Journal of Dermatological Science 72 (2013) 290–295

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Solar-simulated radiation and heat treatment induced metalloproteinase-1 expression in cultured dermal fibroblasts *via* distinct pathways: Implications on reduction of sun-associated aging

Cheng-Che E. Lan [a,b], Ching-Shang Wu [c], Hsin-Su Yu [a,*]

[a] Department of Dermatology, Kaohsiung Medical University Hospital, Department of Dermatology, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[b] Department of Dermatology, Kaohsiung Municipal Ta-Tung Hospital, Kaoshiung Medical University, Kaohsiung, Taiwan
[c] Department of Medical Laboratory Science and Biotechnology, Kaohsiung Medical University, Kaohsiung, Taiwan

ARTICLE INFO

Article history:
Received 7 December 2012
Received in revised form 26 June 2013
Accepted 30 July 2013

Keywords:
Solar aging
Solar radiation
Heat
Fibroblast
MMP-1
ROS
TRPV1

ABSTRACT

Background: Sun exposure is an important environmental factor affecting human beings. Most knowledge regarding solar aging focused on light radiation (photoaging), and little emphasis has been placed on heat, a factor that is also closely associated with sun exposure.
Objective: This study was launched to evaluate the effects of simulated solar radiation (SSR) and environmental heat on skin fibroblasts in terms of dermal aging.
Methods: Cultured human dermal fibroblasts were treated with moderate amount of SSR (200 J/cm^2) and heat (+2 °C). The metalloproteinase-1 (MMP-1) expression was used as a surrogate marker for dermal aging and the involved regulatory mechanisms were explored.
Results: Both treatment conditions did not affect viability but significantly increased the expressions of MMP-1. In parallel, both treatments increased the intracellular levels of reactive oxygen species (ROS), but the increase induced by SSR is much greater than heat. In contrast, transient receptor potential vanilloid 1 (TRPV-1), the sensor of environmental heat, was upregulated by heat but not SSR treatment. Pretreating fibroblasts with antioxidant abrogated the SSR-induced MMP-1 but has limited effect on heat-induced MMP-1. On the other hand, TRPV-1 antagonist pretreatment reduced heat-induced MMP-1 in fibroblasts but not their SSR-treated counterparts.
Conclusion: Both SSR and heat induced MMP-1 expression in dermal fibroblasts but through different pathways. As current strategies for reducing sun-related aging focused on filtering of light and use of antioxidants, future strategies design to reduce solar aging should also incorporate heat-induced aging into consideration.

1. Introduction

Solar radiation is an important component of nature that makes intricate interactions with living organisms. Encompassing ultraviolet (UV), visible, and infrared (IR) regions of light, solar radiation has been recognized to induce photoaging. The clinical characteristics of sun exposure related aging include increased wrinkle formation and loss of skin resilience. Histologic and ultrastructural studies indicate that the pathognomonic changes in sun exposure related skin aging are mainly found at dermal connective tissue in which the collagen fibrils provide mechanical support for the skin [1,2]. More specifically, loss of dermal collagen is the hallmark of sun-related skin aging [3,4]. Mechanistically, photoaging is believed to be caused by photons that penetrate into the dermis and induce reactive oxygen species (ROS) formation in the fibroblasts that modulate various physiologic changes [5]. Increased synthesis and expression of matrix metalloproteinase-1 (MMP-1) by dermal fibroblasts after UV and IR radiation through increased ROS formation are believed to play a critical role in photoaging by enhancing degradation of type-1 and type-3 collagen [6–8]. Therefore, MMP-1 is thought to play a key role in the pathogenesis of photoaging in human skin [9].

In addition to photons, the radiation from the sun also provides heating energy for the surface of the earth. Although sun exposure

Abbreviations: SSR, solar simulating radiation; MMP-1, metalloproteinase-1; ROS, reactive oxygen species; TRPV-1, transient receptor potential vanilloid 1; UV, ultraviolet; IR, infrared; DMEM, Dulbecco's minimal essential medium; FACS, fluorescence-activated cell sorting; MFI, mean fluorescence intensity; 8-OHdG, 8-hydroxy-2-deoxyguanosine; NAC, N-acetylcysteine; RR, ruthenium red.

* Corresponding author at: Department of Dermatology, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung, Taiwan.
E-mail addresses: ec_lan@hotmail.com (C.E. Lan), yup.kmu@gmail.com (H.-S. Yu).

0923-1811/$36.00
http://dx.doi.org/10.1016/j.jdermsci.2013.07.015

naturally includes light radiation that accompanies elevated environmental heat, the current concept for preventing sun-related aging focused on light radiation with little emphasis on the effect of heat. It has been demonstrated, however, that heat shock treatment induces MMP-1 expression through activation of extracellular signal related kinase pathways in cultured human fibroblasts [10]. Mechanistically, the transient receptor potential vanilloid 1 (TRPV1) has been shown to mediate the heat shock induced MMP-1 expression in human keratinocytes [11]. Moreover, it has been shown that environmental heat generated by natural sun slight exposure induces MMP-1 expression in the human skin [12]. Therefore, we hypothesized that light and heat, the two different forms of energy derived from the sun, may both contribute to solar aging but through different pathways. The current study was launched to investigate the effects of moderate solar simulating radiation (SSR) and heat treatment on culture human fibroblasts in terms of MMP-1 expression and explore the molecular events involved.

2. Methods

2.1. Culture of dermal fibroblasts

Normal human dermal fibroblasts were obtained from healthy adult foreskin. Cell culture for dermal fibroblasts were performed as described elsewhere [13]. Briefly, the adipose tissues were removed by a sterilized scissor, and the remaining specimens including epidermis and dermis were sectioned into 5 mm by 5 mm blocks and laid onto 10 cm^2 Petri dishes. The dishes were maintained semi-openly in the laminar flow for 40 min in order to adhere the blocks onto the surface. Once the blocks were fixed, 10 ml of Dulbecco's minimal essential medium (DMEM) (Gibco BRL, Gaithersburg, MD) with 10% fetal bovine serum (Gibco), penicillin (100 U/ml) and streptomycin (100 μg/ml) (Gibco) were added to the dishes. Subsequently, the culture dishes were maintained in a humidified incubator and the culture medium was changed every two days. The fibroblasts gradually crawled out of the minced blocks in 10 days. In this study, the fibroblasts were cultured in the serum-free medium overnight for synchronization prior to the experiments. The cultured fibroblasts from passages six to ten were used for the experiments.

2.2. Treatment of fibroblasts

A SSR device (SAN-EI Electric Co., Osaka, Japan) was used to mimic the effects natural sunlight on dermal fibroblasts. Spectroradiometric assessment of the SSR indicated that relative emission in the 350–700 nm and 700–1100 nm regions were 58.5% and 41.5%, respectively. This radiation spectrum closely resembles natural sunlight. The irradiance of SSR was 100 mW/cm^2, and the fluence used in our study was 200 J/cm^2. Accordingly, the irradiation time required for this fluence was 33 min and 20 s. In our experimental condition, no elevation of temperature was detected during the SSR irradiation. To investigate the effects of environmental heat on fibroblasts, cultured fibroblasts were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h. Then the cells of experimental group were placed in a similar incubator with temperature set at 39 °C. Thirty minutes after elevated heat treatment, the fibroblasts were moved back to the original incubator for indicated duration (37 °C).

2.3. Cell viability assay

The viability of cultured fibroblasts was determined using an MTS cell proliferation kit (CellTiter 96® AQueous One Solution Cell Proliferation Assay kit, Promega, Madison, WI) as described previously [14]. Twenty-four hours after indicated treatment, 20 μl of CellTiter reagent containing tetrazolium compound and electron coupling reagents were added into each well. The plates were then incubated for 4 h and the colorimetric absorbance was recorded at 570 nm by a microplate reader SpectraMax M5 (Molecular Devices, Sunnyvale, CA).

2.4. Determination of intracellular ROS by flow cytometry

Cultured fibroblast cells (4×10^5 cells) were seeded onto 6-well plate and pre-loaded with 10 μM of 2′,7′-dichlorodihydrofluorescein-diacetate (DCF-DA) solution (Sigma, St. Louis, MO) for 30 min. Immediately after indicated treatment, the fibroblasts were harvested and washed three times with PBS. 10,000 cells were then sorted by fluorescence-activated cell sorting (FACS) (FACScan; Becton Dickinson, San Jose, CA, USA) and analyzed (FL-1 channel) by CELLQuest Prosoftware (Becton Dickinson). The mean fluorescence intensity (MFI) of each test was determined by the WinMDI software (http://facs.scripps.edu/index.html).

2.5. 8-Hydroxy-2-deoxyguanosine (8-OHdG) measurement

The 8-OHdG enzyme immunoassay (EIA) kit (Cayman Chemical Company, Ann Arbor, MI, USA) is a competitive assay used for quantification of 8-OHdG. The EIA utilizes an anti-mouse IgG-coated plate and tracer consisting of an 8-OHdG-enzyme conjugate. The supernatants of fibroblast culture were collected immediately after treatments and subjected to 8-OHdG determination according to the manufacturer's guidelines. The development reagent (Ellman's Reagent) was added to each well and the absorbance was read with ELISA reader at 405–420 nm.

2.6. Real-time quantitative polymerase chain reaction (PCR)

The expression of MMP-1 mRNA and TRPV-1 mRNA were determined by real-time PCR. For indicated experiments, cultured fibroblasts were pretreated with 6 mM N-acetylcysteine (NAC, antioxidant) and 10 μM ruthenium red (RR, TRPV-1 inhibitor) for 2 h before the SSR or heat treatment. The fibroblasts were harvested at 16 h after indicated treatment. This time duration was selected based on our preliminary study showing that the increases in mRNA expression were predictable and significant at this time point after stimulation. Total RNA were extracted from treated fibroblasts using the Trizol method (Gibco) and processed as recommended by the manufacturer. Five microgram of RNA were reverse-transcribed to cDNA as the PCR template. The primers used for real-time PCR were the following: MMP-1, 5′-TGTGGCTCAGTTTGTCCTCACT-3′ (sense) and 5′-CAAATCTGGCGTGTAATTTTCAAT-3′ (anti-sense); TRPV-1, 5′-ACACACCTGATGGCAAGGAC-3′ (sense) and 5′-AGACTGCCTATCTCGAGCAC-3′ (anti-sense); β-actin, 5′-AGTGTGACGTTGACATCCGT-3′ (sense) and 5′-GCAGCTCAGTAACAGTCCGC-3′ (anti-sense). Amplification and detection were performed with an ABI Prism 7500 sequence detection system (Applied Biosystems, NJ, USA). The data were analyzed by use of the Delta–Delta CT method (Applied Biosystems).

2.7. Western blotting analysis

Twenty-four hours after indicated treatment, the total cellular proteins were extracted from treated fibroblasts with lysis buffer (1.5% SDS, 0.0625 M Tris–HCl, and 1 mM Na_3VO_4, pH 6.8) containing protease inhibitor cocktail (Roche, Mannheim, Germany). This time duration was selected based on our preliminary study showing that the increases in protein expression were predictable and significant at this time point after stimulation. For Western blot analysis, 50–70 μg of extracted proteins