J. Cosmet. Sci., 63, 359–364 (November/December 2012)

Protection against UVB-induced oxidative stress in human skin cells and skin models by methionine sulfoxide reductase A

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Accepted for publication March 28,2012.

Synopsis

Environmental trauma to human skin can lead to oxidative damage of proteins and affect their activity and structure. When methionine becomes oxidized to its sulfoxide form, methionine sulfoxide reductase A (MSRA) reduces it back to methionine. We report here the increase in MSRA in normal human epidermal keratinocytes (NHEK) after ultraviolet B (UVB) radiation, as well as the reduction in hydrogen peroxide levels in NHEK pre-treated with MSRA after exposure. Further, when NHEK were pre-treated with a non-cytotoxic pentapeptide containing methionine sulfoxide (metSO), MSRA expression increased by 18.2%. Additionally, when the media of skin models were supplemented with the metSO pentapeptide and then exposed to UVB, a 31.1% reduction in sunburn cells was evident. We conclude that the presence of MSRA or an externally applied peptide reduces oxidative damage in NHEK and skin models and that MSRA contributes to the protection of proteins against UVB-induced damage in skin.

INTRODUCTION

Skin is the first line of defense against environmental trauma in humans. When skin is exposed to ultraviolet B (UVB, 295–320 nm), the production of reactive oxygen species (ROS) can lead to cellular disruption and various skin pathologies including photo aging (1). At the cellular level, UV-induced ROS target unsaturated membrane lipids to form lipid peroxides (2), as well as DNA to form oxidative lesions, such as 8-oxo-deoxyguanosine (3). Proteins are also susceptible to oxidative stress due to the formation of carbonyl moieties and sulfhydryl oxidation forming disulfide bonds (4). Another form of protein oxidation that has received less attention is the formation of methionine sulfoxide (metSO) (5).

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Methionines may act like free radical sinks by reacting with ROS to form metSO on the periphery of a protein and thus protect the protein (6). Although methionine is oxidized in the process, the reactive intermediate is disarmed and the overall integrity of the protein is preserved. metSO may then be regenerated back to methionine by methionine sulfoxide reductase A (MSRA) using thioredoxin as a cofactor (7). This strategy may be advantageous to a cell because the time for repair to occur may be a critical factor for cell maintenance or it may be energetically more favorable for the cell to repair itself rather than to resynthesize a new protein.

In this study, MSRA levels were evaluated in normal human epidermal keratinocytes (NHEK) before and after UVB exposure. MSRA's protective effect was also determined by a direct addition of this enzyme to cells before irradiation. Further, NHEK were pretreated with a metSO-containing pentapeptide (mp) in order to determine if MSRA could be induced in a non-cytotoxic manner and provide an enhanced antioxidant defense mechanism. Lastly, this peptide was added to the media of skin models and tested for its ability to inhibit sunburn cell production after UVB treatment.

MATERIALS AND METHODS

CELL CULTURE

NHEK were obtained from Cascade Biologics (Portland, OR) as primary culture male cells. Cells were maintained in EpiLife (calcium-free) medium containing 1% supplemented serum (Cascade).

SKIN MODELS

EFT 400 skin models were obtained from MatTek (Ashland, MA) and maintained with a transwell membrane air/liquid interface as per the manufacturer's instructions. After treatment, histological examination was carried out by hematoxylin and eosin staining (Paragon Bioservices, Baltimore, MD).

REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION

Conventional reverse transcription polymerase chain reaction (RT-PCR) was performed on RNA extracts for *msrA* transcription using primers obtained from Invitrogen (Carlsbad, CA): GTGGTGTTCCAGCCCGAGCACAT (sense) and ATGTCGGTGGTGATCAGGCCGAA (antisense). β-Microglobulin or *gapdh* was used as housekeeping genes. Following annealing, reverse transcription, and amplification, amplicons were separated on 2% agarose gels. Gels were stained with SYBR Gold (Invitrogen) and visualized by UV transillumination. Images were captured with a charge-coupled device (CCD) camera and quantitated with UnScanIt imaging software (Silk Scientific, Orem, UT).

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HYDROGEN PEROXIDE DETERMINATION

Hydrogen peroxide (H_2O_2) was measured as described previously (8). Briefly, cells were treated with 10 μM 2′,7′-dichlorodihydrofluorescein diacetate (Molecular Probes, Eugene, OR) for 6 h and their fluorescence measured ($Ex_{485\ nm}/Em_{530\ nm}$).

CELL VIABILITY

Cell viability was evaluated by an MTS assay as described previously (9).

MSRA AND METHIONINE SULFOXIDE PEPTIDE

MSRA was obtained as an experimental test sample from Promega (Madison, WI). The pentapeptide containing metSO was synthesized and obtained from MMP Inc. (Plainfield, NJ).

RESULTS

NHEK were irradiated in Dulbecco's phosphate-buffered saline, pH 7.4 with 50 mJ/cm^2 UVB, the RNA isolated, and subjected to RT-PCR. The results from this analysis showed an increase in *msrA* expression in response to environmental trauma (Figure 1). The *gapdh*-normalized *msrA* levels (*msrA*/*gapdh* ratio) increased 22.6% from 0.53 to 0.65 (±0.07 S.E.). In order to establish whether increased amounts of MSRA could reduce ROS in NHEK following UVB irradiation, NHEK (2×10^4) were pre-treated with 0.35 and 0.7 mg/ml of MSRA overnight and then exposed to 20 mJ/cm^2 UVB. As shown in Figure 2, H_2O_2 decreased by 24.7% (±3.3% S.E.) and 36.1% (±0.5% S.E.), respectively.

In a next series of experiments, an mp was added to NHEK for 24 h, harvested, and then analyzed by RT-PCR for *msrA* expression as before. Additionally, an identical pentapeptide containing methionine instead of metSO was tested in parallel. The results from these experiments demonstrated an 18.2% (±4.0% S.E.) increase in *msrA* expression in the cells treated with only 0.01 mg/ml mp, whereas a similar increase was not observed in the met-containing mp (Figure 3). Increases were observed at the 0.1 and 0.25 mg/ml concentrations as well. Further, no cytotoxicity was measured for either pentapeptide as determined by the MTS assay for cell viability (data not shown).

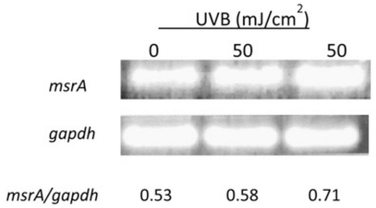

Figure 1. Conventional RT-PCR analysis of UVB-irradiated NHEK showed an average increase in *msrA* expression when normalized to *gapdh* (0.53 vs. 0.65 ± 0.07 S.E.).

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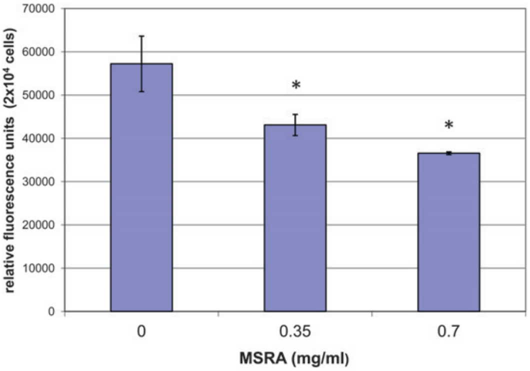

Figure 2. H_2O_2 levels decreased in the presence of 0.35 and 0.70 mg/ml MSRA by 24.7% (±3.3% S.E.) and 36.1% (±0.5% S.E.), respectively (* = Student's *t*-test assuming unequal variances, $p < 0.05$, n = 3).

To test the potential usefulness of boosting MSRA levels in skin, mp was then added to the media of skin models for 48 h, exposed to 100 mJ/cm^2 UVB, and then incubated for an additional 24 h post-irradiation. The skin model samples were fixed, subjected to histological analysis, and stained with hematoxylin and eosin. In Figure 4, light microscopic images of these skin models show an increase in sunburn cells in UVB-exposed skin model samples. Sunburn cells are characterized by increased pyknotic nuclei and eosinophilic staining (10). In contrast, the presence of sunburn cells in skin model samples pre-treated with the mp prior to UVB exposure was significantly diminished by 31.1% (±10.0% S.E.).

DISCUSSION

Human skin has developed many strategies to counteract the effects of environmental exposures and their consequent trauma. In this report, we describe the presence of *msrA* in NHEK and its induction by UVB. Our data indicate a rapid and protective

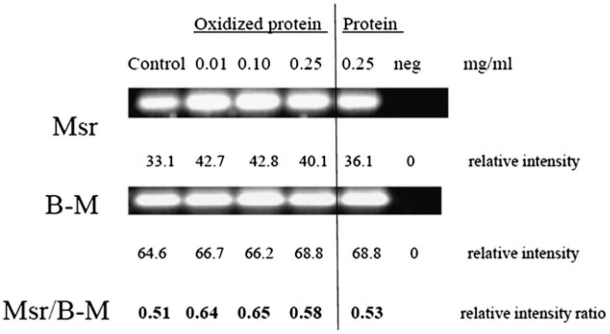

Figure 3. Conventional RT-PCR analysis of NHEK pre-treated with mp. *msr*A expression increased by 18.2% (±4.0% S.E., n = 4) when treated with 0.01 mg/ml mp and normalized to β-microglobulin. Higher levels (0.1 and 0.25 mg/ml) of mp also induced increases in *msrA*, whereas the unoxidized form had no effect.

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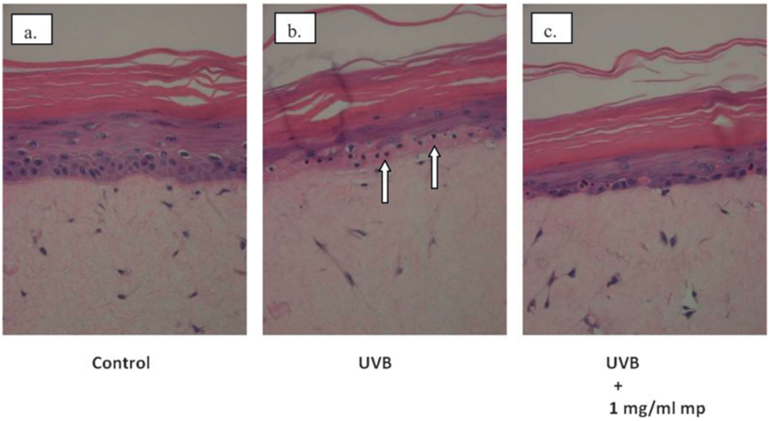

Figure 4. Histological cross-sections of skin models stained with hematoxylin and eosin after 100 mJ/cm^2 UVB irradiation with and without 1 mg/ml mp treatment. A 31.1% (±10.0% S.E.) reduction in sunburn cells (see arrows) was calculated from five contiguous light microscopic fields (40x) in two separate experiments. (a) Unexposed, (b) UVB-exposed, (c) mp + UVB-exposed.

response to UVB-induced ROS, which may be critical for normal protein function. We further show how an mp can trigger this protective response in NHEK. Additionally, when this mp was incubated with a skin model sample, we saw a reduction in UVB-induced cellular damage. Thus, in order to account for the effects observed in skin models, we postulate that proteins sensitive to the presence and repair of metSO are related to the activation of signaling and apoptotic mechanisms. We thus conclude that MSRA plays a protective role at the protein level in NHEK and could also provide protection at the cellular level in skin. Future work in this area should include the application of small interfering RNA to silence *msrA* or the overexpression of transfected *msrA* cDNA in order to further delineate the role played by MSRA in NHEK. Lastly, topical application of small molecular weight peptides containing metSO may provide protection to human skin against UV-induced photodamage leading to improved cutaneous health.

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