Photo-damages by chronic Infrared-A Irradiation for human dermal fibroblasts

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Abstract (Maximum of 250 words)

Background: In this study, we investigated cellular damages by acute and chronic infrared-A rays.

Methods: Two types of fibroblasts were irradiated with a customized solar infrared-A simulator (infrared-A intensity of 42 mW/cm^2) at well-controlled temperature conditions for 4 weeks.

Results: At physiological temperature (34℃), acute infrared-A irradiation on fibroblasts showed negligible effects on cell proliferation while there was slight changes in several gene expression e.g. matrix metalloproteinase-1 (MMP1) and collagen type-1 α-1 genes. Acute infrared-A irradiation only with heat stress (43℃) elicited reduced cell proliferation and decrease of procollagen type 1 C-terminal peptide. On the other hand, repeated infrared-A irradiation for 24 days (chronic infrared-A irradiation) significantly reduced cell proliferation, increased cellular reactive oxygen species, and elicited cellular apoptosis and morphological changes at physiological temperature. In particular, the degree of cellular responses by infrared-A was dependent on types of fibroblasts from different donors. In addition, the harmful effects by infrared-A irradiation on human fibroblasts were greatly protected with titanium dioxide-based emulsion.

Conclusion: Taken together, it is crucial to protect skin from harmful damages by chronic infrared-A itself for healthy skin and well aging during daily life.

Keywords: chronic irradiation; fibroblasts; infrared A ray; photoaging

Introduction.

Infrared rays contribute up to approximately 45% of the total solar light. Particularly, infrared-A ray (700 to 1,400 nm) accounts for ~ 30% of total solar infrared rays [1]. It is well known that infrared-A ray can penetrate deeply to the dermis. In addition, considering total energy by infrared-A ray during whole lifetime, its biological impacts on skin integrity should be examined in detail. However, previous several studies investigated biological effects of infrared-A ray in various irradiation conditions, e.g. light sources with undefined wavelength, unnatural dose of infrared-A ray, and no temperature controls. Accordingly, cellular responses to infrared-A ray during long term irradiation need to be examined at a natural dose of irradiation in a temperature controlled manner.

In our previous study, we formulated hybrid microparticles composed of TiO_2 and $Al(OH)_3$ colloids with rough surface [2]. To evaluate hybrid microparticles as an infrared-A protector from natural level of infrared-A, infrared-A ray was generated with a customized solar infrared-A simulator (infrared-A intensity of 42 mW/cm^2) at physiological temperature conditions (34℃) for 4 weeks in this study. Cellular damages of skin fibroblasts by repeated infrared A ray exposure were investigated at various infrared A doses and different time periods (4, 10, 17, and 24 days) at physiological temperature. In particular, two different human dermal fibroblasts from a differnt donor were used for this study. Cellular apoptosis and reactive oxygen species were determined by annexin V staining and 2′,7′-dichlorofluorescin diacetate (DCFDA) assay, respectively. After repeated irradiation by infrared A ray to fibroblasts, changes in cellular morphology were observed by fluorescence microscopy.

Materials and Methods.

Cell proliferation assay: Fibroblasts were plated on 35-mm dishes at a density of 3×10^4 cells per dish. After 24 h of incubation, infrared A was irradiated on cell plates (42 mW/cm^2 for 4 h per day). Temperature in cell culture media was 34℃ by a water-circulating plate equipped with a circulating water bath (Lab Korea, Korea). After repeated infrared A irradiation (3, 6, 10, and 14 times) for 4~ 24 days, total cell number was counted using the hematocytometer.

Annexin V staining: Fibroblasts were plated on 35-mm dishes at a density of 1.5×10^5 (acute exposure) or 3×10^4 cells (chronic exposure) per dish, 24 h prior to the first irradiation. The cells were then irradiated with infrared-A for acute (three times) and chronic (6 and 15 times) exposure. After the last irradiation, the cells were washed with PBS solution and were stained with FITC-labeled annexin V for 15 min at room temperature in the dark.

After washing cells thrice for 5 min with PBS solution containing 5% FBS, cells were fixed with formaldehyde (3.7%) in PBS solution for 10 min and visualized using inverted fluorescence microscopy (Olympus, Shinjuku, Tokyo, Japan). The fluorescence intensity within the cells was quantified using Image J software (National Institutes of Health, USA; http://rsb.info.nih.gov/ij/).

Reactive oxygen species assay: Fibroblasts were irradiated 3 and 14 times with infrared-A at 34℃, as stated previously. After the last irradiation, the cells were washed with PBS solution and stained with DCFDA (20 μM) for 30 min at 37℃. After washing the cells with PBS solution, fluorescent images of DCFDA was visualized using inverted fluorescence microscopy. The fluorescence intensity within cells was analyzed using Image J software.

Mitochondrial reactive oxygen species was determined by Mitosox™ Red mitochondrial superoxide indicator, according to manufacturer's protocol. After 14 times of infrared-A exposure, fibroblasts were washed with PBS solution and incubated with MitoSOX (5 μM) solution for 10 min. After incubation, fluorescence intensity within fibroblasts were visualized by inverted fluorescence microscopy.

Fibroblast morphology: After fibroblasts were then irradiated with infrared-A for three times and 14 times, cells were washed with PBS solution and stained with CellTracker™ Red dye (18 μM) for 30 min at 37℃. Then, cells were washed with PBS solution and fixed with 3.7% formaldehyde in PBS solution for 10 min. Morphology of each fibroblast was visualized via inverted fluorescence microscopy. The ratios of cell length to cell width were analyzed using Image J software.

Results.

Cell proliferation assay: Fibroblasts with different doubling times were investigated to determine effects of infrared-A radiation on cell proliferation. Several times of infrared-A

radiation showed no changes for all two cells. However, as increasing number of infrared-A radiation upto 14 times, reduced cell proliferation was observed for all two cells. After 14 times of infrared-A radiation, statistically reduced cell proliferation was shown for all two cells.

Annexin V staining: Several infrared-A radiation showed negligible changes in fluorescence intensity after Annexin V staining. However, repeated infrared-A radiation (14-times) exhibited stronger annexin V signals by ~4-folds, compared to control cells.

Reactive oxygen species assay: After infrared-A radiation, cellular and mitochondrial reactive oxygen species were examined by DCFDA assay and MitoSOX staining, respectively. Several times of infrared-A radiation showed no noticeable fluorescence changes, while repeated infrared-A radiation (~14 times) exhibited evidently increased DCFDA fluorescence signal. In addition, after repeated infrared-A radiation (~14 times), stong red fluorescence signals was also observed after MitoSOX staining.

Fibroblast morphology: Size and morphology of fibroblasts showed negligible changes after several times of infrared-A radiation. However, repeated infrared-A radiation elicited morphological changes of fibroblasts to thin and elongated shape.

Discussion.

In this study, natural level of infrared-A intensity (42 mW/cm2) was radiated to fibroblasts at physiological temperature to examine effects of infrared-A intensity on skin cells clearly [3, 4]. Two types of fibroblasts from different donors were used to examine individual responses to repeated infrared-A radiation. Both cells showd evidently reduced cell proliferation by repeated infrared-A radiation. Annexin V staining results clearly indicate that repeated infrared-A irradiation elicited cellular apoptosis. Unnatural morphological changes in fibroblasts by repeated infrared A radiation clearly indicates cellular damages and aging of skin fibroblasts.

Conclusion.

In this study, we clearly demonstrated that repeated infrared-A irradiation evidently reduced cell proliferation and induced cellular apoptosis without heat stress. However, three times of infrared-A irradiation at 34℃ elicited no noticeable changes of cell

proliferation. Additionally, repeated infrared-A irradiation showed significantly high apoptotic signals, elevated reactive oxygen species generation, and morphological changes. Accordingly, repeated infrared-A irradiation played a crucial role on integrity of skin cells, which should be protected for healthy skin.

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Conflict of Interest Statement. NONE.

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