ORIGINAL ARTICLE

Does low intensity direct current affect open fracture wound healing?

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ABSTRACT

Aim To explore the effects of a molecular pathway from the application of low-intensity direct current (LIDC) for wound healing through the pathway signalling growth factor and initiation of fibroblast activation.

Methods This randomized clinical trial included 32 patients with open fracture wounds who came to Hasan Sadikin Hospital in Bandung, Indonesia. The patients were divided in the control and the treatment group. Extensive assessment of wound contractions, FGF2 and FGF7 levels, and fibroblast expression were evaluated before and after the treatment.

Results This study showed a better wound area repair in the treatment group than the standard group, 3.17±0.11 and 0.78±0.07, respectively. The increase of FGF-2 level-(42.69±3.5 and 15.09±1.8, respectively), FGF-7 level–(42.99±3.55 and 14.67±1.9, respectively), and fibroblast group expression (7.62±0.79 and 3.54±0.6, respectively) were found to be higher in the treatment group ($p < 0.05$).

Conclusion Low-intensity direct current accelerates wound healing through the increase of growth factor and fibroblast activation.

Key words: fibroblast, fibroblast growth factor 2, fibroblast growth factor 7, open fracture, wound healing

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INTRODUCTION

Open wounds that are common in cases of open fracture trauma are infection-prone conditions (1). Infection that occurs in these wounds will impact the elongation of the inflammatory phase and inhibit the wound healing process (2,3). The incidence of infection in open wounds due to open fracture trauma is quite high at around 42.6% (1,2). As a result of an infection that may occur in these open wounds causes slower wound healing, which will further increase the risk of further and more severe infection to the extent that delays in the management will cause sepsis and death in patients (3). This condition shows that it is essential to explore more comprehensive therapeutic modalities to accelerate the healing of open wounds in open fracture cases so that the severe effects of the infection can be prevented (1,3).

Current therapies only focus on wound cleansing, and broad-spectrum antibiotics, because the most common cause of the infection in open wounds is gram-positive and harmful bacteria, especially Staphylococcus aureus and Pseudomonas (3).

The steps of healing of infected wound can be classified into three stages: the inflammation phase, the phase of cell addition, and wound healing (4). The infection will prolong the inflammatory phase, and inhibit the transition into the cell addition phase, so that wound healing does not occur (3-5). In the inflammatory phase, macrophages and platelets release several important mediators or growth factors, including FGF-2 protein and FGF-7 protein (5,6). Both proteins play an essential role in the healing process of infected wounds since they have broad-spectrum mitogenic abilities and help regulate migration and target cell changes (7-9).

The proliferation of fibroblasts is essential in the process of wound healing (6,7). When granulation tissue forms in dermal wounds, platelets, monocytes, and other cellular blood elements release various growth factors to stimulate fibroblasts that will migrate to the wound area and proliferate, to repair various connective tissue components (10-12). Electromagnetic pulsation can help wound healing by enhancing new blood vessels, which will form new tissue to accelerate wound healing (8,9). Ways of inducing electric current for wound healing to date are four types: low-intensity direct current (LIDC), alternating current (AC), low voltage pulse current (LVPC) and transcutaneous electrical nerve stimulation (TENS) (10,12). The LIDC is preferred because it has a better effect than other electric currents, but until now there is no clear biomolecular response that can explain the mechanism of healing of infected wounds by using LIDC (13-16).

This study seeks to explore therapeutic modalities electric in the process of accelerating open fracture open wound healing, and the effects of a molecular pathway from the application of LIDC for wound healing through pathway signalling growth factors (FGF2 and FGF7) and initiation of fibroblast activation. This research is increasingly important because the exploration conducted in this study is in clinical trials in human subjects, where exploration is still minimal.

PATIENT AND METHODS

Patients and study design

This randomized clinical trial included 32 patients with open fracture wounds, who came to Hasan Sadikin Hospital in Bandung, Indonesia, during the period between June and November 2019.

Initial assessments by physicians had been carried out to determine the patients with fracture injuries.

Inclusion criteria for the patients were: over the age of 20 years, with infected wounds, had not received definitive therapy for open fracture management, and agreed to participate in the study by signing an informed consent. Exclusion criteria were: patients with severe chronic disorders such as diabetes mellitus, dyslipidaemia disorders, blood clotting disorders, immunocompromised disorders, and autoimmune disorders, and patients who are pregnant or breastfeeding.

The patients were randomly divided (without stratification) into two groups: a control group including the patients who had only got standard antibiotic therapy for open wound of open fracture, and a treatment group, i.e. patients who had got standard antibiotic therapy and LIDC application. Extensive assessment of wound contractions, FGF2 and FGF7 levels, and fibroblast expression was evaluated on day one and day 14.

The study was approved by the Hasan Sadikin Hospital Bandung Ethics Commission No. LB.02.01/X.6.5/189/2019.

Randomization was carried out using computer-generated random sequences to ensure equal allocation between the two groups carried out by the independent data centre's statistician at Hasan Sadikin Hospital in Bandung, Indonesia.

Methods

The antibiotic used in this study was cefazolin (the first-generation cephalosporin).

The LIDC is a method of electromagnetic application by providing electrical current to an area of 500uA using electrical stimulation ITO 320, which is applied to both electrodes continuously within 2 hours using a DC flow battery (10,11)

Wound contraction rate. Aa assessment of rate of wound contraction was done by the Gillman procedure (17), where wound area was measured by a digital calliper (Krisbow, Jakarta, Indonesia) so that the area of the wound was obtained in mm2. Measurements were made every day of observation.

Levels of growth factors. The assessment of FGF2 and FGF7 growth factor levels was carried out using the ELISA (Enzyme-Linked-Immunosorbent Assay) sandwich method according to the procedures contained in the FGF 2 and FGF 7 Human ELISA Kit (Cloudclone, Hangzhou, China) manuals. Briefly, 50 µL of standard diluent or serum samples were added to wells that have been coated with anti-serotonin and incubated at 37 °C for 30 minutes. After the plates were washed, 100 µL of biotinylated antibody solution was added and incubated for 30 minutes at 37 °C. After three times washing, 50 uL avidin-peroxidase complex solution was added and incubated for 15 minutes at 37 °C. Next step, 50 µL of tetramethylbenzidine colour solution was added and incubated in the dark for 15 minutes at 37 °C. Finally, 50 uL stop solutions were added to stop the reaction. Optical density (OD) values were measured using an ELISA reader (Biorad, Singapore), wavelength 450 nm.

Fibroblast expression. Wound tissue samples were inserted into the next fixation fluid, dehydrated using alcohol and xylene, then paraffinized and cut as thick as 5 um using a rotary microtome (Leica, Illinois, USA). The following tissue was placed on the coated-object glass. Then, rehydration was carried out on the tissue using xylene and alcohol with a concentration of 96%, 90%, 80%, and 70% and rinsed with tap water. In the next stage, retrieval antigen was carried out with the HIER (Heat-Induced Epitope Retrieval) method (18), where the slides were put into a citrate buffer solution, then heated at the temperature of 95 ^{o}C for 60 minutes. Then, 1:700 (Cloud Clone, Hangzhou, China) fibronectin antibody was painted, followed by overnight incubation at 4^{o}C. The next stage was to paint with a secondary antibody, Biotinylated-HRP (Horseradish Peroxidase), incubation for 1 hour, at room temperature. Next, the administration of the chromogen was carried out. Next, the dehydration process was carried out again, using concentration and xylene alcohol. Furthermore, mounting and evaluating fibronectin expression using ImageJ Software, the percentage of fibronectin expression as a fibroblast marker would be obtained.

Statistical analysis

Data processing began with the process of inputting, editing, clearing, and coding. First, a descriptive and univariate analysis was performed, followed by a bivariate analysis to see differences in the average growth factor levels, differences in the average expression of fibroblasts, and the average extent of wound contractions. Bivariate analysis was performed using the T-test, with p=0.05.

RESULTS

This randomized clinical trial including 32 patients with open fracture wounds and dividing into the control group (the patients who only received standard antibiotic therapy for open wound of open fracture), and the treatment group (patients who received standard antibiotic therapy with LIDC application) were evaluated for wound contractions, FGF2 and FGF7 levels, and fibroblast expression on day one and day 14.

The wound contraction area was more extensive in the treatment group than in the control group (3.17±0.11 and 0.78±0.07, respectively) ($p<0.05$). The addition of the area of wound contraction in the treatment group was significantly different compared to the control group ($p<0.05$) (Table 1).

The FGF-2, FGF-7 level, as well as fibroblast expression, all were significantly increased after the treatment in the treatment group comparing to the control group: for 42.69±3.5 and 15.09±1.8

pg/mL ($p<0.05$), 42.99±3.55 and 14.67±1.9 pg/mL ($p<0.05$), and 7.62±0.79 and 3.54±0.6 mm^2 ($p<0.05$), respectively (Table 1).

Table 1. Comparison of wound contraction area, FGF-2, FGF-7, fibroblast expression before and after the treatment

Variable	Control group		Treatment group	
	Before treatment	After treatment	Before treatment	After treatment
Contraction area SD (mm^2)	0.430.02	1.210.09	0.450.01	3.620.12
FGF-2 SD (pg/mL)	16.3482	31.4362	16.4372	59.1222
FGF-7 SD (pg/mL)	17.6582	32.3272	17.2244	60.2199
Fibroblast expression SD (%)	3.1122	7.6582	3.2523	10.8702

FGF: Fibroblast Growth Factor

DISCUSSION

This study shows that the application of LIDC in open fracture open wounds had a very positive impact on the wound healing process. The LIDC application can increase wound healing acceleration through increased production of growth factors, FGF-2, and FGF-7. The fibroblast activation process begins with an increase in growth factor production, which then spurs an increase in collagen production. This collagen will later act as a wound covering by forming a layer of web and thread on the damage (19).

The use of LIDC to initiate growth factor production is an exciting subject to study further. Some studies show that the use of electrical devices can influence the electronegativity of cells (20-23). Changes in electronegativity in cells will stimulate the release of sodium ions to extracellular in the epidermis via the Na / K-ATPase pump. The epidermis located on the central wound's edge becomes an electropositive area, while the central wound area is electronegative. This condition will trigger the traction contraction force between the wound's edge with the central wound, which ultimately accelerates wound closure (23-24).

The use of the LIDC application is also believed to increase blood flow to the injured area through the effect of heat regulation on the skin around the wound (23). Adequate blood flow will accelerate the inflammatory process in the wound area by accelerating the migration of various inflammatory cells; both neutrophils and macrophages will produce various growth factors, including FGF (23). Increased levels of growth factors will initiate the process of vascularity and angiogenesis. The angiogenesis process will be followed by the activation of fibroblasts, where fibroblasts are precursor cells that play an essential role in producing collagen. Collagen produced by fibroblasts will act as the primary substance that plays a role in wound closure (25).

However, this study has limitations because it used a minimal number of samples.

In conclusion, low-intensity direct current was effective to accelerate wound healing through increased growth factor and fibroblast activation. Further research needs to be done with a larger sample, so it is expected that low-intensity direct current can be used as an additional therapy for wound healing.

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TRANSPARENCY DECLARATION

Conflict of interest: None to declare.

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