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PROCEEDINGS

THE 6th INDONESIAN BIOTECHNOLOGY CONFERENCE

“ENHANCING INDUSTRIAL COMPETITIVENESS
THROUGH BIOTECHNOLOGY INNOVATION”

Surakarta, 6-7 September 2016

Editors:

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HEALING OF DIABETIC FOOT ULCER USING AUTOLOGOUS PERIPHERAL BLOOD MONONUCLEAR STEM CELLS (STUDY CASE)

Basuki Supartono^{1,2}, Prita Kusumaningsih³, Muzayyana Sakinah³

¹Orthopaedic Department, National Sport Hospital of Indonesia, Jalan Jambore Raya No. 1, Cibubur, Jakarta, 13720, Indonesia

²Stem Cell Research and Tissue Engineering Center University of Pembangunan Nasional "Veteran" Jakarta, Jalan RS Fatmawati, Pondok Labu, Jakarta, 12450, Indonesia

³RSU Al-Fauzan, Jalan Pedati no 3, Jakarta Timur, 13540, Indonesia

Email: drbasuki@gmail.com

Abstract

Diabetic foot ulcer is the most dreaded complications of Diabetes Mellitus (DM), wound healing in patients with DM is a very important issue. Here, we report a case study of stem cell therapy on unhealed diabetic wound. The mononuclear stem cells (MNC) were isolated from patient's peripheral blood with Ficoll gradient. Topically MNC stem cells administration on 47 years old female patient for 2 months, triggered the formation of granules in the cells of the surrounding wound. Eventually, wound were healed completely. In conclusions, MNC stem cells therapy healed end stage diabetic foot wounds, without surgery.

Keywords: *diabetic foot ulcer, mononuclear stem cells.*

1. Introduction

Diabetic foot ulcer was the most dreaded complications of Diabetes Mellitus (DM), wound healing in patient with DM was very difficult. Wound healing was a complex process, involving various aspects for integrity returning and functions of the tissue. Any disruptions in wound healing process caused chronic ulcer, moreover developed into severe. Several factors played a role in delaying of wound healing, namely vascular insufficiency, chronic disease like diabetes, renal disease, local pressure and advanced age [1].

Approximately 15 - 25% DM patients will undergo diabetic foot ulcer, with the most frightening complication was amputation [2]. Despite current clinical care protocols for ulcer treatment have been developed, an amputation rate remained high. Hence, there is an urgent need for new intervention [3].

Delayed or non-healed wound in diabetes mellitus resulted from dysregulation of the normal healings process. The diabetic wound was complex, that usually associated with infection,

neuropathy and vascular disturbances. Several factors contributed for non-healed wound were impaired cellular activity, deficient extracellular matrix (ECM) synthesis, growth factor expression reduction, and local neovascularize diminution [3,4].

The current standard care are debridement, blood flow restoration (in peripheral arterial disease), pressure elimination from the ulcer, and antibiotic administration [3]. Unfortunately, diabetic foot ulcer sometimes occurs in elderly patient which have impairment in most of body function. The management of diabetic foot ulcer particularly focused on lower limb amputation prevention [5]. Recent therapies developed, such as negative pressure therapy, hyperbaric oxygen therapy, and growth factors therapy, had limited success.

Tissue engineering has developed new strategy for diabetic wound repairment. Cell based therapy offered a new treatment strategy to cure non-healed diabetic wound, and prevent amputation. [3]. Stem cells therapy have been issued successfully, to treat both chronic and acute

wounds [1]. Basic science and clinical studies showed that these therapies provided a comprehensive solution by addressing multiple factors (cell proliferation, extracellular matrix synthesis, growth factor release, and vascularization) during wound healing process [4]. Application of stem cell was promising as treatment for diabetic foot ulcer [4].

Stem cells are considered the master cells, capable of both self-renewal and multi-lineage differentiation. Stem cells have ability to regenerate, forming cells and constituent body tissue of organism [6,7]. Stem cell obtained from embryonal cells or human body tissues isolation. Tissue stem cell especially MNC cell, obtained from solid tissue (skin) and liquid tissue (bone marrow aspiration and blood) isolation [8].

The objective of this study was to evaluate MNC stem cells therapy usefulness on non-healed diabetic foot ulcer patient.

2. Methods

In this case, we administrated suspension of autologous peripheral blood mononuclear stem cells (MNC) on end-stage diabetic wound. Suspension applied topically on the wound every 1-2 weeks.

Patients details

We treated a 47 years old woman who suffered type-2 Diabetes Melitus for 12 years. She

had ulcer on her right foot . There was no improvement on her ulcer, and worsen since 4 months before. On physical examination, we found an ulcer with eight like shaped on the dorsal side, untill interdigital space III-IV on the distal plantar of the right foot. The size of the ulcer was 10 cm length, 3 cm width, and 1 cm depth. There was sign of soft tissue infection without bone involvement. Soft tissue infection marked by hyperaemia, discoloration of the foot, swelling, and fragrant odorand pus production from the ulcer. Surgeon at the previous hospital planned foot amputation, to prevent infection extension and further tissue death, which threatened patient's life. The patient refused foot amputation. Patient has controlled type-2 diabetes mellitus with routine oral hypoglycaemic drug consumption. The patient had no history of heart disease and peripheral arterial disease (PAD).

The patient had knowingly signed informed consent. The procedure consists of patient's peripheral blood collection, MNC isolation, wound toilet, MNC application topically and performed wound dressing. The evaluation was performed weekly for 8 weeks. Totally we applied five times peripheral blood MNC into the wound, with various cell counts, and viabilities (Table 1). Patient also received oral antidiabetic and oral antibiotic, and also antibiotic injection when necessary.

Table 1. Characteristic of MNC cell

No	Administration	Cell count (10 ⁶)	Viability (%)
1	I	33	91.6
2	II	46	90
3	III	88	92
4	IV	40	92
5	V	37.9	97

Collection of patient's peripheral blood

Blood was collected from patient, about 50 ml. The donor had knowingly signed informed consent before the procedure held.

Isolation of MNC

Blood specimens from peripheral blood, were diluted with PBS + KCl solution, filtrated with Ficoll and centrifuged. Buffy coat layers were taken and washed, then supernatant was removed,

only mononuclear cells (MNC) were collected. The MNC cell amount and viability was checked.

Wound toilet

Regular wound care was done before and after every MNC administration. The wound cleaned before administration normal saline (NaCl 0.9%), and H₂O₂ solution and / or povidone iodine dressing occasionally. Any necrotic and potential infection tissue were removed, to ensure the wound free from infection.

Topical Administration of MNC

MNC stem cell are instilled topically on the ulcer. Three ml syringe and 22G needle are used to deliver the stem cell by dripping liquid filled cell to the entire surface of the wound. Liquid cell also added to mixed dressing solution.

Wound Dressing

After the wound cleansing and MNC stem cells administration, the wound was dressed with a gauze dressing, impregnated with the antibiotic framycetin sulphate (sofra-tulle), honey, antibiotic solution and finally covered with sterile gauze. Wound was left closed in order to maintain the wound moisture and dressing were changed every week. All procedure was done by orthopaedic surgeon.

Evaluation

Evaluation of treatment was done weekly, treatment is marked with some sign such as:

1. Healing and decrease in the ulcer size
2. Present of fresh granulation tissue
3. No sign of further infection
4. No sign of further indication for any surgical treatment especially amputation

3. Results and Discussion

The result was very satisfying. The wound improved progressively week by week and healed completely after 60 days. The improvement of the wound marked by the absent of soft tissue infection, narrowing wound size, present of granulation tissue, and closure of the wound exactly. The surgical intervention such as surgical debridement, skin graft, even amputation, was not necessary in this case. The early appearance of the wound indicated for amputation, due to wide skin loss and marked infection. But until the end of wound care, no amputation was done. This proved that MNC stem cell therapy healed severe diabetic foot ulcer and prevent amputation. During two months of treatment, we did not found any adverse effect, allergic reaction, related to stem cell administration. The following pictures and table showed the course of disease during stem cell administration, related to parameters evaluation (Figure 1, 2, Table 2).



Figure 1. Comparison of ulcer before and after MNC therapy. A and B: before MNC therapy; C and D: after two months of MNC therapy.



Figure 2. Wound healing progress. A. The wound appearance before stem cell administration. B. After 1st MNC administration. C. After 2nd administration. D. After 3rd administration. E. After 4th administration. F. After 5th administration or 2 months of treatment, the ulcer is completely recovered.

Table 2. Course of Wound Healing During MNC Cell Administration Related to Parameter Evaluation

Time	Healing/ decrease ulcer size	Granulation tissue	Infection	Surgical indication
0 day	(-)	(-)	(+)	(+)
6 days	(-)	(-)	(+)	(+)
9 days	(-)	(+)	(-)	(-)
23 days	(+)	(+)	(-)	(-)
30 days	(+)	(+)	(-)	(-)
37 days	(+)	(+)	(-)	(-)
44 days	(+)	(+)	(-)	(-)
58 days	(+)	(-)	(-)	(-)

Our result is in line with recent study. In our result the healing time is shorter than two others. Many recent study showed that MNC stem cell therapy is promising in diabetic wound recovery (Table 3). Kirana et al. [9] in their randomized clinical controlled trial on 30 diabetic patients, conclude that MNC cells improved the microcirculation and supported wound healing.

Ruiz-Salmeron et al. [10] performed intra-arterial autologous MNC transplantation in 20 diabetic patients with peripheral artery disease. After 3 to 12 months, all patients exhibited clinical improvement with a significant vascular network escalation.

Table 3. Comparison Between Study of Autologous MNC Stem Cell for Diabetic Foot Ulcer

Author	Type of study	Sources of MNC	Subject	Average number of cells (10^6)	Route of Application	Healing time	Result
Kirana et al.	RCT	BM	30 + PAD	306.8	I.m.gastrocnemius	10 - 13 months	wound healed
Ruiz-Salmeron	Prospective	BM	20 + PAD	266.2	Intra-arterial	3 - 12 months	Wound healed + increase vascular network
Basuki	Case study	PB	1	50	Topical	2 months	wound healed

Note: RCT: Randomized Controlled Trial; BM: bone Marrow, PB: Peripheral Blood, PAD: Peripheral Arterial Disease; i.m: intramuscular.

There is similarity and also diversity among their study. Three studies above use the same autologous MNC, but derived from different sources. Two studies utilized bone marrow aspiration, whereas our study utilized MNC from peripheral blood. This is reflected in the number of cells produced, BM aspiration produced more MNC cells. However, it does not take the PB MNC inferior although cell number is fewer, but wound healing time of PB MNC in our study is shorter than bone marrow. Possible reason is no PAD complication in our patient.

Many study on peripheral blood MNC therapy have been published but the mechanism remains debatable. Some studies proposed about elevated expression of angiogenic growth factors, others suggest that MNC cells suppressed inflammation. One explained about biological mechanism in which that progenitor cells circulate in the blood and differentiate into cells or tissues. PB MNC contains a various multipotent progenitor cell, potential to differentiate into various tissue cell such as blood cells, muscle cells, epithelial cells, neural cells, or fibroblast under appropriate conditions [4, 11]. Collecting sources MNC cells from PB is easier than BM, the collection is not invasive and comfortable for patients. Patient should not undergo any operative procedure like bone marrow aspiration collection [11]. So PB MNC is promising therapy for unhealed diabetic foot ulcer.

Stem cells research still developing, to full-fill blank notes in stem cells application. The application of PB MNC seems to show bright spot, likely in this case report however, many question still haunted the usefulness of stem cell therapy. Several questions namely the characteristics of donor, collecting the the sources, isolation method, characteristics of cells, the effective dose, and the parameter evaluation. And also the role of each related factors such as blood sugar level, vascular patency, level of infection etc. All of these should be considered as interrelated factors in the successful of the therapy. Therefore, more advanced basic and clinical trials are required in order to explore the efficacy, and safety of MNC stem cells therapy for diabetic foot ulcer.

4. Conclusion

In this case we proved that administration of autologous PB MNC stem cells can healed diabetic foot ulcer without operation.

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