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Original Article

EXTREMELY LOW-FREQUENCY-PULSED ELECTROMAGNETIC FIELD EXPOSURE IN THE HEALING PROCESS OF SPRAGUE-DAWLEY RATS WITH DELAYED-UNION FEMUR FRACTURE: A STUDY OF THE FAILURE LOAD OF AXIAL FORCE

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ABSTRACT

Objective: Under normal conditions, fractures can heal, but under some conditions, complications can occur, such as delayed union or nonunion. Interaction between the processes of angiogenesis and osteogenesis (the interaction of osteoblast and osteoclast) is the determining factor in the healing process. Exposure to an electromagnetic field, as a physical stimulus, affects osteogenesis both in the developmental stage of the embryo and in the fracture healing process. This study was conducted to determine the healing of delayed-union fractures through exposure to an extremely low-frequency-pulsed electromagnetic field (ELF-PEMF), comparing the failure load scores in experimental animals.

Methods: The study was conducted in the Faculty of Medicine, Universitas Indonesia, with 56 experimental rats during August and September 2018.

Results: There was a significant difference in the failure load score in both groups in the fourth and fifth weeks of the study. There were no differences in clinical improvement in the two groups.

Conclusion: This study concluded that there was an improvement in delayed-union fracture healing after the administration of ELF-PEMF, as seen in the difference in failure load scores.

Keywords: Fracture, Delayed union, Electromagnetic field

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INTRODUCTION

Fracture is among the most common causes of injury in traffic accidents, mostly occurring in productive ages [1-3]. Generally, a person has a 50% risk of a fracture during his or her lifetime [4]. About 9.1% of fractures in Indonesia are caused by traffic accidents [1]. Usually, fractures heal normally, but some pathological healing processes can occur, such as nonunion or delayed union. Previous studies [5] have shown that the prevalence of nonunion and delayed-union fractures was 2.5% and 4.4%, respectively. In cases of open tibial fractures, a delayed union can occur in 31% of cases [5]. Furthermore, an abnormal healing process can cause various long-term problems, such as joint arthritis, decreased joint mobility, immobilization, prolonged treatment, and decreased quality of life in patients.

The diamond concept shows that the healing process comprises osteogenic (cell) components, osteoconductive components (matrix, scaffold), osteoinductive components (growth factors), a stable mechanical/fixation environment, and vascularization [6, 7]. Each component relates to the others, and a deficiency in one component can disrupt bone healing and cause a delayed union, even nonunion.

Studies of biophysical stimulation, including mechanical, ultrasonic, electrical, and electromagnetic stimulations, show some improvement in the fracture healing process. Even though the mechanism is not yet fully known, electromagnetic field (EMF) stimulation increases the expression of osteogenic genes [9, 10]. Bone piezoelectricity or the bio-electric-mechanical phenomena explains this. Mechanical stress on the bone will produce an endogenous electric field in the bone, and collagen, an extracellular component of bone, acts as a transducer, transforming mechanical energy into electricity. This endogenous electric field influences cell proliferation and vascular invasion, facilitates classification, lowers oxygen pressure, increases pH, changes the cyclic activity of Adenosine Monophosphate (AMP), and promotes the osteogenic process [11]. All these conditions primarily occur in two main signaling pathways: wingless-int (Wnt) and bone morphogenetic protein (BMP) [12].

Some previous studies have shown the effect of EMF exposure on the healing process of a bone fracture. A study conducted in 2014 found that 28 d of EMF exposure combined with BMP-2 exposure in cultured cells significantly increased alkaline phosphate activity and accelerated the calcium deposit process, both of which are markers of osteogenesis [13, 14]. Another study [15] has shown that patients with a delayed-union long-bone fracture experienced superior clinical improvement in the first three months when receiving EMF stimulation (38.7% compared to 22.2% for those who did not receive EMF stimulation). At the end of treatment, EMF exposure provided a faster recovery rate (77.4% compared to 48.1% for those who did not receive EMF stimulation) [15].

The objective of this study was to determine the effect of extremely low-frequency-pulsed electromagnetic field (ELF-PEMF) exposure on delayed-union bone-fracture healing. This was done by measuring the strength of the callus formed in the fracture site. The strength of the callus was determined by the load failure score of the axial force measured in Newtons (N).

MATERIALS AND METHODS

An experimental study was conducted with 56 healthy male Sprague-Dawley rats weighting 250-300 g, randomly organized into two groups. The study was conducted for five weeks. In the second to fifth weeks, seven rats in each group were sacrificed for examination. The protocol of the study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia–Cipto Mangunkusumo Hospital.

In each animal, a fracture was made and then fixed with intramedullary K-wire. A delayed-union healing model was created with the circular periosteal stripping method, 5 mm proximal and distal from the fracture line. [16] During surgery, each rat received anesthesia with an intraperitoneal injection of ketamine, 80 mg/kgBB (Ilium Ketamil Injection®, Troy Laboratories, Pty. Ltd., Australia), and Xylazine, 10 mg/kgBB (Ilium Xylazil-100 Injection®, Troy Laboratories, Pty. Ltd., Australia). Both groups were kept in the Animal Laboratory of Research and Development, Indonesian Ministry of Health.

In the intervention group, electromagnetic fields were provided at an intensity of 4 h/day. During the second, third, fourth, and fifth weeks, the subjects were sacrificed with 75 mm/kgBB phenobarbital intraperitoneally. Each week, seven subjects were sacrificed in each group. Later, the femur was cleaned from the surrounding muscle tissue, leaving the soft tissue around the fracture area. Specimens were stored in rectangular container made from plastic. The failure load score was examined with an axial force test (Geotech Al-7000S/2014-02960, Taiwan) with a minimum force of 10 N. The examination was done in Puspitek Bogor, Indonesia. The result of this examination was a graph with a failure load score for each femur. Statistical assessment was performed with IBM SPSS ver. 24, analyzing the test with one-way ANOVA for data with normal distribution and a Kruskal–Wallis test for data with an abnormal distribution. If significance occurred in the one-way ANOVA test, then a post hoc analysis was performed to assess the comparison between groups.

RESULTS

The mean weight of subjects at the beginning of the experiment was 269.70 g. In the t-test analysis for unpaired samples, there were no differences in the characteristics of the experimental animals' weight, femur weight, and femur length in the treatment and control groups. The characteristics of the experimental animals are illustrated in table 1.

Table 1: Characteristics of subjects

Week	Variables	Intervention n = 7	Control n = 7	p value
1	Weight (g)	286.04±41.72	265.5±25.58	0.389
2	Weight (g)	246.57±23.16	265.43±39.30	0.296
	Femur weight (g)	1.21±0.12	1.14±0.36	0.625
	Femur length (cm)	34.06±1.48	35.57±1.52	0.085
3	Weight (g)	247.14±21.97	270.71±29.14	0.113
	Femur weight (g)	1.50±0.25	1.46±0.26	0.793
	Femur length (cm)	33.54±1.21	34.67±1.38	0.128
4	Weight (g)	283.29±40.99	269.00±13.59	0.399
	Femur weight (g)	1.83±0.25	1.68±0.29	0.315
	Femur length (cm)	34.71±1.99	36.26±1.12	0.099
5	Weight (g)	297.14±27.18	280.00±28.08	0.628
	Femur weight (g)	1.70±0.39	1.85±0.08	0.186
	Femur length (cm)	37.49±2.32	36.22±1.49	0.245

A failure load score examination was done to define the rigidity of the bone after the healing process for a delayed-union fracture. The stroke score is defined as a shift in the bone before deformity. We found that there was no difference between the stroke scores in both groups, as shown in table 2. We also found that there were significant differences in the load failure score in the fourth and fifth weeks. Tukey post hoc analysis showed a statistically significant failure load score in each week. We also found that there was no difference in clinical improvement via inspection by the researcher.

Table 2: Failure load score and stroke in both groups

Week	Variables	Intervention n = 7	Control n = 7	p value*
2	Load Score (N)	31.99±6.41	24.89±7.61	0.083
	Stroke (mm)	1.66±0.24	1.45±0.32	0.207
3	Load Score (N)	61.95±22.06	59.42±16.48	0.812
	Stroke (mm)	1.64±0.19	1.52±0.21	0.287
4	Load Score (N)	217.09±20.05	176.43±31.48	0.014
	Stroke (mm)	1.61±0.18	1.72±0.17	0.260
5	Load Score (N)	311.88±21.27	263.26±20.55	0.001
	Stroke (mm)	1.25±0.17	1.23±0.13	0.793

*independent t-test

Table 3: Clinical improvement in both group

Week	Variables	Intervention	Control	p value*
2	Clinically united	1	0	1
	No	6	7	
3	Clinical union	5	4	1
	No	2	3	
4	Clinical union	7	5	0.462
	No	0	2	
5	Clinical union	7	7	N/A
	No	0	0	

*Fisher test

DISCUSSION

An experimental study with 56 healthy male Sprague-Dawley rats, aged 3-4 mo, was conducted for five weeks. During the study, no animals had an infection in the trauma area, experienced implant protrusion, or died. There was no difference in subject weight at the beginning of the trial in both groups. Weight control was one of the biases controlled in this experiment. There were no differences in femur weight and length in either group.

The bone healing process involves bone cortex, periosteum, connective tissue, and bone marrow. The process begins with a chondrogenesis from days 7-10. Later, on day 14, cartilage calcification begins, and bone formation occurs under the periosteum. In the third week, calcified cartilage begins to form callus, which is then degraded by chondroblast to be replaced by bone. The calcified trabeculae of bone becomes more prominent on the fourth and fifth weeks [17].

The failure load score relates to the rigidity and calcification on the callus to deal with a given axial force. We found that there was a significant difference between the failure load score on the fourth and fifth weeks, even though there were no stroke differences in both groups. Turner *et al.* [18] stated that fracture's occurence relates to the stiffness and calcification of the bone. When the axial force was over the capacity of the bone to absorb the force, the bone was fracture. The rigidity of the bone relates to bone mineral density. The more flexible the bone is, the less rigid it becomes [18, 19].

Electric and electromagnetic fields stimulate the bone mechanically. They change the force gradient, causing the interstitial fluid to move through canaliculi. This process increases the osteocytes. Various *in vitro* studies show a stimulation of cell proliferation, increased extracellular matrix synthesis, and calcification after exposure to an electric field. Primarily, electromagnetic stimulation influences the osteoblast and periosteal cells. This stimulation also increases bone strength and synthesis of prostaglandin and collagen. Furthermore, electromagnetic field stimulation causes early cartilage formation and an increase in the number of chondrocytes [20, 21].

Various studies support the results of this experiment. One study found that PEMF exposure in patients with delayed union of long-bone fractures had better fracture healing (77.4%) than the control had (48.1%) ($p = 0.029$) [22]. Other studies support this finding [9-11]. A later study, conducted in 2012, also found that PEMF stimulation causes perfect bone healing in 77.3% of cases with delayed-union and nonunion tibial fractures [23]. It was elsewhere shown that PEMF stimulation causes significant nonunion bone healing, with a cure rate of 81% [24]. Finally, a meta-analysis determined that significant differences in fracture healing occurred in a group stimulated by electromagnetic waves when compared to a group not stimulated by electromagnetic waves [21].

CONCLUSION

Exposure to ELF-PEMF in experimental animals with delayed-union fractures can accelerate the process of bone healing, based on a comparison with a control group. Even though the failure load scores were different in the fourth and fifth weeks, the stimulation of ELF-PEMF increased the bone healing of a fracture in delayed-union cases.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

All authors have none to declare

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