The Efficacy of Human Amniotic Membrane Mesenchymal Stem Cells (hAMMSC) Plus Vitamin C for Treating Chronic Plantar Ulcer in Leprosy Patient: A Case Report

Riyana Noor Oktaviyanti,[1] Rahmadewi,[1] Medhi Denisa Alinda[1], Bagus Haryo Kusumaputra[1], Linda Astari[1], M. Yulianto Listiawan, Indropo Agusni[1], Fedik A. Rantam[2,3] and Cita Rosita Sigit Prakoeswa[1]

[1] *Departement of Dermatology Venereology, Faculty of Medicine Universitas Airlangga, Dr Soetomo General Hospital, Surabaya, Indonesia*

[2]*Stem Cell Research and Development Center, Universitas Airlangga, Surabaya, Indonesia.*

[3]*Virology and Immunology Laboratory, Department of Microbiology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia*

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Abstract: Chronic plantar ulcer in Leprosy is a still a problem in term of patient's medical and social aspect. Amniotic membrane stem cell (AMSC) conditioned medium provide growth factors and sitokin for the chronic ulcer's healing. Vitamin C as antioxidant, anti inflamatory, dan collagen synthesis can be useful for wound healing.

1 INTRODUCTION

Chronic plantar ulcers in Morbus Hansen (MH) including level 2 defects, are the most common cause of disability in MH patients. The prevalence of level 2 deficiency in MH patients in Southeast Asia increased from 2010 to 2011 by 2.5%. In Indonesia, MH patients with disability level 2 also increased during the year 2011-2012 by 10.11% (World Health organization, 2012; Metro Tempo Co., 2013). Approximately 30% of patients with Leprosy have peripheral nerve damage, and 10-20% of them have neuropathic ulcers due to peripheral nerve damage. Chronic ulcers are a serious medical and social problem. Patients experience decreased quality of life, so wound healing management continues to be investigated to deal with chronic ulcers (Halim and Menald, 2010; Bauman, Girling, and Brand, 1963). In chronic ulcers there is a decrease in growth factors such as platelet derived growth factor (PDGF) and growth factor transformation (TGF) -β required in the healing process, there is a high level of reactive oxygen species (ROS), which can damage cells and collagen degradation (Diegelmann and Evan, 2004).

Nowadays start developed therapy using metabolite product from stem cell without the stem cell. The use of this metabolite product is easier and safer because it is aseluler. The AMSC metabolite product was obtained at culture from AMSC, which in this metabolite product contained several cytokines and growth factors required for wound healing (Lindenmair *et al*., 2012; Ennis, Sui, and Bartholomew, 2013).

Vitamin C known as antioxidants and play role in collagen synthesis. These functions are believed to also be useful in wound healing. As an antioxidant vitamin C neutralizing ROS that we could found in chronic ulcers. Vitamin C also directly stimulates collagen synthesis. One derivative of vitamin C that is stable is sodium ascorbyl phosphate (SAP) (Telang, 2013).

2 CASE

38- year-old female patient, working as a housewife came with chief complaint ulcer at her right foot since 1 years ago, painless and no itchy sensation. At first the ulcer is small, then became bigger and deeper by the time. Patients have been frequently treated since the last 8 months for her complaints,

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she got treatment for treating wounds with sterile normal saline, antibiotics ointments and framycetin gauze dressing (FGD) but no improvement.

The patient was suffering from borderline leprosy since 3 years ago. She had already got multibacillary multi-drug therapy for 12 months and had finished the treatment 1 year ago. The result of microbiology *Mycobacterium leprosy* examination was bacterial index (BI) 0 and morphological index (MI) was negative.

The initial treatment for the ulcer was sterile normal saline and surgical debridement to remove necrotic tissue, callus, and debris. After obtaining a clean ulcer, wound area and wound depth measurements were performed. Then the ulcer was given FGD and sterile gauze above the FGD. On subsequent visit, the ulcers begin to be given topical hAMMSC-CM + Vit C gel and covered with transparent film dressings. Application of gel was done every 3 days, and weekly evaluation included measurement of the size and the depth of the ulcer was done. Initial measurements of the ulcer was 2 x 2 x 0.4 cm. Patient received hAMMSC-CM + vitamin C gel for 7 weeks. After the therapy with hAMMSC-CM + Vit C gel for 7 weeks, the ulcer healed compeletely.

3 DISCUSSION

Since the early 20th century, amniotic membranes have been used for the treatment of widespread injuries, burns, chronic ulcers, deep ulcers, and surgical wounds that are difficult to heal completely. Studies on the human amniotic membrane mesenchymal stem cells (hAMMSC) for the repair and regeneration of cells showed good results. The growth factors and cytokines can be found in the tissue medium of stem cells in *in vitro* conditions through its metabolite product so it can be utilized as a useful modality in the process of cell regeneration (Bauman, Girling, and Brand, 1963; Diegelmann and Evan, 2004).

The previous research has proved that injuries that are difficult to heal in humans have high levels of oxidative stress. Wounds or chronic ulcers often show the increasing levels of reactive oxygen species (ROS) or free radicals that can delay wound healing process, inhibit the formation of granulation tissue and epithelialization. Free radicals are formed in response to tissue damage which then inhibits the recovery process by attacking DNA, membranes, proteins, and lipids from cells. Antioxidants are believed to repair wounds by reducing the damage caused by free radicals released by neutrophils in the inflammatory phase of the wound healing process Ennis, Sui, and Bartholomew, 2013; Telang, 2013).

Plantar ulcers in leprosy are neuropathic ulcers, occurring due to prior nerve damage or sequelae. Chronic ulceration results from one or more extinctions of the wound healing phase (WHO, 2012). In a chronic wound, one may detect a prolonged inflammatory response, elevated protease activity and decreased growth factors. These findings may account for the delayed wound healing process. The prolonged inflammatory response may be caused by infection or just inflammation. The FGD is an antibiotic wound dressing that works in the inflammatory phase of wound healing by treating the infection and contra-acting the colonisation. It is not effective in an inflammatory phase of wound healing that is not caused by infection. It is also not effective in the proliferative phase of wound healing (Halim and Menald, 2010; Metro Tempo Co., 2013). Prakoeswa *et al* (2018), study analytical experimental approach comparing the topical hAMMSC-CM and the framycetin gauze dressing (FGD) applied every 3 days up to 8 weeks on the healing of CPUL. Ulcer healing in the hAMMSC-CM group was significantly better than that in the FGD group with significant clinical and statistical differences ($p < 0.005$ and $p< 0.005$).

The hAMMSCs contains many cytokines and growth factors, which may be effective in the inflammatory and the proliferation phase of the wound healing. In the inflammatory phase of wound healing, the pro-inflammatory cytokines in hAMMSCs may influence the infection and colonisation via its antimicrobial effect and the anti-inflammatory cytokines in hAMMSCs will diminish the inflammation (immunomodulatory effect). In the proliferation phase of the wound healing, the growth factors in hAMMSCs willstimulate proliferation and migration of cells, induce angiogenesis, and stimulate the formation of tissue granulation and epithelialization (Bauman, Girling, and Brand, 1963; Diegelmann and Evan, 2004).

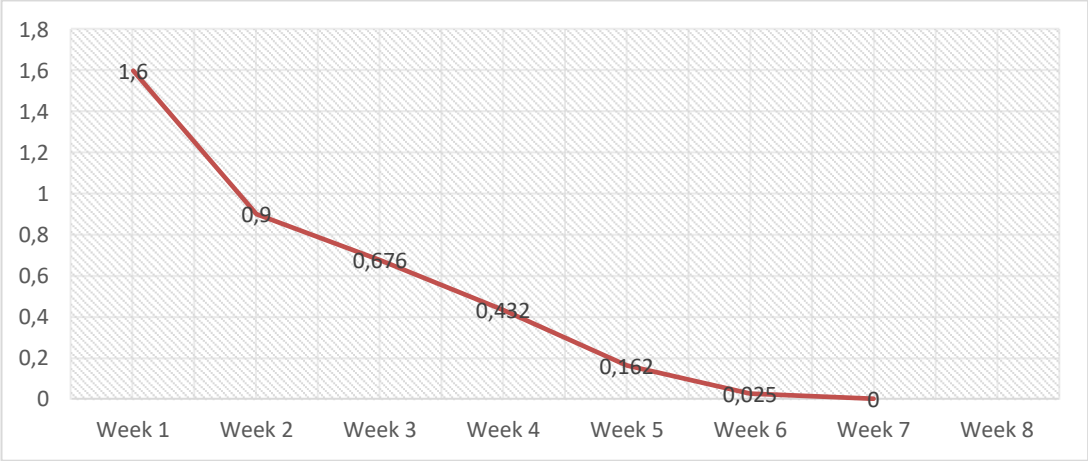

Figure 1: Healing progression. Ulcer treated hAMMSC-CM + Vit C healed completely at week 7

In the dermatology Vitamin C known as antioxidants, and collagen synthesis. These functions are believed be useful in wound healing. The function of vitamin C as an antioxidant is beneficial in neutralizing ROS present in chronic ulcers. Vitamin C also directly stimulates collagen synthesis. One derivative of vitamin C that is stable, is sodium ascorbyl phosphate (SAP). This derivative has the same function as vitamin C but has better stability. The use of vitamin C and metabolite products simultaneously is expected to accelerate wound healing, with faster healing times that cost less and the quality of life of patients increases (Lindenmair *et al*., 2012; Ennis, Sui, and Bartholomew, 2013).

Function of ascorbic acid as an antioxidant is useful in inhibiting free radicals produced in the inflammatory process (Moro, 1999; Telang, 2013). Another study by Sarpoosh HR and colleagues assessed the effectiveness of topical vitamin C in necrotic tissue cause burns grade II. There were 30 patients with burns gr II divided into control groups receiving sulfadiazine ointment and treatment group receiving sulfadiazine ointment accompanied with vitamin C. In the group of treatment the initial necrotic tissue volume was 1.033 ± 0.182 and on day 14 the average volume was 1.233 ± 0.430, and in the initial necrotic tissue volume control group was 1,000 ± 0,000 and at day 14 the average volume became 1,700 ± 0.794. There was a significant difference between the two groups, in the treatment group, the mean difference of necrotic tissue volume was less than the control group (Singh and Singh, 2012; Sarpooshi, Vaheb, Tabarayee, Sabzevar, and Mortazavi, 2016)

4 CONCLUSIONS

In this case the function of vitamin C is SAP as antioxidant, anti-inflammatory, antibacterial and collagen synthesis can help wound healing where in chronic injury disorders occur in the inflammatory phase, and the proliferation phase. The combination of PM-AMSC which contained several cytokines and growth factor with vitamin C seemed to give good results on the healing of chronic plantar ulcers of leprosy, and in this case there were no side effects in mixing PM-AMSC with vitamin C in chronic leprosy ulcers.

The ulcer improvement within 7 weeks of AMSC + vitamin C. There are several other factors that also play a role in ulcer healing, age, type of work and duration of ulcers may contribute to ulcer healing.

REFERENCES

Bauman, J.H., Girling, J.P., Brand, P.W., 1963. Plantar Pressure and tropic ulceration. *The Journal Of Bone And Joint Surgery*, 4, 652-673.

Diegelmann, R.F., Evan, M.C., 2004. Wound healing : an overview of acute, fibrotic and delayed healing. *Frontiers in Bioscience*, 9, 283-289.

Ennis, W.J., Sui, A., Bartholomew, A., 2013. Stem cells and healing: impact on inflammation. *Adv Wound Care*, 7, 369-378.

Halim, L., Menald, S.L., 2010. Tata Laksana Komprehensif Ulkus Plantar pada Pasien Lepra. *Maj Kedokt Indon*, 60, 237-244.

Lindenmair, A., Hatlapatka, T., Kollwig, G., Hennerbichler, S., Gabriel, C., Wolbank, S., *et al*.,

2012. Mesenchymal stem or stromal cells from amnion and umbilical cord tissue and their potential for clinical applications. *Cells*, 1, 1061-1088.

Metro Tempo Co., 2013. (Internet). Jakarta. (cited Oktober 2015). Available from:http://metro.tempo.co/read/news/2013/02/14/214461169/penderita-kusta-indonesia-tertinggi-ketiga-dunia

Moro, O., 1999. Biological activities of stable ascorbic acid derivative, 2-0-a-D- glucopyrasonyl-L-ascorbic acid (AA-2G) in cosmetics. *Shiseido Research Center. J ApplCosmetol*, 18, 154-163.

Prakoeswa, C.R.S. *et al*., **(**in press). Efficacy of topical human amniotic membrane mesenchymal stem cell-conditioned medium (hAMMSC-CM) on the healing of chronic plantar ulcers in leprosy: a controlled prospective clinical trial.

Sarpooshi, H.M., Vaheb, M., Tabarayee, Y., Sabzevar, A.V., Mortazavi, F., 2016. The effects of topical vitamin C solution on the necrotic tissue volume of burn wounds. Journal of Nursing and Midwifery Science, 3(3), 11-17.

Singh, P., Singh, P., 2012. Role of topical ascorbic acid in management of refractory corneal ulcer. *IOSR Journal of Pharmacy*, 2, 1-4

Telang, P.S., 2013. Vitamin C in dermatology. *Indian Dermatol Online J*., 4: 143–6.

World Health organization, 2012. *Weekly Epidemiological Record,* 87, 317–328.