Skin barrier before and after topical Adipose Stem Cell-Conditioned Medium (ASC-CM) treatment in photoaging

Winawati Eka Putri, Anang Endaryanto, Damayanti Tinduh, Fedik Rantam, Hari Basuki Notobroto, Cita Rosita Sigit Prakoeswa

ABSTRACT

Background: Photoaging is a type of aging mainly caused by ultraviolet irradiation. The skin barrier is affected in photoaging and it will result in coarseness, roughness and dry skin. It is probably because Ultraviolet (UV) affects the extracellular matrix in the skin. Adipose Stem Cell-Conditioned Medium (ASC-CM), which contains many growth factors, becomes a potential agent as a novel anti-photoaging treatment by repairing the skin barrier. This study aims to evaluate the skin barrier function before and after treatment with ASC-CM in photoaging skin by evaluating changes in Transepidermal Water Loss (TEWL) and skinfold thickness.

Methods: A true experimental study was conducted among 28 male Wistar rats aged 10-12 weeks. They were acclimated for 1 week and randomly divided into 4 groups: UV irradiated group with topical ASC-CM (Group P1); UV irradiated group with topical vehicle only (Group P2); only topical ASC-CM, non-UV irradiated (Group P3), and non-UV irradiated and no topical treatment (Group P0). The ASC-CM was given twice a day for four weeks and the total irradiance dose for 6 weeks was 4.2 J/cm². Data were analyzed using SPSS version 20 for Windows.

Results: There was a statistically significant difference of TEWL between pre- and post-treatment in group P3 (p=0.043) and P0 (p=0.018). In addition, a statistically significant difference in skin thickness was also found between pre- and post-treatment in group P2 (p=0.005) and P3 (p=0.003). There was a statistically significant difference in skin thickness post-treatment (p=0.000); however, there was no statistically significant difference of TEWL post-treatment by One-Way ANOVA test (p=0.138).

Conclusion: ASC-CM could be a potential option for photoaging skin based on the TEWL and skin thickness evaluation. However, a longer study period was needed to know the further effect of ASC-CM.

Keywords: ASC-CM, Skin Barrier, Transepidermal Water Loss, Skinfold Thickness.

INTRODUCTION

Photoaging or extrinsic aging is a type of aging caused by repeated exposure to the skin by ultraviolet (UV) irradiation. It is different from intrinsic aging, although both occur simultaneously. The histological features of photoaged skin are epidermal and dermal changes, varied epidermal thickness, and degeneration of collagen and elastin. The stratum corneum contributes to skin barrier properties and plays a role in maintaining the skin’s barrier function and water-holding capacity.

Transepidermal Water Loss (TEWL) measurement is the most widely used measurement for assessing the skin’s barrier function. The skin barrier stratum corneum has many protective functions, including contribution to the control of transcutaneous water loss. Ultraviolet B irradiation causes skin damage, leading to skin dehydration and an increase in TEWL. In this study, we used adipose stem cell-conditioned medium (ASC-CM), which contains a lot of soluble factors, such as cytokines, chemokines, and Growth Factors (GF). These become a promising treatment for skin barriers in photoaged skin through their paracrine effect.

Based on those mentioned above, limited studies have been conducted to improve skin barrier function with the secretome of ASC in photoaging treatment. So, this study aims to investigate the effect of ASC-CM on photoaged skin.

METHODS

There were 28 male Wistar rats (Rattus norvegicus) aged 10-12 weeks with an average body weight between 150-200 grams from the Laboratory of the Institute of Tropical Disease, Universitas Airlangga, Surabaya included in this study. They were
carried out for two weeks of acclimation. The Ethics Committee of the Faculty of Veterinary, Universitas Airlangga, Surabaya, Indonesia, also approved standard animal care and experimental procedures. Rats were randomly divided into four groups; each was placed in individual cages. Those groups were: UV irradiated with topical ASC-CM group (P1); UV irradiated with topical vehicle only group (P2); only topical ASC-CM, non-UV irradiated group (P3), and non-UV irradiated and no topical treatment group (P0). Rats are sheared every week.

Before UV irradiation, the back part of rats was shaved approximately 4x4 cm² in size. The 14 rats (P1 and P2) were photoaged using UV lamps (Ultraviolet B Broadband TL lamps. Phillips TL, 20W/01 RS) for 6 weeks. The emission wavelength is between 290-315 nm. A UV meter (UV light meter, UV 340B) was used to measure the UV irradiance. The distance between the lamp and the animal’s back was 30 cm. First three weeks, the irradiation was conducted 3 times a week with gradually increased doses. We use different Radiation intensity expressed in minimal erythema dose (MED), 1 MED (100 mJ/cm²; 9 minutes 15 seconds) in the first week; increased by 2 MED (200 mJ/cm²; 18 minutes 30 seconds) in the second week; 3 MED (300 mJ/cm²; 27 minutes 45 seconds) in the third week. For the next three weeks, the irradiation was conducted only 2 times a week with a dose of 4 MED (400 mJ/cm²; 37 minutes). The total irradiance dose for 6 weeks was 4,2 J/cm².

A topical ASC-CM was obtained from the Stem Cell and Tissue Bank Department of Dr. Soetomo General Hospital, Surabaya, Indonesia. The procedures for obtaining adipose tissue from the donor, culture process and separation of secretome were performed legally in accordance with international standards for tissue donor and stem cell culture from humans. After 6 weeks of UV irradiation, topical ASC-CM was applied on the back part skin rats, which were shaved previously, twice a day for 4 weeks.

At the beginning and the end of the study, TEWL and skinfold thickness were measured using the Cutometer MPA 580 with the Tewameter® TM 300 probe and a digital caliper, respectively, on the back part skin rats, which were shaved previously. TEWL and skinfold thickness are measurements used to assess the skin barrier.

Data were collected from the research status, then performed cleaning, editing and coding. The data collected were entered in the Statistical Package for the Social Sciences (SPSS) version 20.0 for Windows. The statistical test used was the normality test with the Shapiro-Wilk test because the sample number was smaller than 30 per group and the data were also normally distributed (p>0.05). A Paired-Samples T test was conducted to compare data before and after treatment. In addition, a One-Way ANOVA test was also carried out to compare data between groups if the data were normally distributed.

## RESULTS

This study reported that TEWL value before and after treatment with ASC-CM in UV irradiated with topical ASC-CM group was decreased by 21.38% but not significant (p>0.05). The results of TEWL value were reduced by 54.97% in UV irradiated with topical vehicle alone group, but it was also not significant (p>0.05). The results of the TEWL level were significantly decreased in non-UV irradiated and no treatment group (P3) (p=0.043) and non-UV irradiated and no treatment group (P0) (p=0.018) after treatment compared to before treatment with ASC-CM (p<0.05) (Table 2). Analysis with One-Way ANOVA showed no significant difference between groups after treatment with ASC-CM (p=0.138) (Table 1).

This study reported a significant difference in skin thickness between groups after treatment with ASC-CM (p=0.000) (Table 2). Skinfold thickness before and after treatment in UV irradiated with topical ASC-CM group was increased by 13.18%, but it was not statistically significant (p>0.05). The results of the skinfold thickness measurement were significantly decreased in UV irradiated with topical vehicle alone group (p=0.005) and non-UV irradiated with topical ASC-CM group (p=0.018).

### Table 1. Comparison between TEWL intragroup; and also, intergroup treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>TEWL Pre-treatment (Mean±SD)</th>
<th>TEWL Post-treatment (Mean±SD)</th>
<th>p*</th>
<th>Difference between Pre- and Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>10.51±5.036</td>
<td>8.26±2.355</td>
<td>0.463</td>
<td>-2.250</td>
</tr>
<tr>
<td>P2</td>
<td>10.31±2.902</td>
<td>10.65±1.662</td>
<td>0.599</td>
<td>0.333</td>
</tr>
<tr>
<td>P3</td>
<td>20.40±8.698</td>
<td>9.18±1.613</td>
<td>0.043*</td>
<td>-11.280</td>
</tr>
<tr>
<td>P0</td>
<td>15.47±8.479</td>
<td>8.51±1.635</td>
<td>0.018*</td>
<td>-6.957</td>
</tr>
<tr>
<td>p</td>
<td>0.024*</td>
<td>0.138</td>
<td>0.062</td>
<td></td>
</tr>
</tbody>
</table>

P1: UV irradiated with topical ASC-CM group; P2: UV irradiated with topical vehicle only group; P3: Only topical ASC-CM and non-UV irradiated group; P0: non-UV irradiated and no topical treatment group; *Paired-Sample T-Test; : One-Way ANOVA; : Statistically significant if p-value less than 0.05.

### Table 2. Comparison between SkinThickness intragroup; and also intergroup treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Skin Thickness Pre (Mean±SD)</th>
<th>Skin Thickness Post (Mean±SD)</th>
<th>p*</th>
<th>Difference between Pre- and Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1.286±0.163</td>
<td>1.456±0.422</td>
<td>0.404</td>
<td>0.1700</td>
</tr>
<tr>
<td>P2</td>
<td>1.408±0.203</td>
<td>0.850±0.108</td>
<td>0.005*</td>
<td>-0.5583</td>
</tr>
<tr>
<td>P3</td>
<td>1.278±0.179</td>
<td>0.876±0.087</td>
<td>0.003*</td>
<td>-0.4020</td>
</tr>
<tr>
<td>P0</td>
<td>1.111±0.218</td>
<td>0.752±0.141</td>
<td>0.062</td>
<td>-0.2586</td>
</tr>
<tr>
<td>p</td>
<td>0.010</td>
<td>0.000*</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

P1: UV irradiated with topical ASC-CM group; P2: UV irradiated with topical vehicle only group; P3: Only topical ASC-CM and non-UV irradiated group; P0: non-UV irradiated and no topical treatment group; *Paired-Sample T-Test; : One-Way ANOVA; : Statistically significant if p-value less than 0.05.
CM group (p=0.003) after treatment compared to before treatment with ASC-CM (Table 2).

**DISCUSSION**

Photoaging is one of the skin damages caused by sun exposure, mainly UV irradiation. Loss of surface smoothness and increased coarseness often called dry skin, is one of the changes in photodamaged skin. The histological features in photoaged skin include an increase in compaction of the stratum corneum, thickened granular cell layer in the epidermis, and thinned epidermis. Those will affect skin barrier function and result in dry and rough skin. There are various methods to assess skin barrier function. The barrier function of the skin mainly depends on the water and lipid content of the stratum corneum. Both Corneometer and the skin surface hygrometer provide objective, reliable, and easy methods for measuring the water content of the stratum corneum. Another technique that measures skin barrier examines the rate of trans-epidermal water loss using a device called Evaporimeter, a hygrometer that measures the amount of water vapor lost at a given time. Transdermal Water Loss (TEWL) is the most widely used objective measurement for assessing the skin’s barrier function. It is affected by properties of the surrounding environment such as humidity, temperature, and airflow and should be measured under controlled conditions. It varies significantly depending on anatomical site, sweat gland activity, skin temperature, and corneocyte properties. Wang YN et al., researched that the highest TEWL value, we also evaluated skinfold thickness before and after treatment with ASC-CM. The result showed that skinfold thickness before and after treatment in UV-irradiated with topical ASC-CM group was increased although not statistically significant. However, a longer study timeline is needed to observe further the effect and mechanism of this secretome.

Both Conditioned Medium (CM) showed improvement in all facial indexes, including TEWL value and hydration. Almost similar to the above studies, Amirthalingam M et al., and Kwon TR et al., showed decreased TEWL value after treatment with ASC-CM but using bone marrow stem cells. Our study reported a decrease in TEWL after four weeks of treatment compared to before treatment with ASC-CM, although not significantly different, compared with previous studies. Besides TEWL value, we also evaluated skinfold thickness before and after treatment with ASC-CM. The result showed that skinfold thickness before and after treatment in UV-irradiated with topical ASC-CM group was increased although not statistically significant. However, a longer study time period is needed to observe further the effect and mechanism of this secretome.

**CONCLUSION**

We demonstrated that the application of ASC-CM for four weeks improved skin barrier function of photoaged skin through improving skinfold thickness and TEWL value. This secretome has the potential effect of improving other parameters of photoaged skin.

**CONFLICT OF INTEREST**

The authors declare that they have no competing interests.

**ETHICS CONSIDERATION**

This research has been declared ethically feasible by The Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, prior to the study being conducted.

**FUNDING**

None

**AUTHOR CONTRIBUTIONS**

All authors are equally responsible for the initial conceptualization, study design, intellectual content, literature search, clinical studies, data acquisition, data analysis, manuscript preparation, and review of the earliest draft of the manuscript.

**REFERENCES**