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The Effect of Human Bone Marrow Mesenchymal Stem Cells on Epidermal Growth Factor and Epidermal Growth Factor Receptor Expression in Re-epithelialization Process in the Healing of Burns on Experimental Rats

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Abstract

BACKGROUND: Research on human bone marrow mesenchymal stem cells (hBM-MSCs) for burns healing has been known to increase the percentage of integrin expression of α2β1, type I collagen, transforming growth factor-β, and matrix metalloproteinases-9, but research on giving hBM-MSCs to growth factor expression in the process of re-epithelialization of burn healing has not been done.

AIM: This study aims to the effect of hBM-MSCs given on the expression of epidermal growth factor (EGF) and EGF receptor (EGFR) in the process re-epithelialization in the healing of burn experimental rat.

MATERIALS AND METHODS: This research is experimental with the post-test only control design, using 30 Wistar rats. Rats were divided into two groups, namely, control (phosphate-buffered saline), and the treatment was given hBM-MSCs, and stem cells were given subcutaneous doses of 2×10^6 cells/ml. Before being treated rats were anesthetized using xylazine and ketamine then the rats were burned in the dorsal (spine) with full-thickness. On the 3, 7, and 14 days, skin tissue was taken to see the expression of EGF and EGFR by immunohistochemical methods. This study was approved by the Ethics Commission of the Faculty of Medicine, Andalas University, Padang. The results of the study were analyzed by the t-test.

RESULTS: Immunohistochemical examination of EGF and EGFR expressions after hBM-MSCs administration has significantly increased epithelialization compared with controls. Increased EGF expression was found on days 3 and 7 with moderate positive internal revenue service (IRS) assessment and on day 14 strong positive EGF expression, as well as EGFR expression on days 3 and 7 with moderate positive IRS assessment and on day 14 robust positive EGFR expression.

CONCLUSION: This study concluded that giving of hBM-MSCs can increase the expression of EGF and EGFR which enhances the process of re-epithelialization thereby accelerating the healing of burns of experimental rats.

Introduction

The re-epithelialization process begins few hours after the skin tissue is injured. In the case of re-epithelialization migration and proliferation of keratinocytes that occur forming back layer epidermis. During the re-epithelialization process, keratinocytes will be stimulated by growth factors, namely, transforming growth factor-β, epidermal growth factor (EGF), and EGF receptors (EGFR). Enzymatic factors that play a role are matrix metalloproteinases (MMPs) such as MMP1 and MMP10 and extracellular matrix, namely, laminin and collagen Types I and IV [1].

EGF is secreted by platelets, macrophages, and fibroblasts and these growth factors on keratinocytes function as paracrine. Research *in vitro* has shown that the EGF is upregulated after acute injury and significantly improve re-epithelialization and tensile strength in the wound. The mechanism of action of the EGF is to increase the expression of K6 and K16 keratin involved in the proliferative signal trajectory which will increase the proliferation and migration of keratinocytes. Other studies have shown that increasing EGF expression in the epithelium will increase the synthesis of EGFR, thus accelerating the proliferation signaling and migration of keratinocytes and accelerating wound healing and preventing scarring [2].

The EGFR a tyrosine kinase transmembrane protein found in all healthy human epidermis, although it is most abundant in the basal layer. *In vitro* studies have shown that activation of EGFR plays an important role in re-epithelialization. In EGFR, it is found that ligands are bound and will be synthesized in the form of anchored membranes, which are processed proteolytically into a soluble bioactive form. Ligands stored in EGFR are important for keratinocyte migration which will increase re-epithelialization during wound healing.

Wound healing, including burns, is greatly affected by good and proper handling. Handling of

deep burns has been done in various ways including skin grafting, administration of growth factors, and currently, the researcher's attention to the use of stem cells [3].

Human bone marrow mesenchymal stem cells (hBM-MSCs) are stem cells that are multipotent progenitor and can differentiate into chondrocytes, osteoblasts, adipocytes, myocytes, fibroblast, myofibroblast epithelial cells, endothelial, and neuronal cells [4] and release chemical mediators that are paracrine. This chemical mediator is very helpful in the proliferation, migration, and differentiation of cells that play a role in wound healing [5]. BM-MSCs have strong potential in skin tissue regeneration [6], [7], but research into the giving of BM-MSCs for skin wounds is currently in the research stage [8].

MSCs research has been conducted on a burn that is derived from human umbilical cord MSCs (hUC-MSCs) indicate that the hUC-MSCs can accelerate wound healing burn by increasing epithelialization and cannot cause infection. This epithelialization involves a matrix including type 1 collagen and MMP1. In the study, the effect of BM-MSCs in burns rat known that stem cells can significantly increase the expression of collagen type 1 and levels of MMP 1 compared to control [9], [10], however, other mechanisms for accelerating epithelialization of the skin tissue after molecular administration of BM-MSC needs to be carried out the research.

Based on the background above, a study was conducted on the effect of hBM-MSCs on the expression of growth factors that play a role in the process of re-epithelialization in the healing of burn experimental rat.

Materials and Methods

MSCs from hBM

hBM-MSCs used are from the Indonesian Medical Education and Research Institute, Faculty of Medicine, University of Indonesia. The use of hBM-MSCs for one rat in this study was 2×10^6 cells/ml.

Preparation of experimental animals (rat) for burns

The rat was anesthetized with xylazine and ketamine (1:1 ratio), then back hair was shaved. To make a full-thickness plate burn heated in boiling water for 30 min and placed on the back of the rat for 20 s. After the injection of phosphate-buffered saline (PBS) for the control group, and hBM-MSCs for the treatment group rat are given analgesics. On days 3, 7, and 14 rats were sacrificed by means of ether burn tissue taken to see the expression of EGF and EGFR output (R&D system) with immunohistochemical methods.

Immunohistochemical staining of EGF and EGFR

Deparaffinization by dipping the slide into the xylol liquid 3 times, each for 5 min, then being rehydrated begins by inserting the slide into the xylol 2 times each for 3–5 min and in sequence in absolute ethanol for 3 times each for 2 min, and 70% ethanol 2 times each for 2 min then rinse with aquadest 3 times and clean the edges of the slide with tissue. Slides were put into 3% H_2O_2 in methanol for 5 min then rinse with distilled water and PBS 3 times. The cleaned slides are put into anti-EGF and EGFR (mouse anti-rat 1:50) for 30 min at room temperature, then rinse with PBS 3 times each of 2 min. Mark around the pieces of tissue that you want to daub with a Pap pen. The slide is inserted into a secondary antibody (rabbit anti-mouse biotinylated antibody label) for 30 min, and then rinsed with PBS solution 3 times each for 2 min. Put the slides into the HRP streptavidin label for 30 min, then rinse in PBS solution 3 times, each for 2 min. Enter chromogen substrate for 3–10 min, and rinse with PBS solution 3 times, each for 2 min then rinses with Aquadest. Put in hematoxylin Mayer for 6–15 min then rinse with running water and mounting.

Research ethics

This research has been conducted clearance ethics and has been approved by the Committee of the Research Ethics of the Faculty of Medicine, Andalas University.

Data analysis

To analyze the effect of hBM-MSCs on the expression of internal revenue service EGF and EGFR values, an analysis using t-test was performed.

Results

Isolation results and determination of hBM-MSCs according to the markers tested are shown in Figure 1.

The difference EGF expression from each treatment is shown Table 1.

Table 1: EGF expressions by IRS scoring assessment in rat skin tissue burns after hBM-MSCs administration

Group	IRS scoring EGF expressions/day		
	3	7	14
Control	2.60	4.80	6.00
Treatment	4.60	6.80	9.00

hBM-MSCs: Human bone marrow mesenchymal stem cells, IRS: Internal revenue service, EGR: Epidermal growth factor.

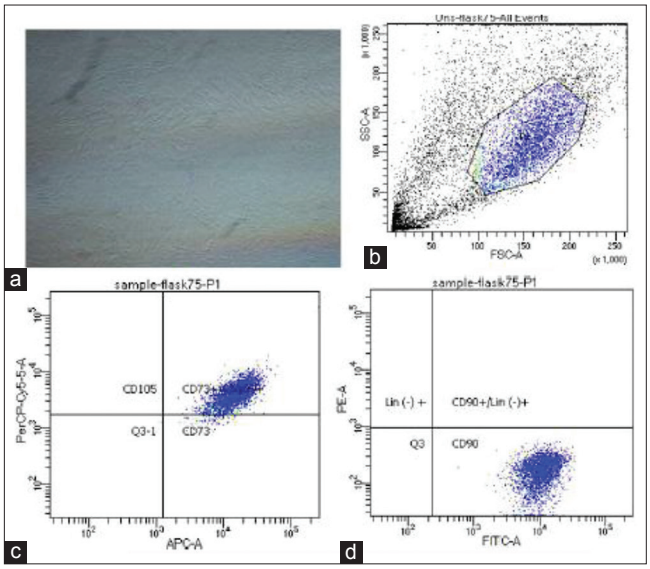

Figure 1: Data on characteristics of human bone marrow mesenchymal stem cells (hBM-MSCs) (a) Cells hBM-MSCs reach confluence. Scale bar: 500 µM. Photographs of cells taken using a Nikon Ti-S microscope. (b) Data flow cytometry. Forward scatter plot and side scatter plot. Population gated events (P1): 20,000. (c) Cell surface markers expression: CD73-APC 100% and CD105-PerCP-Cy5.5 96.8%. (d) Cell surface markers expression: CD90-FITC 100% and Lin (-) - PE 0.0%

Table 1 shows that the rats were given hBM-MSCs an increase in the expression of EGF started on days 3, 7 to 14 days compared with the control, showed a statistically significant difference (p = 0.000).

The results of the research giving hBM-MSCs to rat burn skin tissue found that stem cells can accelerate wound healing, including an increase in the process of re-epithelialization in rat skin tissue, thus accelerating the wound healing process; as shown in Figure 2.

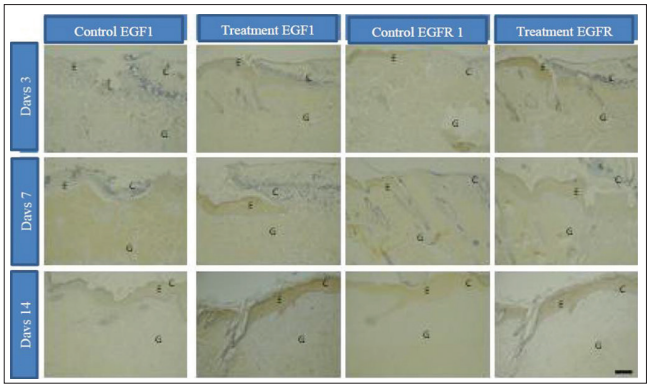

Figure 2: Increased epithelialization process in skin tissue burns in the rat in the control and treatment group given human bone marrow mesenchymal stem cells on days 3, 7, and 14 after burn. In the visible image area burns (C), normal skin, epithelialization (E), and granulation tissue (G) below area burn, scale bar 400 µm

Figure 2 shows that there is an improvement in each treatment. The difference EGF expression from each treatment is shown in Table 2.

Table 2 shows that the rats that were given hBM-MSCs increased expression of EGFR started at days 3, 7 to day 14 compared to control, and statistically showed a significant difference (p = 0.000).

Table 2: EGFR expression with IRS scoring assessment in rat skin tissue burn after hBM-MSCs administration

Group	IRS scoring EGFR expressions/day		
	3	7	14
Control	2.60	4.67	6.75
Treatment	6	7.2	9

IRS: Internal revenue service, EGFR: Epidermal growth factor receptors, hBM-MSCs: Human bone marrow mesenchymal stem cells.

Increasing the expression of EGF and EGFR for each group for immunohistochemical examination in burns injured skin tissue in mice is shown in Figure 3.

In Figure 3, it can be seen that in each treatment days there was an improvement in the burn.

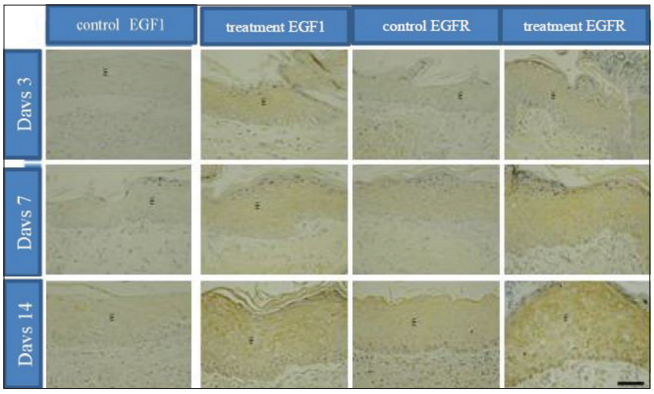

Figure 3: Epidermal growth factor and epidermal growth factor receptors expression with immunohistochemical staining in skin tissue rat burn in the control and treatment group treated with human bone marrow mesenchymal stem cells on days 3, 7, and 14 after burn, bar scale 200 µm

Discussion

The results of the study of injecting hBM-MSCs into the skin tissue of rat burns are known to accelerate wound healing with the good healing quality compared to control. This research is focused on looking at the effect of hBM-MSCs on growth factors, EGF and EGFR which play a role during the re-epithelialization process. The results showed that the expression of these two growth factors significantly increased (p = 0.05) after the administration of hBM-MSCs compared to controls in skin burns of experimental rats. Increased levels and expression of EGF after allogenic hBM-MSCs administration in incision wounds and excision in mice were also known to be an increase [11], [12].

An increase in these two growth factors would increase re-epithelialization during wound healing. EGF wound healing will affect the growth of cells around the wound, namely, epithelial cells, fibroblasts, and endothelial cells [13], so this growth factor will affect inflammation, re-epithelialization, and angiogenesis. EGF has a role to stimulate growth, proliferation, and differentiation of cells around the wound by binding to EGFR on the cell surface [14]. Stem cells are also known to be able to regenerate by way cells proliferate and differentiate.

In the research Tamama *et al*., EGF was shown to augment MSC proliferation while preserving early progenitors within MSC population. Tethered EGF can also be utilized to direct MSC toward osteogenic lineage both *in vitro* and *in vivo* [15]. Keratinocytes, a major cellular component of the epidermis, are responsible for restoring the epidermis after injury through a process termed epithelialization. Epithelialization is an essential component of wound healing used as a defining parameter of successful wound closure [1]. Transdifferentiation of hBM-MSCs into epidermal cells needed including keratinocytes has been widely demonstrated [16], [17], [18], [19] and keratinocytes formed in significant amounts many. Research on the re-epithelialization phase that has been done Lau, it is known that the hBM-MSCs can be found in the epidermis of rat, and here stem cells will differentiation into epithelial cells. Besides, that cell fusion can also occur between hBM-MSCs and epithelial cells by increasing epithelialization through paracrine signals; however, transdifferentiation is predominant in MSCs [20].

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