

α -Lipoic acid can not prevent effect on cell viability, collagen synthesis inhibition, collagen degradation induction in ultraviolet A irradiated human fibroblast cell

Putu Dyah Ayu Saraswati^{1*}, Soedirman Sastrodiprojo², Yohanes Widodo Wirohadidjojo²

¹Department of Dermatovenereology, Wangaya Distric General Hospital, Denpasar, Bali,

²Department of Dermatovenereology, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia

ABSTRACT

Ultraviolet A (UVA) irradiation on human skin can generate free radical and stimulate matrix metalloproteinase production resulting in collagen degradation and transforming growth factor beta (TGF- β) inhibition. It causes synthesis collagen inhibition and induces cell death. α -Lipoic acid (ALA) is an universal antioxidant and a powerful scavenger of free radicals. In this study we investigated the effect of ALA on viability, collagen synthesis and degradation in UVA irradiated human fibroblasts. Normal human skin fibroblasts cell culture were irradiated with UVA for three times with each dose of 3000 mJ/cm² UVA. α -Lipoic acid in various concentration was added to the culture following UVA irradiation and incubated for 48 hours. The cell viability was determined by MTT-assay while collagen synthesis and degradation were determined by Sirius red binding assay. The difference of cell viability and collagen synthesis and degradation between fibroblasts cell after and without UVA irradiation were analyzed using paired-t test with 95% confidence interval ($p < 0.05$). The results showed that UVA irradiation decreased cell viability, inhibited collagen synthesis and induced collagen degradation in fibroblasts cell. However, ALA was not sufficient to increase viability, to increase collagen synthesis and to inhibit collagen degradation in fibroblasts cell due to UVA irradiation. In conclusion, ALA can not prevent UVA irradiation effect on human skin fibroblasts cell.

Key words: UVA – irradiation - human skin fibroblasts – antioxidants – α -lipoic acid

ABSTRAK

Pajanan sinar ultraviolet A pada kulit dapat menghasilkan radikal bebas dan memicu produksi matrik metalloproteinase sehingga menghambat sintesis kolagen dan transforming growth factor beta (TGF- β). Hal ini menghambat sintesis kolagen dan memicu kematian sel. Asam α -lipoat (ALA) merupakan antioksidan dengan kemampuan kuat menangkap radikal bebas. Dalam penelitian ini telah dikaji pengaruh ALA terhadap viabilitas, sintesis dan degradasi kolagen sel fibroblas kulit yang dipajani sinar UVA. Kultur sel fibroblas kulit manusia normal dipajani sinar UVA sebanyak tiga kali dengan dosis masing-masing 3000 mJ/cm². Asam α -lipoat pada berbagai konsentrasi ditambahkan pada kultur fibroblas diikuti pajanan sinar UVA selanjutnya diinkubasikan selama 48 jam. Viabilitas sel ditetapkan dengan MTT assay sedangkan sintesis dan degradasi kolagen ditetapkan dengan Sirius *binding assay*. Perbedaan viabilitas sel, sintesis dan degradasi kolagen sel fibroblas setelah dan tanpa pajanan UVA dianalisis dengan uji t pasangan dengan tingkat kepercayaan 95% ($p < 0.05$). Hasil penelitian menunjukkan pajanan sinar UVA menurunkan viabilitas, menghambat sintesis dan memacu degradasi kolagen sel fibroblas. Namun demikian, ALA tidak cukup kuat meningkatkan viabilitas, memacu sintesis dan menghambat degradasi kolagen sel fibroblas akibat pajanan sinar UVA. Dengan demikian dapat disimpulkan bahwa ALA tidak dapat mencegah efek pajanan sinar UVA pada sel fibroblas kulit manusia.

Kata kunci: UVA – iradiasi – fibroblas kulit manusia- antioksidan – asam α -lipoat

* corresponding author: dyahramadi@yahoo.com

INTRODUCTION

Fibroblasts are important component of skin. Fibroblast cells produce and organize extracellular matrix in dermis which consists of collagen type I type III, elastin, proteoglycans and glycosaminoglycans, and play an important role in tensile strength of the human skin.¹ Meanwhile, ultraviolet A (UVA) irradiation in skin fibroblasts cell up regulates collagen-degrading matrix metalloproteinase (MMPs) and inhibits collagen synthesis.^{2,3} UVA Irradiation also induces cell damage through generation of reactive oxygen species (ROS) which causing deoxyri-bonucleic acid (DNA) mutation, lipid peroxidation and finally cell death.⁴ These conditions may lead to functional changes of cell as shown in the photoaging process.³

Human cells have antioxidants property designed to detoxify harmful effect of reactive oxygen species (ROS) molecules before DNA and other cell components damages occurred. However, UVA-irradiation suppresses the cell's defense against ROS damage by reducing antioxidants activity.⁴ In this case, supplementation of exogenous antioxidant may support the endogenous antioxidants and prevent generation of free radical.⁵

α -Lipoic acid (ALA) and dihydrolipoic acid (DHLA) have been acknowledged as universal antioxidant since they have solubility both in lipid and water resulting in their ability to reach all portion of the cell to provide antioxidants protection.⁶ ALA prevents stress-induced lipid peroxidation⁷, scavenges ROS and prevents cellular senescence.³ This study was conducted to investigate the effect of ALA on collagen degradation and synthesis and also on cellular viability on UVA-irradiated human skin fibroblasts cells.

MATERIALS AND METHODS

Cell culture

Fibroblasts sub cultured passage 3 were established from foreskin of 2 healthy human donors aged 11 years and were grown in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% bovine serum, 1000 μ g/100 unit Penstrep-Gibco™/mL, amphotericine B-Gibco™ 1000 μ g/

mL, and 100 mg/mL ceftriaxone at 37°C in 5% CO₂. Fibroblasts used were 2×10^4 cells for each column.

Irradiation of fibroblasts with UVA

The cultures were divided into two groups. In the first groups, fibroblasts cell culture was irradiated with UVA (PAST Sun B-LIPI™) for three times with each dose of 3000 mJ/cm². α -Lipoic acid in various concentration was added to the culture following UVA irradiation and incubated for 48 hours before the culture was given another UVA irradiation. This procedure was repeated until the fibroblasts cultures were irradiated with UVA 9000 mJ/cm². In the second group, fibroblasts cell culture was added with ALA of various concentration similar to first group but without following UVA irradiation.

α -Lipoic acid

α -Lipoic acid (Mecola Forte® -PT. Lapi. Indonesia) was dissolved in dimethyl sulfoxide (DMSO) and added to the medium in final concentration of 50, 100, 200 and 400 μ M. The highest concentration of organic solvent (DMSO) used in this study was no more than 0.2%. α -Lipoic acid was added on fibroblasts cell culture immediately after UVA irradiation.

Cell viability, collagen deposition and collagen degradation

Forty-eight hours after fibroblasts cell culture and ALA incubated, cell viability was determined by 3,-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay according to method used by Freshney.⁸ Collagen degradation and synthesis were determined using Sirius red binding assay according to method used by Heng *et al.*⁹ and Taskiran *et al.*¹⁰, respectively.

Statistical analysis

Statistical analysis were performed using computer software. Paired t-test with 95% confidence interval (p<0.05) was used to determine the statistical significance of differences of cell viability, collagen degradation and synthesis between fibroblasts cell after and without UVA irradiation.

RESULTS

The effect of UVA irradiation on cell viability of human skin fibroblasts is presented in FIGURE 1. Cell viability of human skin fibroblasts decreased after UVA irradiation as expressed by the significant decrease of OD of fibroblasts cell after UVA irradiation compared with those without UVA irradiation ($p < 0.05$). More over, UVA irradiation also

significantly decreased collagen synthesis ($p < 0.05$). The mean of OD of collagen synthesis after UVA irradiation was lower than those without UVA irradiation (FIGURE 2). In related to the decrease of collagen synthesis, the collagen degradation significantly increased after UVA irradiation ($p < 0.05$). The mean of OD of collagen degradation after UVA irradiation was higher than those without UVA irradiation (FIGURE 3).

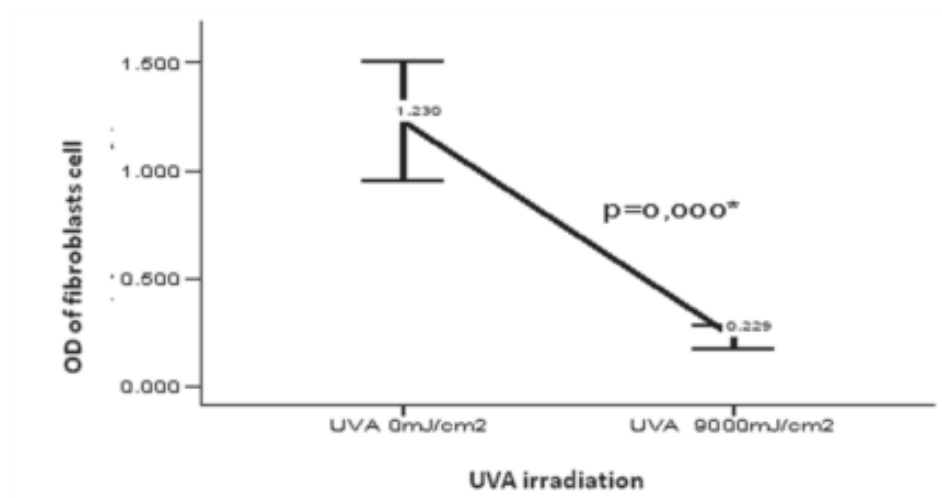


FIGURE 1. Effect of UVA irradiation on cellular viability of human skin fibroblasts

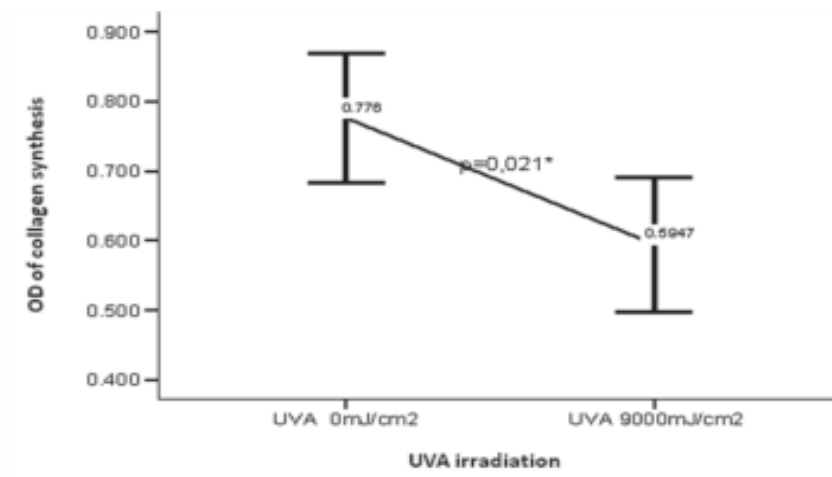


FIGURE 2. Effect of UVA irradiation on collagen synthesis of human skin fibroblasts

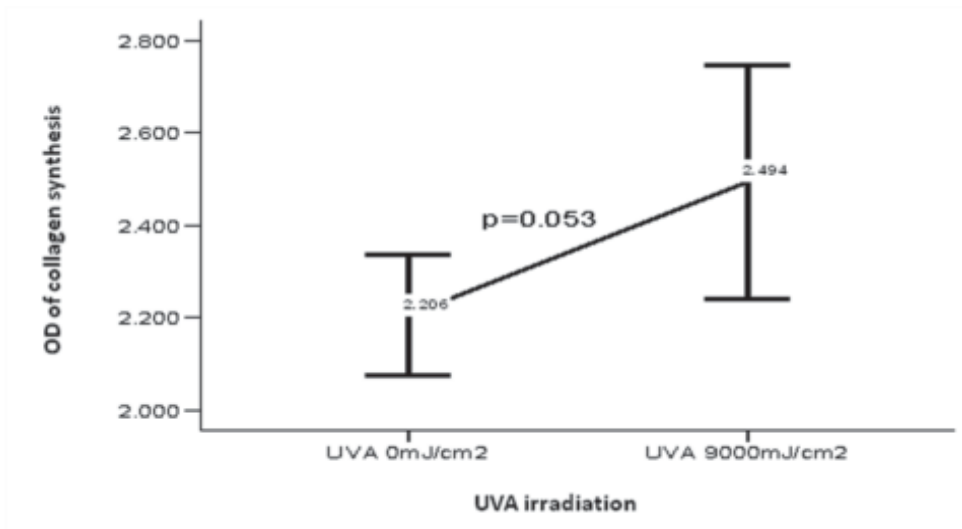


FIGURE 3. Effect of UVA irradiation on collagen degradation of human skin fibroblasts

The cellular viability of human fibroblasts cell after ALA supplementation in various doses and UVA irradiation resulted in significant decrease of

fibroblasts viability compared with the control ($p < 0.05$) as shown on FIGURE 4.

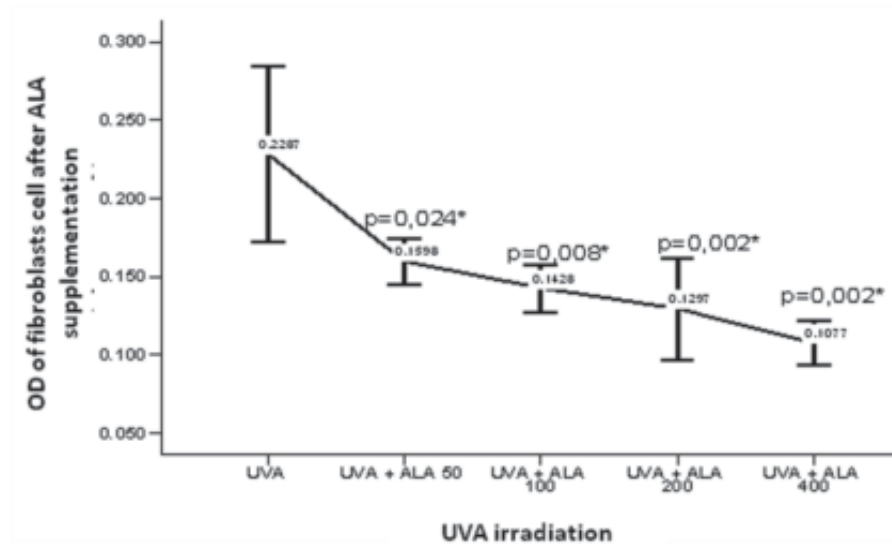


FIGURE 4. Effect of ALA on cellular viability of UVA irradiated human fibroblasts

Supplementation of ALA on UVA irradiated human fibroblasts also resulted in significant decrease of collagen synthesis compared with the control (FIGURE 4) and resulted in increase of collagen degradation compared with the control (FIGURE

5). However enhancement doses of ALA resulted in decreasing cell viability and collagen synthesis and increasing collagen degradation as shown in FIGURE 3, 4 and 5.

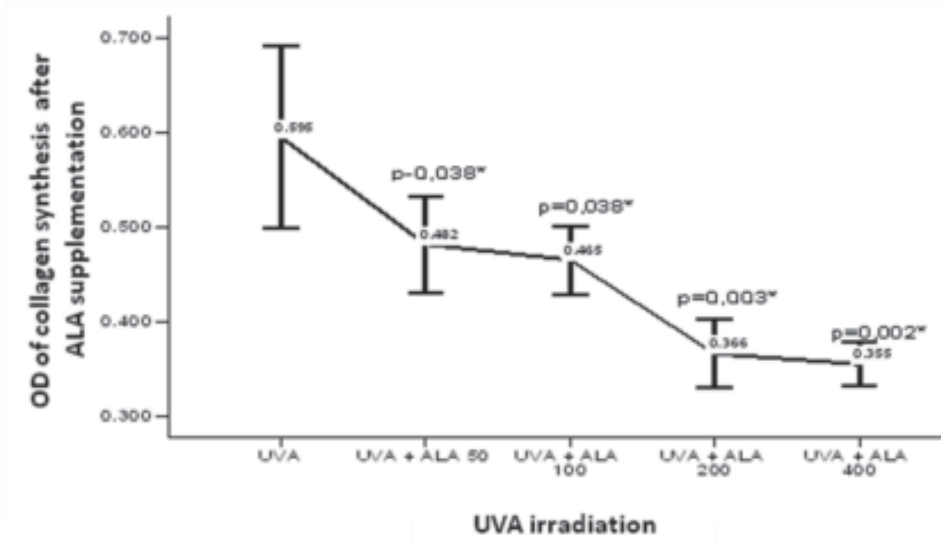


FIGURE 5. Effect of ALA on collagen synthesis of UVA-irradiated human fibroblasts

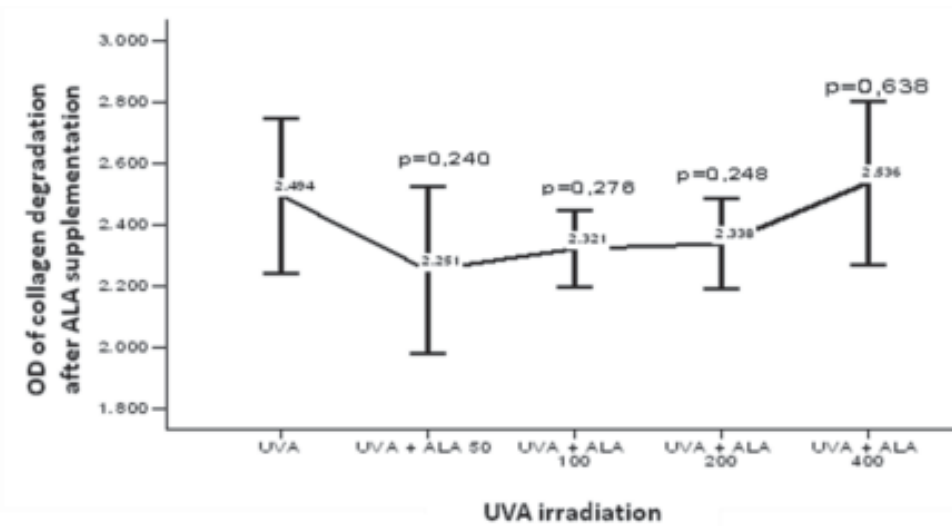


FIGURE 6. Effect of ALA on collagen degradation of UVA irradiated human fibroblasts

DISCUSSION

UVA irradiation generates free radicals resulting in collagen degradation, inhibits collagen synthesis and finally induces cell death.^{11,12} Cell has an ability to repair damage caused by UVA exposure. Reduction of cell's ability to repair generates damage and accumulation of cell death that creates functional changes as shown in the photoaging process.^{4,13} The decrease of cell viability on UVA-irradiated human fibroblasts cell is also seen in FIGURE 1.

α -Lipoic acid and DHLA are universal antioxidant and act as a scavenger of ROS.^{6,14} However, several studies showed that ALA and DHLA also have pro-oxidant effect.^{15,16} The effect of ALA supplementation in various doses among UVA-irradiated human fibroblasts cell resulted in decrease of cell viability compared with human fibroblasts cell without UVA irradiation or the control ($p < 0.05$) as presented in FIGURE 3, decrease in collagen synthesis (FIGURE 4) and increase in collagen degradation (FIGURE 5). These results did not in accordance with the ALA properties as universal antioxidant that could repair oxidative damage of cell due to UVA irradiation.¹⁴

The pro-oxidant properties of ALA may play role in the inability to repair oxidative damage. As pro-oxidant, ALA can induce mitochondrial permeability transition (MPT) resulting in rupture of cell membrane and release proapoptotic factors.¹⁷ α -Lipoic acid and DHLA can also lead to cell death through internucleosomal DNA fragmentation and induce caspase 3 cleavage that causes apoptosis of cells with higher cytotoxicity found in DHLA compared with ALA.¹⁸ In addition, level of intracellular oxidant of ALA increased in parallel with ALA concentrations.¹⁹

UVA irradiation causes alternations in dermal collagen through two primary pathways: 1). stimulation of collagen breakdown and 2). inhibition of procollagen synthesis, resulting in loss of collagen content.²⁰ UVA stimulates collagenase directly and causes tissue damage through induction on protein kinase C (PKC) on fibroblast.²¹ UVA also activates the activator protein 1 (AP-1) and nuclear factor kappa beta (NF- κ B) which stimulates the production of MMPs that play a role in photoaging.^{4,11} Transforming TGF- β is also inhibited by over expression of the AP-1 family and causes reduction

of procollagen expression.^{3,12} In this study, UVA irradiation inhibits collagen synthesis and increases collagen degradation as shown on FIGURE 2 and 3.

Evaluation of collagen synthesis and cell viability of UVA-irradiated human fibroblast was in 48 hours. This evaluation time was based on study by Fisher *et al.*¹² which stated expression of procollagen of UVA-irradiated returns to their original level 48-72 hours. However, supplementation of ALA among UVA-irradiated human fibroblasts was not able to up-regulate collagen synthesis (FIGURE 4). The collagen synthesis analysis has not been optimized yet due to lack of follow up evaluation and required longer evaluation time.

The photo protective effect of topical antioxidants applied on human skin before UV irradiation has been recognized. Topical application of antioxidants, such as vitamin E significantly reduces acute skin responses such as erythema and edema, sunburn cell formation, lipid peroxidation, DNA adduct formation, immunosuppression as well as UVA-induced binding of photosensitizers.^{22,23} Application of various antioxidants such as vitamin C, vitamin E, and melatonin 30 minutes before UV-irradiation protects skin from UV-induced erythema.²³ The effect of antioxidants administered after irradiation is less obvious. Diminished erythema formation was reported when antioxidants were topically administered after UVA irradiation.²⁴ However, these findings were in contrast to other human studies which were not able to demonstrate any diminished UV-related skin damage when antioxidants were applied to UV-irradiated in humans.²⁵ In this study no significant effect of ALA was observed when ALA was supplemented immediately to UVA irradiated human fibroblasts.

CONCLUSION

ALA was not successful in up-regulating collagen synthesis, enhancing viability of fibroblast and inhibiting collagen degradation because of the pro oxidant properties of ALA. Therefore, the finding of this study indicates that supplementation of ALA to UVA irradiated human fibroblasts was not able to increase the cell viability, to up-regulate collagen synthesis and to inhibit collagen degradation.

ALA has no impact when it is supplemented among UVA irradiated human fibroblasts.

ACKNOWLEDGMENT

The authors would like to thank Head of Department of Dermatovenereology, Faculty of Medicine, Gadjah Mada University/Dr. Sardjito General Hospital, Yogyakarta for his permission to perform this study.

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