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/fixed 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 bioelectric-mechanical phenomena explains this. Mechanical stress on the bone will produce an endogenous electric field in 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 screw 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 (Biun Ketamin 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 Hz/day. During the second, third, fourth, and fifth weeks, the group received ELF-PEMF stimulation, consisting of a low-frequency-pulsed electromagnetic field (ELF-PEMF), comparing the failure load scores in experimental animals.
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.

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 1: Characteristics of subjects

| Week | Variables   | Intervention n = 7 | Control n = 7 | p value* |
|------|-------------|-------------------|---------------|----------|
| 1    | Weight (g)  | 268.0±41.72       | 265.5±25.58   | 0.389    |
| 2    | Weight (g)  | 246.5±72.31       | 265.4±39.30   | 0.296    |
|      | Femur weight (g) | 1.2±0.12     | 1.1±0.36      | 0.625    |
|      | Femur length (cm) | 34.06±1.48   | 35.7±1.52     | 0.085    |
| 3    | Weight (g)  | 247.1±21.97      | 270.7±29.14   | 0.113    |
|      | Femur weight (g) | 1.5±0.25     | 1.4±0.26      | 0.793    |
|      | Femur length (cm) | 33.5±1.21   | 34.6±1.38     | 0.128    |
| 4    | Weight (g)  | 283.2±40.99      | 269.0±13.59   | 0.399    |
|      | Femur weight (g) | 1.8±0.25     | 1.6±0.29      | 0.315    |
|      | Femur length (cm) | 34.7±1.99    | 36.2±1.12     | 0.099    |
| 5    | Weight (g)  | 297.1±27.18      | 280.0±28.08   | 0.628    |
|      | Femur weight (g) | 1.7±0.39     | 1.8±0.08      | 0.186    |
|      | Femur length (cm) | 37.4±2.32    | 36.2±1.49     | 0.245    |

*Independent t-test

Table 2: Failure load score and stroke in both groups

| Week | Variables   | Intervention n = 7 | Control n = 7 | p value* |
|------|-------------|-------------------|---------------|----------|
| 2    | Load Score (N) | 31.9±6.41     | 24.8±7.61     | 0.083    |
|      | Stroke (mm)  | 1.66±0.24       | 1.45±0.32     | 0.207    |
| 3    | Load Score (N) | 61.9±22.06    | 59.4±16.48    | 0.812    |
|      | Stroke (mm)  | 1.6±0.19        | 1.5±0.21      | 0.287    |
| 4    | Load Score (N) | 217.0±20.05  | 176.4±31.48   | 0.014    |
|      | Stroke (mm)  | 1.6±0.18        | 1.7±0.17      | 0.260    |
| 5    | Load Score (N) | 311.8±21.27  | 263.2±20.55   | 0.001    |
|      | Stroke (mm)  | 1.25±0.17       | 1.23±0.13     | 0.793    |

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          | 3            | 3       |          |
| 4    | Clinical union | 7           | 5       | 0.462    |
|      | No          | 2            | 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
peristeum. 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 occurrence 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.

ACKNOWLEDGEMENT

This article was presented at The 3rd International Conference and Exhibition on Indonesian Medical Education and Research Institute (ICE on IMERI 2018), Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. The study was supported by Hibah PITTA UI 2018. We thank the 3rd ICE on IMERI Committee who had supported the peer review and manuscript preparation before submitting to the journal.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

All authors have none to declare

REFERENCES

1. Riyadina W, Suhardi, Permana M. Pola dan determinan sosiodemografi cedera akibat kecelakaan lalu lintas di Indonesia [Sociodemographic patterns and determinants of injuries from traffic accidents in Indonesia]. Maj Kejakatan Indon 2009;59:464-72.

2. Singer BR, McLauchan GJ, Robinson CM, Christie J. Epidemiology fractures in 15,000 adults: the influence of age and gender. J Bone Joint Surg (Br) 1998;80:243-8.

3. Ganveer GB, Tiwari RR. Injury pattern among non-fatal road traffic accident cases: a cross-sectional study in Central India. Indian J Med Sci 2005;59: 9-12.

4. Brinker MR, O’Connor DP. The incidence of fractures and dislocations referred for orthopaedic services in a capitatted population. J Bone Joint Surg Am 2004;86A:250-7.

5. Phieffer LS, Goulet JA. Delayed unions of the tibia. Instr Course Lect 2006;55:389-401.

6. Giannoudis PV, Einhorn TA, Marsh D. Fracture healing: the diamond concept. Injury 2007;38(Suppl 4):S3-6.

7. Giannoudis PV, Einhorn TA, Schmidmaier D, Marsh D. The diamond concept-open questions. Injury 2008;39(Suppl 2):S5-8.

8. Lu C, Midlau T, Hu D, Marcucio RS. Ischemia leads to delayed union during fracture healing: a mouse model. J Orthop Res 2007;25:51-61.

9. Tewen CM, Greives M, Natale RB, Su Y, Luo Q, He BC, et al. Differentiation of osteoprogenitor cells is induced by high frequency pulsed electromagnetic field. J Craniofac Surg 2012;23:586-93.

10. Einhorn TA, Gerstenfeld LC. Fracture healing: mechanism and interventions. Nat Rev Rheumatol 2015;11:45-54.

11. Bilezikian J, Lawrence GR, Rodan GA, editors. Principles of bone biology. 2nd ed. Portland: Academic Press; 1996.

12. Marcelini S, Henriquez JP, Bertin A. Control of osteogenesis by the canonical Wnt and BMP pathway in vivo. Bioessays 2012;34:953-62.

13. Ongaro A, Pelliati A, Bagheri L, Fortini C, Setti S, De Mattei M. Pulsed electromagnetic fields stimulate osteogenic differentiation in human bone marrow and adipose tissue derived mesenchymal stem cells. Bioelectromagnetics 2014;35:426-36.

14. Yu FC, Lin CC, Chang JK, Chen CH, Tai IC, Wang JW, et al. A novel single pulsed electromagnetic field stimulates osteogenesis in bone marrow mesenchymal stem cells and bone repair. PLoS One 2014;9:e91581.

15. Shirley D, Marsh D, Jordan G, McQuaid S, Li G. Systemic recruitment of osteoblastic cells in fracture healing. J Orthop Res 2005;23:1012-21.

16. Utvåg SE, Grundnæs D, Reikeras O. Effects of periosteal stripping on healing of segmental fractures in rats. J Orthop Trauma 1996;10:279-84.

17. Mooney MP, Siegel MI. Animal models for bone tissue engineering of critical-sized defects (CSDs), bone pathologies, and orthopedic disease states. In: HoBinge J0, Einhorn TA, Doll BA, Sfeir C, editors. Bone tissue engineering. Boca Raton: CRC Press; 2005. p. 217-44.

18. Turner CH. Bone strength: current concepts. Ann NY Acad Sci 2006;1068:249-6.

19. Einhorn TA. The science of fracture healing. J Orthop Trauma 2005;19:94-6.

20. Victoria G, Petrisor B, Drew B, Dick D. Bone stimulation for fracture healing: what’s all the fuss? Indian J Orthop 2009;43:117-21.

21. Aaron RK, Combror DM, Simon BJ. Treatment of nonunions with electric and electromagnetic fields. Clin Orthop Relat Res 2004;419:21-9.

22. Shi HF, Xiong J, Chen YX, Wang FJ, Qiu XS, Wang YH, et al. Early application of pulsed electromagnetic field in the treatment of postoperative delayed union of long-bone fractures: a prospective randomized controlled study. BMC Musculoskeletal Disord 2013;14:35.

23. Assiotis A, Sachinis NP, Chalidis BE. Pulsed electromagnetic fields for the treatment of tibial delayed unions and nonunions. A prospective clinical study and review of the literature. J Orthop Surg Res 2012;7:24.

24. Gossling HR, Bernstein RA, Abbott J. Treatment of united tibial fractures: a comparison of surgery and pulsed electromagnetic fields (PEMF). Orthopedics 1992;15:711-9.