

The Effect of Mesenchymal Stem Cell Conditioned Medium (Adipose-Derived and Wharton's Jelly-Derived) on the Prevention of Hypertrophic Scar Formation

Gita Hening Bunga¹, Retno Dwi Utami¹, Erlina Pricilla Sitorus¹, Novan Adi Setyawan², Indah Julianto^{1,3}, Moerbono Mochtar¹

¹ *Dermatoveneerology Department Faculty of Medicine Sebelas Maret University, Surakarta, Indonesia*

² *Pathology Anatomy Department Faculty of Medicine Sebelas Maret University, Surakarta, Indonesia*

³ *Dermama Biotechnology, Surakarta, Indonesia*

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Abstract: The process of wound healing can lead to the formation of varied outcomes, from scarless healing to excessive fibrosis (hypertrophic) or atrophic scar. Mesenchymal stem cell (MSC) had been shown to prevent the growth of fibrosis tissue. Conditioned media (CM) is the medium where stem cells are cultured. Adipose-Derived Stem Cell Conditioned Medium (ADSC-CM) and Wharton's Jelly Stem Cell Conditioned Medium (WJSC-CM) are MSC-CM reported to contain growth factors, some of which plays a role in the formation of hypertrophic scar such as TGFβ1 and VEGF, and some in the prevention of scar formation, such as bFGF. This in vivo study conducted using 24 mice, divided into 4 groups: group I given Dulbecco's Modified Eagle Medium (DMEM), group II ADSC-CM, group III WJSC-CM, and group IV without any treatment (control). Before the injection, wound was made on the back of each mice with a 1 cm punch biopsy. On day 28th, rebiopsy was done on the scar area, the tissue stained with hematoxyllin eosin staining to assess the Scar Elevation Index (SEI). The SEI score showed that WJSC-CM group showed the lowest percentage of hypertrophic scar formation (50%) compared to ADSC-CM (66.67%), DMEM (83.33%) or control group(100%), although statistically the difference was not significant. This study showed that WJSC-CM injection had a greater potential in preventing the formation of hypertrophic scar than ADSC-CM. It is thought to be related to higher bFGF levels as well as lower TGFβ1 in WJSC-CM.

1 INTRODUCTION

The wound healing process can cause the formation of fibrosis tissue, also known as scar. Scar tissue consist of a collection of cells (especially fibroblasts) and an irregular extracellular matrix (mainly composed of collagen) (Gurtner et al., 2008). Hypertrophic scar is a fibroproliferative dermis disorder with typical features of excessive collagen deposition in the dermis and subcutaneous layer. The occurrence of hypertrophic scar indicates an excessive wound healing process, including migration and cell proliferation, inflammation, increased of synthesis and secretion of cytokines and extracellular matrix proteins, also remodeling of new matrices that form to excessive deposition of extracellular matrix (Meenakshi et al., 2005).

Several experimental and pre-clinical studies have reported the potential of mesenchymal stem cells (MSCs) to prevent the growth of fibrosis tissue (Dong et al., 2015; Li, Zhang and Fu, 2016). Mesenchymal stem cells may be present in both embryonic and adult tissue such as adipose tissue, muscle, peripheral blood, lung, heart, corneal stroma, dental pulp, placenta, endometrium, amniotic membrane and Wharton's jelly (Kalaszczynska et al., 2015).

The use of stem cell-conditioned media, compared to direct stem cells, provides a better solution in overcoming the limitations of cell-based therapies (Jayaraman et al., 2013). Potapova et al proved that the media used to culture stem cells, so-called conditioned media, was useful for survival, proliferation, invasion of extracellular matrix, and in vitro endothelial cell migration (Potapova et al., 2007). The conditioned media also contains a number of cytokines and growth factors that are directly

related to the wound healing process (Jayaraman et al., 2013).

Zhang et al showed that adipose-derived stem cell (ADSC) intralesional injection can reduce the formation of hypertrophic scar in rabbit ears by decreasing the expression of α -SMA genes and collagen type I, as well as reducing collagen deposition (Zhang et al., 2015). Yun et al also conducted research using ADSC injection on full-thickness defects made on pig's back and found that ADSC injection reduce the size, color and soften the scars that arise. The ADSC injection also decreases mast cell activity and inhibits the transformational growth factor β (TGF β), and stimulates scar remodeling by increasing the expression of matrix metalloproteinase (MMP) (Yun et al., 2012).

Kitajima et al conducted studies using umbilical cord blood (UCB-MSC) mesenchymal stem cells and wharton's jelly mesenchymal stem cells (WJ-MSC) injected in full thickness wounds in nude rats. Although the results showed no significant difference between injection of UCB-MSC and WJ-MSC on scar formation, the scar tissue in the WJ-MSC group showed smaller and thicker areas (Kitajima et al., 2016).

The various descriptions above underlay the researcher to examine the effect of injection of mesenchymal stem cell conditioned-medium

(adipose-derived and wharton's jelly-derived) in preventing hypertrophic scar formation.

2 RESEARCH METHODS

This experimental research using mice and was conducted at the Animal House of Pharmacology Department Faculty Of Medicine Gajah Mada University Yogyakarta between April-Mei 2016. As many as 24 mice were used divided into 3 treatment groups and 1 control group. Group I was given Dulbecco's Modified Eagle Medium (DMEM) injection 0.2 ml perilesion, Group II injected with ADSC-CM 0.2 ml perilesion, Group III given WJSC-CM 0,2 ml perilesion, and group IV as negative control not given any injection. Before the injection was performed, wound were made on the back of each mice with a 1 cm diameter punch biopsy (day 0). On day 28th, re-biopsy was done on the scar area, the tissue taken was stained with Hematoxyllin Eosin in Pathology Anatomy Laboratory of Gajah Mada University and analyzed with Image J to assess the Scar Elevation Index (SEI) of each scar. If the SEI > 1 = hypertrophic, while if SEI < 1 the scar is normal (eutrophy). The result were statistically analyzed with one way analysis of variance (ANOVA) with the significance value of $p < 0.05$.

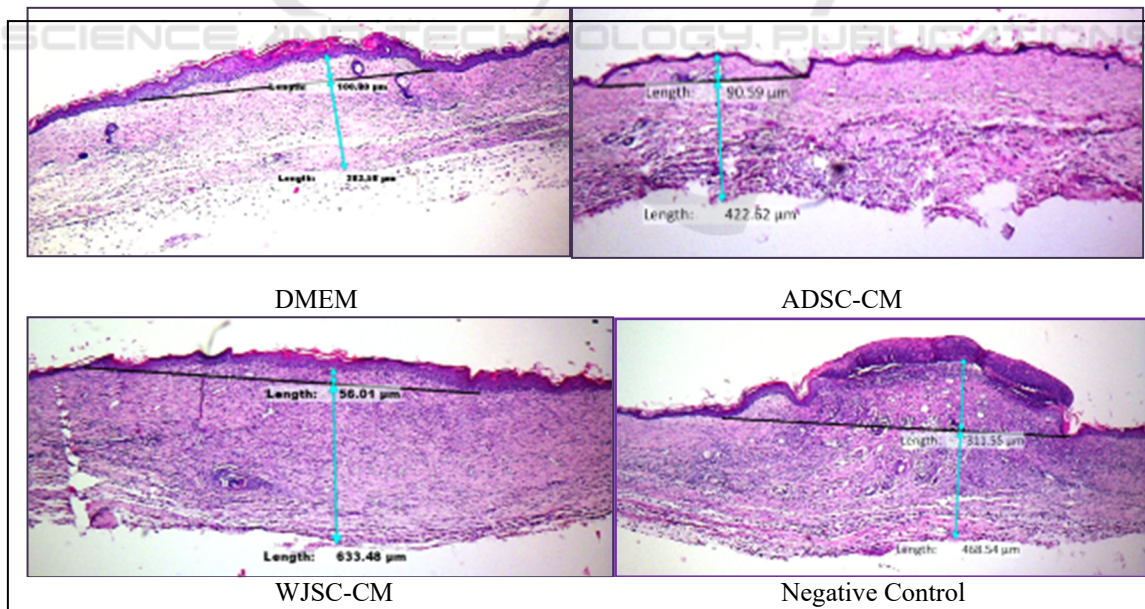


Figure 1. Histopathology Image and Scar Elevation Index (SEI) Measurement.

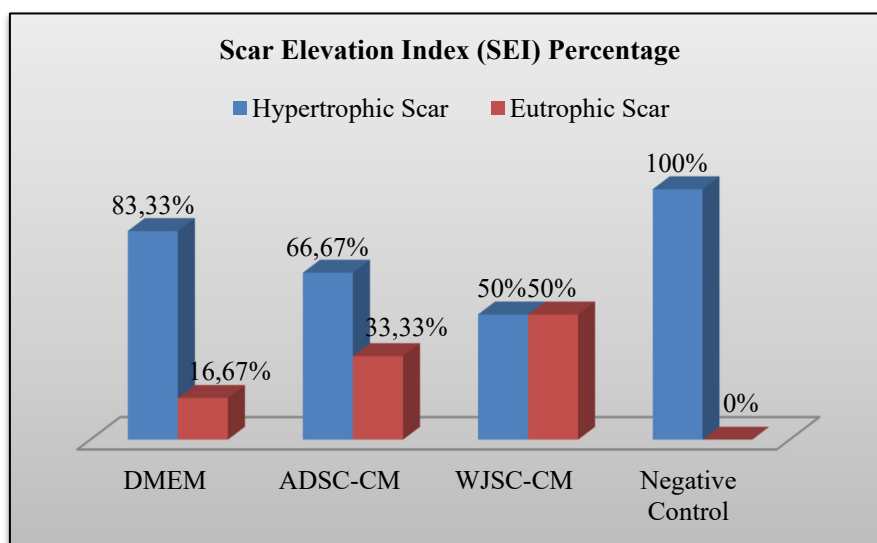


Figure 2. Scar Elevation Index Percentage.

3 RESULTS

ANOVA analysis from SEI measurements showed no significant differences between groups ($p > 0.05$). For the percentage of scar formed the result showed that in group I (DMEM) hypertrophic scar formed as much as 83,33%, group II (ADSC-CM) 66,67%, group III (WJSC-CM) 50% and in group IV (control) all of the scar was hypertrophic. 100%. The data showed that WJSC-CM had the lowest percentage of hypertrophic scar formation compared to ADSC-CM, DMEM or without treatment, although statistically the difference was not significant.

4 DISCUSSION

In the SEI measurements there was no significant difference between the treatment groups statistically. However, it can be seen that the percentage of hypertrophic scar most in group IV (without treatment) equal to 100% and smallest in group III (given WJSC-CM) as much as 50%.

The Enzym-Linked Immunosorbent Assay (ELISA) examination of growth factor content contained in ADSC-CM and WJSC-CM revealed the content of bFGF, TGF β 1 and VEGF in both of the conditioned media. The content of bFGF at WJSC-CM (7,141 pg / ml) is higher than ADSC-CM (6.376 pg/ml), whereas the TGF β 1 content of WJSC-CM (7.596 pg/ml) is lower than ADSC-CM

(8,176 pg/ml). Similarly, the WJSC VEGF content (3.645 pg / ml) is lower than ADSC-CM (7.287 pg / ml).

Several studies have proven the role of bFGF in the process of wound healing and prevention of hypertrophic scar formation (Spaccapelo, 2016). Ono et al injected basic fibroblast growth factor (bFGF) in postoperative wounds and showed that bFGF injection may inhibit the formation of hypertrophic scars and inhibit the widening of postoperative scar size (Ono et al., 2007). In this study it appears that the administration of WJSC-CM, which contains higher bFGF levels, showed a lower percentage of hypertrophic scar formation slightly compared to ADSC-CM, DMEM media or without treatment. This is in accordance with the theory that bFGF can prevent the formation of hypertrophic scars.

Transforming growth factor β 1 (TGF β 1) is known to have an important role in the formation of hypertrophic scars (Lu et al., 2005). WJSC-CM-conditioned media known to contain lower levels of TGF β 1 than ADSC-CM. It is estimated that the lower TGF β 1 levels are also a factor that plays a smaller percentage of WJSC-CM hypertrophic scarred than ADSC-CM.

Vascular endothelial growth factor (VEGF) is a growth factor that played a role in angiogenesis of wound healing process. In angiogenesis process it is estimated that dermis endothelial cells can stimulate expenditure of TGF β , CTGF or other profibrotic factors that can stimulate scar tissue formation by fibroblasts. The process of remodeling the

extracellular matrix that accompanies angiogenesis also indirectly can stimulate the formation of scar tissue. Thus VEGF can act as a link between angiogenesis and scar formation, by directly stimulating both endothelial cells and dermal fibroblasts (Wilgus et al., 2008). In this study WJSC-CM, which contained lower VEGF, showed the lowest percentage of hypertrophic scar. It is estimated that low levels of VEGF also affect these results, in addition to higher levels of bFGF and lower TGF β 1.

5 CONCLUSION

WJSC-CM injection has a greater potential in preventing the formation of post-wound hypertrophic scarring than ADSC-CM. It is thought to be related to higher bFGF levels, as well as lower TGF β 1 in WJSC-CM used in this study.

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