Wistar Rat as Photoaging Mouse Model

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Original Article

Wistar Rat as Photoaging Mouse Model

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Abstract

Background The increasing of human life expectancy may cause aging problems, including photoaging. Photoaging raises new problems in community, because its influence in human self-esteem and quality of life. Studies about photoaging have been extensively performed to enhance the basic mechanism of photoaging. Mouse skin is usually used as cutaneous aging model, because it is genetically similar to human skin.

Methods This study was true experimental study, post test only-control group design that was aimed to evaluate the role of ultraviolet (UV) B irradiation to produce photoaging mouse model. Twenty-four Wistar rats were divided randomly allocation into 2 groups, namely non-UV irradiated group and UV irradiated group. Ultraviolet B irradiation were performed for 5 weeks until reaching the total dose 3100 mJ/cm^2. Dermal collagen density and transepidermal water loss (TEWL) level were evaluated after the intervention.

Results Dermal collagen density in non-UV irradiated group and UV irradiated group were 38.21±2.67% and 27.73±0.77%. TEWL level in non-UV irradiated group and UV irradiated group were 7.55±0.89 g/m^2/h and 17.53±3.94 g/m^2/h. There was significant difference between the groups in dermal collagen density and TEWL level ($p<0.05$).

Conclusion Wistar rat in UV irradiated group that were given total dose 3100 mJ/cm^2 of UV B for 5 weeks has been proven to become photoaging mouse model.

Key words
Photoaging, mouse model, wistar rat, UV B, life expectancy.

Introduction

Photoaging is caused by long term effect of ultraviolet (UV) irradiation. Photoaging and chronological skin aging speed up the occurrence of skin aging with dry skin, lack of elasticity, lack of firmness, and lack of skin smoothness as the clinical manifestation. Photoaging is 80-90% of all skin aging, that can be prevented. The increasing of human life

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expectancy may cause aging problems, including photoaging. Photoaging raises new problems in community, because its influence in human self-esteem and quality of life.[1-3]

Studies about photoaging have been extensively performed to enhance the basic mechanism of photoaging and to develop its prevention. Several studies were done in *in vivo* study using mouse model. Mouse model in photoaging studies is used as cutaneous aging model, because mouse skin is genetically similar to human skin.[4]

Hwang *et al*. showed that collagen synthesis velocity in the skin of 24 months-old mouse decreased 10 times, comparing with 1 month-old mouse. Meanwhile, collagen degradation velocity in mouse model increased by the age. Collagen degradation velocity was 6.4% in 1 month-old mouse and 56% in 24 months-old mouse.[4]

Photon energy from ultraviolet B was 1000 times higher than ultraviolet A. Ultraviolet B is the main cause of DNA destruction and inflammation (activation of prostaglandin (PG) via activation of cyclooxygenase-2 (COX-2)). Ultraviolet B induce the production of reactive oxygen species (ROS), that activate keratinocyte to express transcription factor, namely Nuclear Factor kappa B (NF-κB) and Activator Protein-1 (AP-1). NF-κB and AP-1 induce the activation of matrix-metalloproteinase (MMP), that cause collagen degradation cascade.[1,2,5-7]

Movement of water from stratum corneum to the atmosphere is called transepidermal water loss (TEWL). The definition of TEWL is water content per skin surface area unit and per duration of time which loss from the skin to the atmosphere through diffusion and evaporation, with normal value 0-20 g/m^2/h. Skin barrier regulates water evaporation from the skin. The disruption of skin barrier may cause disruption of these mechanisms. ROS production caused by ultraviolet irradiation may cause the destruction of lipid in stratum corneum, corneocyte proteins, and skin barrier. These process induce inflammatory response by increasing the production of proinflammatory cytokines, which cause photoaging manifestation, showed by the elevation of TEWL level.[8-10]

Methods

This study was true experimental study, post test only-control group design, aimed to prove the role of ultraviolet B irradiation to produce photoaging mouse model. Ultraviolet-irradiated male Wistar rats were used in this study as photoaging animal model. Male Wistar rats (*Rattus norvegicus*) were used to avoid the effect of estrogen level reduction in photoaging. Ten to twelve weeks-old Wistar rats were used in this study to avoid effect of intrinsic skin aging before ultraviolet B irradiation, in order to get photoaging animal model from the only effect of ultraviolet B irradiation.[11]

Twenty-four Wistar rats were divided randomly allocation into 2 groups, namely non-UV irradiated group and UV irradiated group. All subjects were given the same intake and environment. The fur on dorsal area of the rat was shaved in 3x3 cm^2 size. No intervention was performed in non-UV irradiated group. Dermal collagen density and transepidermal water loss (TEWL) level were evaluated after intervention.

Ultraviolet irradiation

In UV irradiated group, Wistar rats were performed ultraviolet B irradiation using Ultraviolet B Broadband TL lamps 20W. Ultraviolet B irradiation were performed for 5 weeks until reaching the total dose 3100 mJ/cm^2. The dose of ultraviolet B were increased

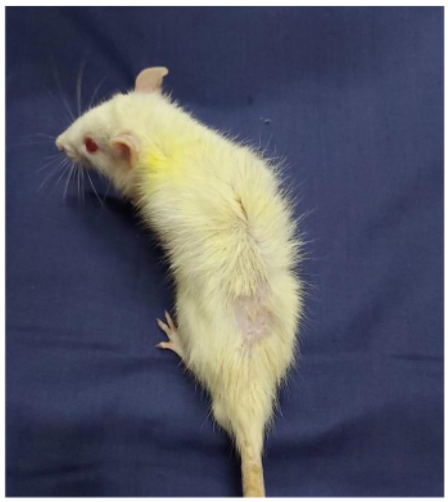

Figure 1 Ten to twelve weeks-old Wistar rats.

Table 1 Dermal collagen density differences between UV irradiated and non-UV irradiated Wistar rat group.

Groups	*Dermal collagen density (%)*	*p*
Non-UV irradiated group	38.21±2.67	0.0000
UV irradiated group	27.73±0.77	

gradually from 100 to 400 mJ/cm^2. On the first week, ultraviolet B irradiation were performed 3 times (100 mJ/cm^2/time). On the second and third week, ultraviolet B irradiation were performed 3 times per week (200 mJ/cm^2/time). On the fourth and fifth week, ultraviolet B irradiation were performed 2 times per week (400 mJ/cm^2/time).[12,13]

Histopathology examination

The skin on the back part of the male Wistar rats were obtained from sacrifice animal under anesthesia at the end of the study. The skin specimens were fixed in formalin buffer 10% and cryosections were prepared. Masson Trichrome staining is staining technique using acid fuchsin and methyl blue in order to evaluate the dermal collagen density. Dermal collagen will be blue stained. All of these evaluation used light microscopy Nikon H600L completed with digital camera DS Fi2 300 megapixel and image analyzer software Nikon Image System. Dermal collagen density was evaluated by calibrated Image J software. The evaluation was performed in 200x magnification.[14]

TEWL level evaluation

Evaluation of TEWL level in this study was performed with Cutometer MPA 580 and Tewameter® TM 300 probe. TEWL level evaluation was performed in 20-22°C room temperature and 40-60% room humidity. The evaluation was performed 3 times in 30 seconds duration in each evaluation.[15-17]

Results

The mean of dermal collagen density in non-UV irradiated group and UV irradiated group were 38.21±2.67% and 27.73±0.77%. There was significant difference between the groups ($p<0.05$) (**Table 1**). **Figure 2** showed histopathology result in Masson Trichrome staining.

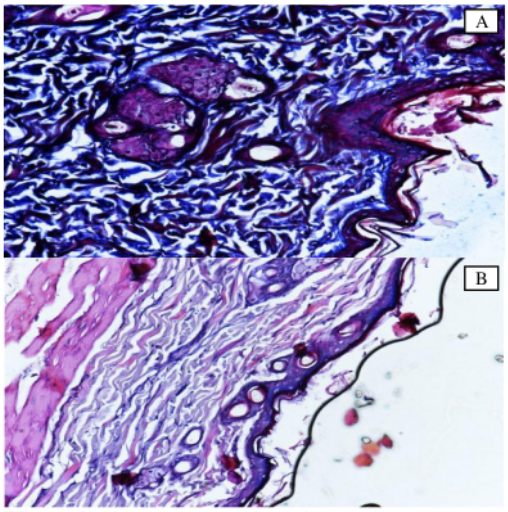

Figure 2 Histopathology result in Masson Trichrome staining showing dermal collagen density (200x magnification). A. In non-UV irradiated group. B. In UV irradiated group.

Table 2 TEWL level differences between UV irradiated and non-UV irradiated Wistar rat group.

Groups	*TEWL level ($g/m^2/h$)*	*p*
Non-UV irradiated group	7.55±0.89	0.0324
UV irradiated group	17.53±3.94	

There was high density of dermal collagen in non-UV irradiated group, meanwhile the dermal collagen density in UV irradiated group was low.

The mean of TEWL level in non-UV irradiated group and UV irradiated group were 7.55±0.89 $g/m^2/h$ and 17.53±3.94 $g/m^2/h$. There was significant difference between the groups ($p<0.05$) **(Table 2)**.

Discussion

Photoaging is one of new problems that arise in community due to the increase in life expectancy. Photoaging is the process of skin aging caused by external factors, mainly due to exposure to ultraviolet radiation. Unlike intrinsic aging, photoaging has better respond to interventions and preventive measures. Typical clinical features of photoaging which are mostly caused by ultraviolet radiation include wrinkle, weakness, roughness, increased fragility, and telangiectasia. In addition, the skin will appear darker and have a mottled pigmentation.[1,2,4]

Sunlight is consist of infrared light, visible light, and ultraviolet (UV) light, with most UV rays are blocked by the earth's atmosphere. UV irradiation that reaches the earth's surface, consists of UVA (320 to 400 nm) in more than 95% and UVB (280 to 320 nm) in about 5%. UVA and UVB contributes to skin aging. UVB, which spreads to the epidermis and upper dermis, is major source of direct DNA damage, inflammation, and immunosuppression. On the other hand, UVA penetrate the skin to the lower dermis, and considered a greater contributor to skin aging than UVB, due to the greater penetration depth and higher percentage of surface sunlight.[2,5]

Many studies on photoaging have been carried out to learn about the basic mechanisms of photoaging and to develop agents for the prevention and treatment of photoaging, which often starting with *in vivo* studies, using mouse models.[1,2,4]

Collagen is the majority structural protein in dermal layer. Two main collagen regulators are transforming growth factor β (TGFβ) and AP-1. TGFβ increase the synthesis of procollagen and collagen; while AP-1 inhibit synthesis of procollagen and collagen. Ultraviolet induce ROS production, that activate AP-1 and inhibit TGFβ. These mechanisms result in decreasing of dermal collagen count.[18-21]

This study showed decreasing of dermal collagen count in group receiving 3100 mJ/cm^2 ultraviolet B irradiation for 5 weeks, significantly ($p<0.05$). Study of Kim *et al.* (2015) showed decreasing of dermal collagen density, elevation of JNK and elevation of MMP-9 in hairless mice after receiving ultraviolet irradiation significantly ($p<0.05$).[12] Ultraviolet B irradiation decrease dermal collagen count via MMP activation, especially MMP-1, that was showed by study of Dong *et al*. (2008).[22]

Ultraviolet can increase mitochondrial DNA mutations which will decrease mitochondrial function and the formation of reactive oxygen species (ROS). Ultraviolet can cause damage to the basement membrane at the dermal-epidermal junction. In addition, photoaging can increase matrix metalloproteinase (MMP).[2,5]

Structural changes in the aging skin layer,

including collagen as the main component of the dermis, increase rigidity and impaired wound healing process. Reduction of collagen type I and III in intrinsic skin aging will be accelerated by the photoaging process. Collagen fragmentation also plays a role in the downregulation of collagen synthesis in intrinsic skin aging and photoaging. Collagen degradation by MMP, increases with the increasing age. MMP-1, MMP-3, and MMP-9 were elevated by the effect of acute UV irradiation, which mainly acts on the epidermis layer. Meanwhile, MMP-1, MMP-2, MMP-3, MMP-9, MMP-11, MMP-17, and MMP-27, which produced by dermal fibroblasts, were elevated in chronically photodamaged skin. This will cause a decrease in the amount of collagen due to increased collagen degradation and decreased collagen production, because fragmented collagen interferes with fibroblast function.[2,5,21]

Evaluation of TEWL level in this study was performed with Cutometer MPA 580 and Tewameter® TM 300 probe. The elevation of TEWL level in photoaging was proved in this study, showed by higher TEWL level in UV irradiated group significantly. Ultraviolet B irradiation induced TEWL level in Wistar rats, which elevated the TEWL level 1.5 times higher than in non-UV irradiated group. This result was similar to studies from Hung *et al*. (2015) and Choi *et al*. (2019), which showed higher TEWL level in ultraviolet B-irradiated hairless mice group ($p<0.001$).[15,23]

Skin is the first body protection from environment, protects the body from mechanical destruction, pathogen penetration and ultraviolet irradiation. Skin also play role in homeostatic mechanism in controlling TEWL through stratum corneum. TEWL is water content per skin surface area unit and per duration of time, which loss from the skin to the atmosphere, through diffusion and evaporation. Water evaporation from the skin is regulated by the skin barrier. The disruption of skin barrier function may cause disruption of water retention and increasing the water evaporation. TEWL level describe the skin barrier function, that was used as the parameter in evaluating photoaging process in this study.[8,9,24]

Ultraviolet irradiation results in disruption of skin barrier function, caused by ROS production. Disruption of skin barrier function may induce inflammatory response through elevation of proinflammatory cytokines and decreasing of water binding capacity in ultraviolet-exposed area. These mechanisms manifest as dry skin in photoaging, that could be evaluated from elevation of TEWL level.[10,23]

Conclusion

The total dose 3100 mJ/cm^2 of ultraviolet B for 5 weeks was able to decrease the dermal collagen density and increase the TEWL level significantly, as the parameters in photoaging in this study, and has been proven to be photoaging mouse model.

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