Comparison of the histological features in the photoaged animal model

Winawati Eka Putri¹, Meidyta Sinantryana Widyaswari¹, Rizki Amalia², David Sajid Muhammad³, Nadia Nisaussholihah⁴, Deny Febriwijaya Romadhani⁵

ABSTRACT

Background: Photoaging is a type of aging primarily caused by exposure to radiation, such as Ultraviolet B (UVB). This radiation penetrates the upper epidermis and papillary dermis leading to sunburn, tanning, photoaging, and photocarcinogenesis. Its light is a significant cause of direct deoxyribonucleic acid (DNA) damage. Furthermore, it induces transcription factors, such as NF-κ and other pro-inflammatory cytokines, the expression of matrix metalloproteinase (MMP), and generates reactive oxygen species (ROS). Therefore, this study aimed to determine the differences in the histologic features of the photoaged animal models.

Methods: There were three groups, including P1, P2, and P3. Group P1 consists of male Wistar rats (Rattus norvegicus), aged 10-12 weeks, with average body weight between 100-150 grams. Group P2 consists of C57BL aged 5-6 weeks with an average weight of 50-75 grams. P3 comprises BABL/c mice aged 5-6 weeks with an average weight of 50-75 grams. They were photoaged for 6 weeks using UV lamps (Ultraviolet B Broadband TL lamps. Phillips TL 20W/01 RS) with a 290-315 nm wavelength.

Results: The results showed a significant difference in epidermal thickness, dermal thickness, and sunburn cell (SBC) between groups after exposure to UVB radiation (p<0.05). Meanwhile, there was no significant difference in their blood vessels (p>0.05).

Conclusion: The exposure to UVB for six weeks affects epidermal and dermal thickness, amount of SBC, and blood vessels in the photoaged animal model.

Keywords: photoaging, epidermal thickness, dermal thickness, sunburn cell, blood vessel.

INTRODUCTION

Exposure to sunlight, specifically ultraviolet rays, can lead to changes in human skin, later known as photoaging.¹,² This is caused by infrared (IR) (52-55%), visible light (44%), and UV light (3%) with wavelengths of 760 nm–1 mm, 400-760 nm, 290–400 nm, respectively.³,⁴ The histological changes caused by ultraviolet irradiation depend on the wavelength.⁵ Ultraviolet B (UVB) is very efficient at irradiation depend on the wavelength.⁶ It stimulates transcription factors, such as NF-κ and other pro-inflammatory cytokines, the development of matrix metalloproteinases (MMPs), and produces reactive oxygen species (ROS), which is the main cause of direct DNA damage.⁷,⁸ This radiation penetrates the upper epidermis and papillary dermis, causing sunburn, tanning, photoaging, and photocarcinogenesis.⁹ Sunburn cell (SBC) keratinocytes are differentiated from the normal type by overexposure to UVB irradiation.¹⁰,¹¹ It features a shrunken glassy, eosinophilic cytoplasm and a pyknotic nucleus.¹² Johnson et al. (1972) showed that the total SBC is related to UV irradiation dose in human epidermal.¹² Following exposure to UVB, the amount rises for 10–24 hours before falling off after 36–48 hours.¹¹

Keratinocyte turnover in the epidermis is accelerated by injury or inflammation. When exposed to UV light, these cells are stimulated to release cytokines from the interleukin (IL-) 1 family, specifically IL-1, IL-1, IL-18, and IL-33.¹³ A previous study showed that in photoaged skin, there were epidermis, atypical and dysplastic keratinocytes, vacuolization of epidermal cells, scattered cell necrosis, and Langerhans cell loss.¹³,¹⁴ Furthermore, the other features of photoaged skin were fibroblast hyperplasia, irregular collagen fibers, thickened vessels, and increased glycosaminoglycans and inflammatory cells.¹³,¹⁵,¹⁶ Intrinsically aging and photoaging of skin showed a decrease in vessel size. The amount of skin covered by vessels and the number of vessels were significantly reduced in the photoaging skin.¹⁷ Even though mice and rats are frequently employed as models for skin diseases, their skin structures differ from those of humans. These variations include skin thickness, melanocyte location, cell signaling, oncogene function profiles, and tumor suppressor gene expression.¹⁸ Because of how closely their skin resembles that of humans, hairless mice are frequently employed as animal models for photoaging.¹ In Indonesia, hairless mice such as SKH-1 are challenging to find and
SBC and blood vessels were counted in 10 high-power fields of 5 sections for each animal.

**Statistical analysis**

Data from the study status were gathered, cleaned, modified, and encoded. Then, they were entered into the Statistical Product for the Social Sciences data format version 20.0 (SPSS, Inc., Chicago, Illinois). Because there were fewer than 30 samples per group and because the data were normally distributed (p>0.05), the normality and Shapiro-Wilk tests were applied. Kruskal-Wallis was further used to compare data between groups.

**RESULTS**

**Effect of UVB irradiation on the epidermal and dermal thickness**

The epidermal and dermal thicknesses were not normally distributed and not homogenous. Hence, Shapiro-Wilk and Levene’s test was performed. Kruskal-Wallis test was conducted to determine the differences between the groups. This study reported a significant difference in epidermal (p<0.05) and dermal thickness (p<0.05) between groups after UVB irradiation (Table 1). The Mann-Whitney test was performed to determine the differences in each group. The result showed differences in dermal thickness between P1 and P2 and P1 and P3 Groups (p<0.05). Meanwhile, only P2 and P3 Groups had differences in epidermal thickness (Figure 1-4).

**Effect of UVB irradiation on SBC and blood vessels**

Blood arteries and SBCs were evenly and normally distributed throughout the body. To identify the differences between each group, a one-way ANOVA was used. The findings revealed that only SBC differed considerably (p<0.05; Table 2), while the blood vessels were unaffected (p>0.05; Table 1).

**Table 1. Comparison of epidermal and dermal thickness between groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Epidermal Thickness (mean ± SEM)</th>
<th>Dermal Thickness (mean ± SEM)</th>
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<tbody>
<tr>
<td>P1</td>
<td>165.40 ± 101.99 mm</td>
<td>443.47 ± 164.86 mm</td>
</tr>
<tr>
<td>P2</td>
<td>175.55 ± 15.49 mm</td>
<td>169.29 ± 13.75 mm</td>
</tr>
<tr>
<td>P3</td>
<td>120.42 ± 13.38 mm</td>
<td>148.68 ± 36.28 mm</td>
</tr>
<tr>
<td>p-value</td>
<td>0.032*</td>
<td>0.006*</td>
</tr>
</tbody>
</table>

Note: *p < 0.05

**Histopathology analysis**

For histological analysis, the back skin tissue of rats and mice was fixed in 10% buffered formalin for 24 hours, sectioned into 3-5 mm thick pieces, embedded in paraffin, and stained with hematoxylin and eosin (H&E). Epidermal, dermal, SBC and blood vascular thickness are all evaluated by H&E staining. Five different epidermal and dermal locations were selected, and values were presented as mean ± SEM.

**Figure 1.** Epidermal thickness (a) P1, (b) P2, and (c) P3 groups on H&E staining with 400x magnification.
DISCUSSION

Photoaging is skin damage caused by UV irradiation. Histological signs of photoaged skin include thinning of the epidermis, thickened granular cell layer in the stratum corneum, and increased stratum corneum compaction. C57BL, SKH1, and BALB/c are the main strains used for photoaging and photocarcinogenesis investigations.\(^{13}\)

The most used mice for the photoaging study were SKH-1. Kligman (1996) discovered that skinfold thickness was increased by UVA, UVB, or UVB + UVA in hairless SKH-1 mice.\(^{20}\) Lesnik et al. (1992) showed that UVB radiation made the epidermis hyperplastic and skin thickening.\(^{21}\) Sharma et al. (2011) observed that UVB-irradiated C57BL/6J skin is similar to human photoaging.\(^{22}\) You et al. (2019) stated that the effects of UVB irradiation were epidermal thickening, increased inflammatory cells, and decreased collagen.\(^{25}\) However, the Balb/c mice showed no epidermal thickening after UVB irradiation. Similar to the previous study, Moloney et al. (1992), Moon et al. (2000), Sharma et al. (2011), and Kwak et al. (2018) reported that epidermal thickness with UVB exposure was higher than control.\(^{22,24-26}\) Sharma et al. (2011), Fan et al. (2015), and You et al. (2019), using hairless BALB/c female mice, observed a substantial increase in epidermal thickness in the UVB-exposed group.\(^{22,23,27}\) Hwang et al. (2014) used male hairless mice (SKH: HR-1) exposed to UVB irradiation 3 times a week for 10 weeks.\(^{28}\) They discovered significant skin thickening and that epidermal thickness was greater in the group. Choi et al. (2019) used HR-1 hairless mice exposed to UVB radiation 3 times a week for 8 weeks.\(^{29}\) An increase in epidermal thickness and a decrease in the intensity of Masson trichrome staining were reported. Like Choi et al. (2019), Kim et al. (2016) reported thicker epidermal and dermal than the control group.\(^{29,30}\) Bora et al. (2018) and Wibisono et al. (2020) employed Wistar rats subjected to UVB irradiation and observed that the thickness of the epidermis was more significant than the treated groups.\(^{31,32}\) No previous report compared epidermal thickness between Wistar rats with C57BL and BABL/c mice. Therefore, this study reported a significant difference in epidermal thickness among groups (p<0.05).

Moloney et al. (1992) and Fan et al. (2015) used female nude Babl/c and hairless SKH-1 mice as photoaged animal models, respectively. They reported a
study concluded that the total doses and schedule for irradiation were essential in producing a photoaging effect on the skin. No previous report compared dermal thickness between Wistar rats, C57BL, and BABL/c mice. Therefore, this study reported a significant difference in dermal thickness among groups (p<0.05).

UVB irradiation increases oxidative stress, activates the apoptotic pathway, and produces SBC.\textsuperscript{11,33} SBC has a condensed nucleus and eosinophilic cytoplasm.\textsuperscript{33} In vitro study by Shin et al. (2013) showed that caspase-3, -8, and -9 were activated in a epidermal skin model exposed to UVB, thereby increasing SBC.\textsuperscript{18} Soetrisno et al. (2020) and Suyono et al. (2020) conducted an in vivo investigation using UVB-irradiated Balb/c mice. They discovered that the number of SBCs was higher in untreated mice than in the treated counterpart.\textsuperscript{11,33} No previous report compared the amount of SBC in Wistar rats, C57BL, and BABL/c mice. However, this study’s results showed a significant difference in the amount of SBC between groups.

Keratinocytes in UV-exposed skin can regulate dermal angiogenesis by secretion of VEGF and essential fibroblast growth factor. Toyoda et al. (2001) reported the formation of new vessels or angiogenesis in photodamaged human skin.\textsuperscript{34} Jung et al. (2010) and Karthikeyan et al. (2019) observed that UV-exposed mice showed increased proangiogenic proteins such as iNOS and VEGF expression.\textsuperscript{35,36} Also, they reported an increase in the size of blood vessels. No previous report compared the number of blood vessels between Wistar rats, C57BL mice, and BABL/c mice. This study reported that the number of blood vessels did not differ significantly between the groups.

This is the first study that compares the histological features of photoaged animal models, including Wistar rats, C57BL, and BABL/c mice. UVB irradiation for six weeks was discovered to affect the epidermal and dermal thickness, number of SBCs, and blood vessels of the photoaged animal model. This study was limited to experimental rats and mice so that only an initial description of the effect of UVB on the parameters tested in this study was obtained, while to obtain results

**Figure 4.** Differences in dermal thickness among groups.

**Table 2.** The number of SBC between P1, P2, and P3 groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Sunburn cells (mean ± SEM)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>P1</td>
<td>6.4 ± 1.14</td>
<td>0.000*</td>
</tr>
<tr>
<td>P2</td>
<td>10.4 ± 1.14</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>12 ± 0.7</td>
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Note: *p < 0.05

**Figure 5.** The difference in the number of SBC between (a) P1, (b) P2, and (c) P3 groups on HE staining with 400x magnification.
CONFLICT OF INTEREST
All authors declare no conflict of interest regarding this study publication.

ETHICAL CLEARANCE
The Ethics Committee of the Faculty of Veterinary, Universitas Airlangga, Surabaya, Indonesia, approved the standard animal care and experimental procedure on 27 July 2020 with Number: 2.KE.071.07.2020.

REFERENCES

AUTHOR CONTRIBUTION
All authors contributed equally to this study's preparation, execution, and manuscript writing.