuickPrep™ Micro mRNA purification kit (Amersham Pharmacia Biotech). Total RNA (10 μg) in sample buffer added with 0.1% (w/v) ethidium bromide was loaded on a 1.2 (w/v) agarose gel containing 1.2% (v/v) formaldehyde solution and separated with Mops buffer. The RNA was blotted overnight by capillary elution on a nylon membrane (Hybond-N^+, Amersham, Uppsala, Sweden) with 20 × SSC and cross-linked by UV irradiation. Blots were alternatively hybridized to the ^{32}P-labeled MT-IIA probe and the labeled glyceraldehyde phosphate deshydrogenase (^{32}P-GAPDH) cDNA (Clontech laboratories, Inc., Palo Alto, CA). Probes were radiolabeled with Nonaprimer Kits (Appligene Oncor). After 18 h at 42°C the membranes were washed twice in SSC buffer with increased molarity.

Statistics

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

RESULTS

Comparison of Cellular MTs Localization in HaCaT Cells Depleted or not in GSH After Zn and or SSL Irradiation

Immunostaining (Fig. 1) was used to compare the localization of MTs in HaCaT cells treated with BSO (Fig. 1-2) and without BSO (Fig. 1-1).

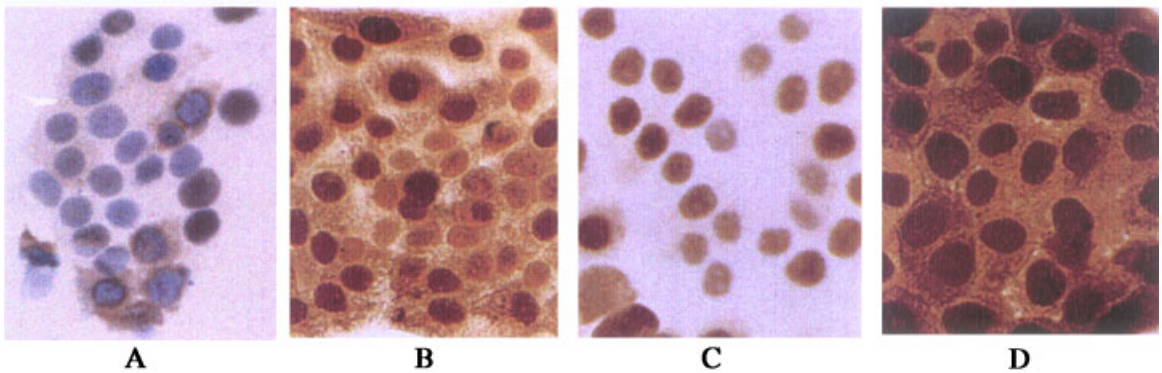

Fig. 1-1.

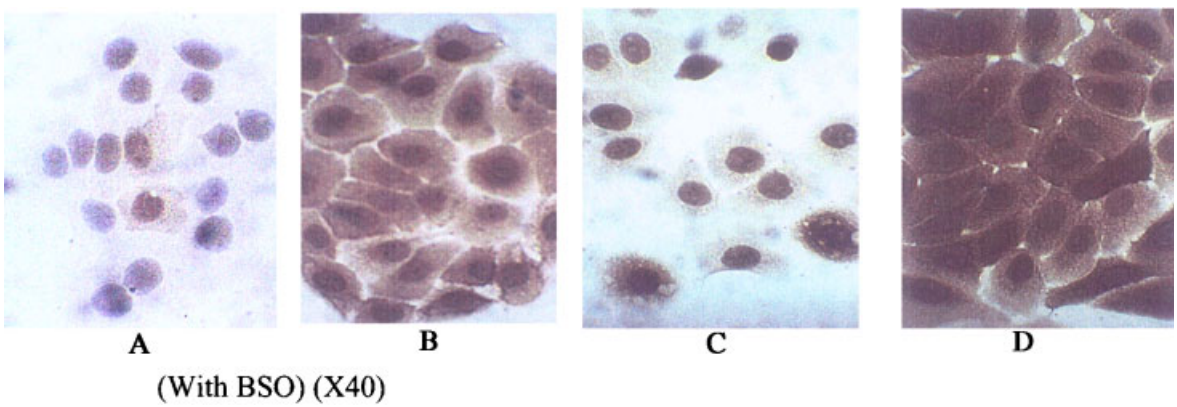

Fig. 1-2.

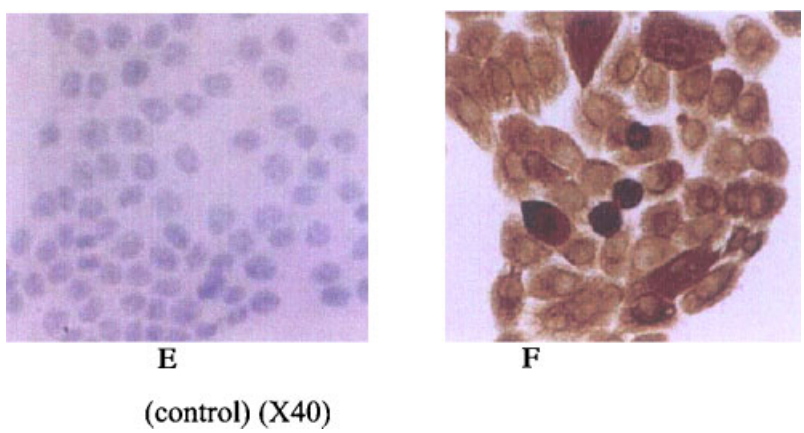

Fig. 1-3.

Fig. 1. Intracellular MTs localization in HaCaT cells after immunostaining. MTs were visualized using light microscopy (×40). Representative results are shown. **1-1**: Cells without BSO treatment. **1-1A**: Cells with basal medium, (**1-1B**) Zn treatment (100 μM, 24 h), (**1-1C**) SSL irradiated cells (0.75 J/cm^2), (**1-1D**) Zn treated (100 μM, 24 h) and SSL irradiated cells (0.75 J/cm^2). **1-2**: Cells treated with BSO (80 μM, 18 h). **1-2A**: Cells with basal medium and BSO, (**1-2B**) Zn treatment (100 μM, 24 h), (**1-2C**) SSL irradiated cells (0.75 J/cm^2), (**1-2D**) Zn treated (100 μM, 24 h) and SSL irradiated cells (0.75 J/cm^2). **1-3**: Immunostaining controls. **1-3E**: Negative control (no primary antibody), (**1-3F**) Positive control (cells labeled with KL1 primary antibody) for details see ''Material and Methods.'' [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

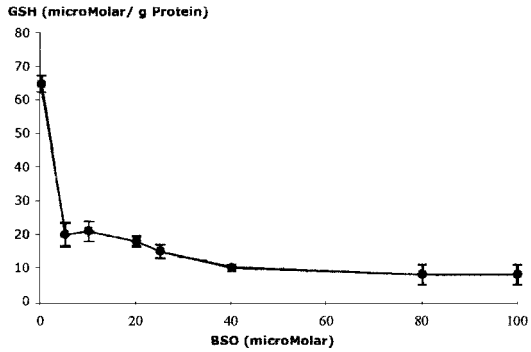

Fig. 2. Determination of GSH content in HaCaT cells after BSO treatment at several non toxic doses. GSH content was measured after 18 h of treatment by the method previously described [Parat et al., 1997a]. Each experiment was repeated three-times. All data expressed as mean ± one standard deviation.

Several conditions were tested, basal condition (Fig. 1-1A,2A); Zn treated cells (Fig. 1-1B,2B), SSL irradiated cells (Fig. 1-1C,2C), both Zn and SSL irradiated cells (Fig. 1-1D,2D).

The presence of constitutive MTs was observed (Fig. 1-1A,2A). In basal culture conditions, a very low staining was visualized; this staining was only localized in the cytoplasm of the cell. Controls (Fig. 1-3E,F) indicated the high specificity of the two antibodies used. As previously demonstrated a single solar irradiation at the dose of 0.75 J/cm^2 (Fig. 1-1C) induced the synthesis of MTs and a complete redistribution of the protein in the nucleus. Interestingly, cytoplasms of HaCaT cells were not stained. Conversely, in Zn treated cells (100 μM for 24 h) a very intense staining was observed both in cytoplasm and nucleus, indicating a new distribution of the protein (Fig. 1-1B).

Moreover, the increased staining observed in Figure 1-1D suggests a synergic effect of UV irradiation and Zn treatment on MTs synthesis. The comparison of Figure 1-1 and 1-2 demonstrated that no modification of the cellular MTs localization could be observed in cells treated or not with BSO. For each condition the same MTs localization and staining intensity was seen. GSH depletion has no effects on cellular MTs localization.

Role of intracellular GSH levels on MTs induction and Zn protective effect against SSR injury.

GSH Depletion of HaCaT Cells With BSO

Some studies indicated that a decrease in intracellular GSH level could induce MTs. To test the hypothesis of a link between GSH decrease and MTs induction in SSR exposed cells, keratinocytes were treated with non toxic doses of BSO for 18 h and the GSH levels were determined (Fig. 2). BSO treatment (5–10 μM) for 18 h caused a dramatic decrease in GSH content of HaCaT cells. For 10–80 μM we obtained a slight decrease in GSH content of cells. A cytotoxic effect of BSO appeared after 18 h of treatment with 120 μM (data not shown).

MTs Induction by Zn Treatment in GSH Depleted Cells

In a first step, we quantified the MTs level in BSO treated cells by Western blotting. Figure 3 showed that GSH depletion has no significant effect on MTs synthesis. The levels of MTs were similar in Zn treated cells for 24–72 h, with or without BSO treatment. A slight but not significant enhancement of MTs expression was found in BSO treated cells (lane 2) compared to control cells (lane 1). A very low amount

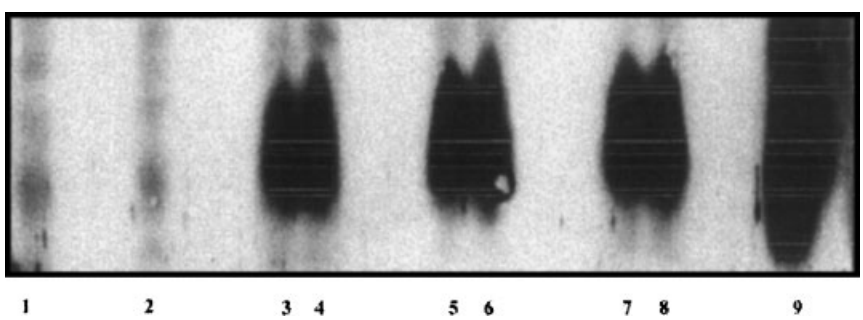

Fig. 3. Comparison between the kinetics of MTs induction in HaCaT cells exposed to Zn ($ZnCl_2$, 100 μM) or exposed to both BSO (80 μM, 18 h) and Zn. Western blot was performed as described in ''Materials and Methods.'' **Lane 1**: Control cells (basal medium containing 3.4 μM of Zn), (**lane 2**) cells treated with BSO, (**lane 3**) Zn treatment for 24 h, (**lane 4**) Zn treatment for 24 h and BSO, (**lane 5**) Zn treatment for 48 h, (**lane 6**) Zn treatment for 48 h and BSO, (**lane 7**) Zn treatment for 72 h, (**lane 8**) Zn treatment for 72 h and BSO, (**lane 9**) control, 6.25 μg of commercial MT-I and MT-II was loaded.

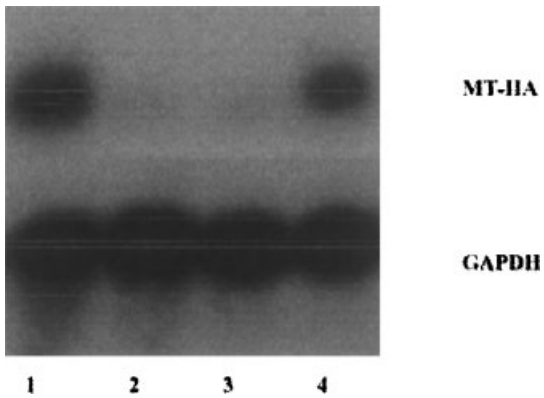

Fig. 4. Northern blot analysis of MTIIA mRNA expression in HaCaT cells. **Lane 1**: Zn treated cells for 3 h, (**lane 2**) control cells (basal medium with 3.4 μM of Zn), (**lane 3**) BSO treated cells, (**lane 4**) cells treated by both BSO (80 μM, 18 h) and Zn (100 μM, 3 h).

of basal MTs expression can be visualized in HaCaT keratinocytes control cells (lane 1). The same amount of MTs was detected in HaCaT treated for 24 h with Zn (lane 3) or treated both with Zn and BSO (lane 4). We have identical results for 48 h of Zn with or without BSO (lane 5 and 6) and 72 h (lane 7 and 8).

These data were confirmed by the Northern blot analysis (Fig. 4). In control (lane 2) and BSO (80 μM, 18 h) treated cells (lane 3) we did not visualize MT-IIA mRNA. We can conclude that GSH depletion in HaCaT cells is not sufficient to induce MT-IIA mRNA whereas MT-mRNA were clearly identified in Zn treated cells (lane 1). On the other hand, GSH depletion did not modify significantly MT-IIA mRNA induced by Zn in HaCaT cells (lane 4).

Zn Genoprotection in Both Solar Irradiated and GSH Depleted Cells

Zn could protect cells by stacking thiol groups of GSH. To understand the potential interaction of Zn-MT and GSH to protect cells against UV damage we used comet assay.

DNA damage was determined in cells treated both with Zn and BSO (BSO + Zn). Figure 5 showed that the GSH depletion had no significant effects on DNA damage induced by low dose of solar radiation. As previously demonstrated [Jourdan et al., 2002], Zn treatment caused a significant decrease of the TM in SSL irradiated cells, due to nuclear MTs redistribution. Moreover, we showed that BSO treated cells also show a significant decrease of the TM after SSL irradiation, indicating that Zn genoprotective effects are not only linked to the GSH level.

DISCUSSION

If the preventive role of Zn in photoprotection is well known, the mechanism is unclear. Thus we focused on the relation between Zn, GSH, and MTs, a cysteine-rich protein, widely distributed in a broad range of eucaryotic species. For a long time MTs were considered only as a stress protein induced by heavy metals or other stimuli. Recent studies have shown the constitutive expression of this protein in a variety of human cells, especially in epidermal

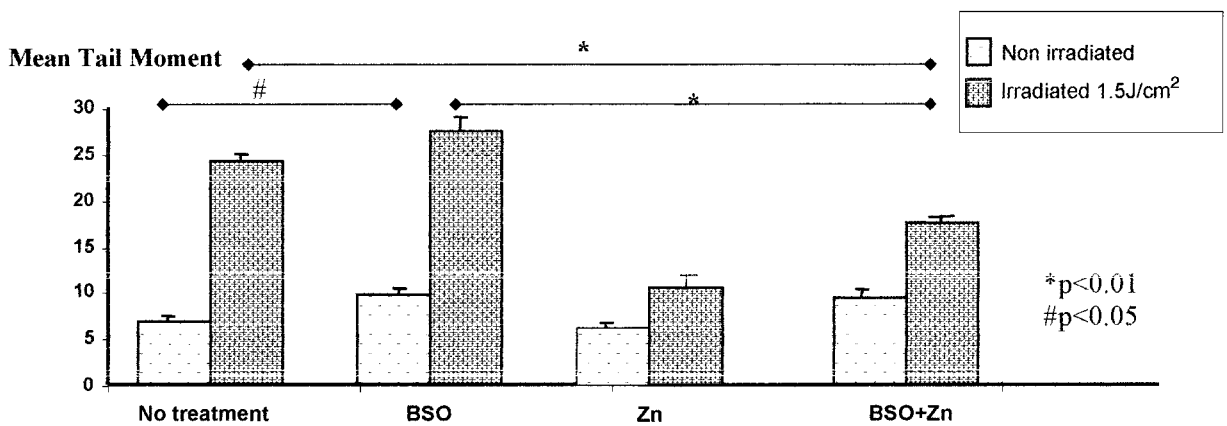

Fig. 5. Comparison of DNA damage after a single SSL irradiation (1.5 J/cm^2) in HaCaT cells treated by Zn (100 μM, 72 h) or both exposed to BSO (80 μM, 18 h) and Zn (100 μM, 72 h). The comet assay was performed as described in ''Materials and Methods.'' Each experiment was repeated three-times. All data expressed as mean ± one standard deviation, were processed statistically using one way analysis of variance (ANOVA) and a Newman–Keuls test. Differences were considered significant when $P < 0.05$.

keratinocytes in vivo [van den Oord and De Ley, 1994; Anstey et al., 1996; Parat et al., 1999]. But their precise role in human skin has not been elucidated. Moreover, more and more studies point out the overexpression of MTs in a great variety of cancers indicating an important role in cell survival and more precisely in the control of cell proliferation [Woo et al., 1996; Hishikawa et al., 1999; Schmidt and Beyersmann, 1999; Tan et al., 1999].

Zn and MTs are both described as important antioxidants protecting cells against DNA damage and lipid peroxidation, maintaining intracellular antioxidant defenses, and preventing apoptosis. This study, which extended our previous findings on cellular antioxidant protection mechanisms afforded by Zn and MTs, demonstrates that Zn prevents DNA genomic lesions induced by solar irradiation (UVA + UVB) at physiological doses. This Zn-genoprotection is an inducible mechanism, independent of the GSH content of HaCaT cells, and strongly correlated with both overexpression and nuclear localization of MTs. This nuclear localization in GSH depleted cells is very important as DNA is very sensitive to oxidative stress and to the deleterious effects of UV, whereas the nucleus is lacking in antioxidant enzymes such as catalase and superoxide dismutase (SOD). The induction of Zn-MTs could be considered as an important adaptive mechanism for the irradiated cells. These proteins prevent DNA lesions due to UV even if the cells are depleted in GSH. Then Zn-MT could maintain the nuclear redox potential and facilitate DNA repair mechanisms by exchanging Zn atoms with Zn-dependant DNA repair enzymes.

Another hypothesis, to understand the genoprotective effect of Zn could be through the maintenance of the reduced thiol groups [Nakatani et al., 2000]. Zn could bind to vicinal sulfhydryl groups, therefore, preventing intramolecular disulfide formation [Seehra and Jordan, 1981]. UV is known to decrease the intracellular GSH levels [Lautier et al., 1992]. Some previous data indicated that GSH depletion could induce MTs in vivo [Sato et al., 1995]. Zn-MTs induced by SSR could prevent the GSH consumption by stacking their numerous thiol groups.

To test this hypothesis HaCaT Keratinocytes were treated by BSO. In our model GSH depletion did not induce MTs overexpression per se. Zn treatment and UV irradiation induced similar levels of intracellular MTs both in control and GSH depleted cells indicating that Zn genoprotective effects appeared not to be related to the intracellular GSH content.

A previous study suggested that under normal conditions, irradiated cells could be protected against oxidative stress by GSH [Quesada et al., 1996].

Here, for the first time, we demonstrated that in absence of GSH, Zn-MTs could protect DNA after SSL irradiation and that GSH depletion has no effect on MTs induction and localization. This observed genoprotection was carried out by nuclear Zn-MTs. So the GSH/Zn and the MT/Zn systems could be two independent but interacting mechanisms of cellular protection against SSL injury.

Some work showed that intracellular redox cycle of GSH and MTs was coupled by catalytic selenols [Chen and Maret, 2001], here we postulated that GSH and MTs genoprotective effects were coupled by the essential metal, Zn.

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