The Aplication of Topical Wharton’s Jelly Mesenchymal Stem Cell Conditioned Medium (Wjmsc-Cm) Inhibit the Increase in Expression of Matrix Metalloproteinase-1 and Increased the Amount of Collagen in the Skin Of Wistar Mice Exposed to Ultraviolet-B Rays

Suarni¹, Wimpie Pangkahila¹,², I Gusti Made Aman¹,³

ABSTRAK
Introduction: Ultraviolet B exposure is one of the factors causing extrinsic skin Aging called photoaging. In this study, stem cell derived media growth factors conditioned from umbilical cord was used. The purpose of this study is to prove that topical administration of Wharton’s Mesenchymal Stem Cell Conditioned Medium Jelly (WJMSC-CM) could inhibit the increase in expression of matrix metalloproteinase-1 and increases the amount of collagen in the skin of wistar rats exposed to ultraviolet-B light.

Methods: An animal experimental with Post-test Only control group design study was conducted to evaluate the aforementioned hypothesis. The number of samples used in this study was 36 wistar rats, aged 3-4 months and were divided into 2 groups: the control group consisted of 18 rats were given placebo glycerin gel and exposed to UV B rays, while the treatment group was given WJMSC-CM and exposed to UV B rays. All treatments were given UV B rays with a total irradiation dose of 840mj / cm² for 4 weeks. The study was carried out at the Laboratory Animal Unit Unit of Pharmacology, Faculty of Medicine, Udayana University, Denpasar.

Results: By using the Shapiro Wilk test for numerical data with normal distribution, Levene's Test showed that the distribution of data and variants are homogeneous groups for both groups. The results of the comparative analysis of the two groups using independent t-test showed significant differences between the two groups on MMP-1 expression and the amount of collagen (p <0.05).

Conclusion: The conclusion of this study is the administration of WJMSC-CM topically inhibits the increase in MMP-1 expression and increases the amount of collagen in wistar rats exposed to UV B light.

Keywords: Wharton's topically conditioned (WJMSC – CM) Mesenchymal Stem Cell Jelly, UV B light, MMP-1 expression, amount of dermal collagen

INTRODUCTION
The aging process causes a gradual decline in the ability to maintain homeostasis and regenerate all tissues and organs of the body. Aging is influenced by intrinsic factors that originated from the body, including race, genetic, hormonal, glycosylation, methylation, apoptosis, and decreased immune system. Extrinsic factors are including ultra violet rays, environmental pollution, cigarette smoke pollution, unhealthy lifestyles, unhealthy diets and stress.¹

One of the extrinsic factors that plays a role in the photoaging process is ultraviolet light exposure, which causes a change of two regulatory factors that play an important role in collagen synthesis, namely activator protein-1 (AP-1) which is a transcription factor that inhibits the production of collagen 1 and III and causes degradation collagen through matrix metalloproteinase (MMPs) enzymes. MMPs that is mostly induced by UV light exposure, such as MMP-1 (collagenase, MMP-3 (stromelysin-1), and MMP-9 (92-kDa gelatinase), especially MMP-1 and MMP-2, is common in aging because ultraviolet light exposure. MMP-1 is an indicator for photoaging process due to ultraviolet light exposure because it is the initiator of triple helix collagen breakdown (type I and III procollagen). The study revealed that the dermis fibroblast also plays a role in producing MMP through an indirect paracrine mechanism by releasing growth factors and cytokines that trigger the production of MMP by keratinocytes in response to ultraviolet light exposure.²
Ultraviolet light radiation triggers the formation of reactive oxygen species (ROS), which activates the receptor epidermal growth factor and cytokine to release intracellular signals such as Mitogen - activated protein kinase (MAP) kinase which will stimulate the regulator protein activator protein -1 (AP-1) which activates nuclear factor kappa beta (NF-κB), a transcription factor that induces the expression of pro-inflammatory cytokines such as IL-1 and IL-6, vascular endothelial growth factor (VEGF), and tumor necrosis factor alpha (TNF-α). Regulatory proteins play a significant role in stimulating the expression of matrix metallo-proteinase and induce the degradation of collagen which plays an important role in the aging process. In addition to inducing the expression of pro-inflammatory cytokines, activator protein-1 suppresses the expression of transforming growth factor-β (TGF-β) receptor, which suppresses the synthesis of procollagen I and III, resulting in reduced dermal collagen. Reactive oxygen species (ROS) also inhibits leucocyte elastase which causes a decrease in elastin degradation resulting in an increase in dermal elastin buildup called elastosis.

Photoaging is clinically characterized by dermal elastosis, inflammation, collagen fragmentation, epidermal thickening, dry skin, roughness, uneven sedimentation, and teleangiectasia. The stem cell derivative media which is conditioned in several studies is used as a therapy to accelerate the regeneration of the skin on aging or injured skin. In this study, the conditioned stem cell derived media growth factors derived from the newborn human umbilical cord was used.

The secretion of cultured Wharton jelly mesenchymal stem cells is called the conditioned medium (WJMSC-CM) which contains various growth factors such as keratinocyte growth factor (KGF), transforming growth factor β (TGF-β), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF), transforming growth factor-a (TGF-α), Monocyte chemoattractant protein-1 (MCP-1), stem cell factor (SCF), insulin like growth factor-1 (IGF-1), molecular vascular cell adhesive (VCAM), interleukin-8 (IL-8), interleukin-10 (IL-10), inducible protein-10 (IP-10) which will induce proliferation, cell migration, extracellular matrix redistribution, angiogenesis, and tissue remodeling.

The aim of this study was to prove that the topical application of Wharton’s conditioned mesenchymal stem cell jelly could inhibit the increase of matrix metalloproteinase expression and increase collagen in ultraviolet B rays exposure condition which will prevent photoaging.

**METHODS**

Wharton jelly mesenchymal stem cell medium was made by the laboratory of the Kalbe Farma stem cell and cancer institute. This study was conducted on 36 Wistar rats (aged 3-4 months). Subjects were divided into two groups: the control group consisted of 18 wistar rats treated with placebo glycerin gel and exposed to UVB and the experimental group which was treated with WJMSC-SM and exposed to UVB. The two groups exposed to UVB with the total dose of radiation received were 840 mJ / cm² for four weeks.

**RESULTS**

At the end of the study, decapitation was carried out in both groups to obtain subject skin tissue for histological preparation to check the amount of collagen in the dermis and MMP-1. The administration of Mesenchymal Stem Cell Wharton’s jelly growth factor showed a significant difference in both groups.

On immunohistochemical examination for the control group (placebo), it appeared that MMP-1 expression (brown color) was increased. In the treatment group which received growth factors, MMP-1 expression (brown color) was decreased compared to the Placebo group.

![MMP-1 expression in rat dermis tissue with IHC staining](image-url)

In the Picro Sirius red examination for the control group (placebo), damage to the composition and structure of the collagen was observed with red collagen fibers that appeared thin. In the treatment group the amount of collagen with red collagen fibers appeared wider and thicker.
Figure 2. Collagen expression in the tissues of mouse dermis by Picro-Sirius Red staining

Comparative analysis of collagen number and MMP-1 is conducted by independent t-test as shown in tables 1 and 2 below.

Table 1 shows that the mean collagen dermis in the control group was 73.20 ± 6.79 and the mean treatment group was 80.89 ± 7.04. Analysis with the t-independent test showed that the value of t = 3.34 and the value of p = 0.002. This means that the mean dermal collagen in both groups after treatment was significantly different (p <0.05).

Treatment effect analysis was tested based on the mean MMP-1 expression between groups after being given topical treatment of WJMSC-CM gel. The results are presented in Table 1 and 2 below.

Table 2 shows that the mean MMP-1 expression in the control group was 20.31 ± 2.36 and the mean of the treatment group was 9.52 ± 2.29. Meaning analysis with t-independent test shows that the value of t = 13.92 and p = 0.001. This means that the mean MMP-1 expression in both groups after being given treatment differed significantly (p <0.05).

Figure 4 shows that there was a decrease in MMP-1 expression in the treatment group compared to the control group. The control is a group that is given glycerin gel (placebo) while the treatment is a group given WJMSC-CM.

DISCUSSION

This study found that the exposure to UV B rays with a total dose of 840mj / cm2 for 4 weeks was enough to cause collagen tissue damage in the dermis of Wistar rats. AP-1 will suppress the expression of procollagen-1 gene and procollagen-3 and TGF-β fibroblast dermis cells, as well as induce an increase in MMP-1 which cause decrease in collagen synthesis and increasing the degradation rate.

Topical administration of Wharton’s Mesenchymal Stem Cell Conditioned Medium Jelly (WJMSC – CM) provide a protective effect on dermis collagen damage to Wistar rats exposed to UV B light. This is evidenced by a significant increase in collagen in treatment group compared to the control group that treated by placebo glycerin. Wharton's Mesenchymal Stem Jelly Cell Conditioned Medium (WJMSC – CM) contains growth factors, chemokines and cytokines which have a protective effect on various cell types in overcoming the effects of inflammatory reactive oxygen species caused by UVB exposure. WJMSC-CM increase the expression of genes that play a role in the repair process such as the TGF-β coding gene and PDGF (platelet derived growth factor) that stimulate fibroblasts to proliferate, migrate and prevent damage to the extracellular matrix as well as alleviate the suppression of the type I procollagen gene and type III.

TGF-β inhibits macrophage and lymphocyte proliferation which inhibit the proinflammatory effect, prevent the increase in nuclear factor-kappa beta (NF-kB) thus preventing induction of IL-1, IL-6, VEGF, and TNF-α expression. TGF-β also inhibits AP-1 and MMP-1 through increased proteinase inhibitor activity, TIMPs (tissue inhibitor metalloproteinase), which prevents and reduces damage to collagen tissue so that collagen synthesis increases. FGF (fibroblast growth factor) and IGF-1 (Insulin like growth factor) play a role in increasing fibroblast proliferation and increasing collagen 1 production in fibroblasts.
Table 2. The difference in mean dermis collagen mean between groups after topical administration of WJMSC-CM gel

<table>
<thead>
<tr>
<th>Kelompok Subjek</th>
<th>n</th>
<th>Rerata Ekspresi MMP-1 (%)</th>
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<tr>
<td>Kontrol</td>
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<td>20,31</td>
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<tr>
<td>Perlakuan</td>
<td>18</td>
<td>9,52</td>
<td>2,29</td>
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</tbody>
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Figure 4. Comparison of MMP-1 Expressions between Control Groups and Treatment Groups

In addition, Wharton Jelly Mesenchymal Conditioned Medium (WJMSC-CM) can reduce the number of cells undergoing apoptosis due to UVB radiation, thus providing therapeutic potential for healing skin damaged by UVB radiation and oxidative stress.\(^\text{11,12}\) Thus there will be an increase in collagen mRNA expression and a decrease in MMP-1.

CONCLUSION

Based on the results of topical WJMSC-CM gel administration in Wistar rats exposed to UVB rays it was concluded that the administration of Wharton’s jelly mesenchymal stem cell conditioned medium was proven to increase the amount of collagen in dermis and was shown to reduce expression of MMP-1 UVB exposed Wistar rat skin.

REFERENCES