Effect of electroacupuncture on the healing process of tibia fracture in a rat model: a randomised controlled trial

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ABSTRACT
Background Electrical stimulation is used to promote bone reunion, and is most effective when applied directly to the fracture site.
Objective To examine the effects of electroacupuncture (EA) on the healing process of tibia fracture in a rat model.
Methods Thirty 12-week-old male Wistar rats underwent unilateral open osteotomies of the tibiae. The rats were then assigned randomly to three groups: EA group (n=10), sham group (n=10) and control group (n=10). In the EA group, a cathodal electrode was connected to an acupuncture needle percutaneously penetrated directly at the surgery site, while an acupuncture needle inserted at 15 mm proximal to the surgery site was used as an anodal electrode. EA (50 Hz, 20 μA, 20 min) was performed daily for 3 weeks. In the sham group the acupuncture needles were inserted at the same sites but no electrical stimulation was given and in the control group, no treatment was given. The response was evaluated at 1, 3, 4 and 6 weeks after surgery by radiographic, macroscopic and biomechanical examinations.
Results The EA group showed accelerated bone healing (EA group 29.92±4.55 mm², sham group 26.46±5.21 mm², control group 26.19±2.81 mm², p<0.05 at 3 weeks) and accretion of the callus (radiographic evaluation: EA group 35.66±4.37 mm², sham group 32.60±5.73 mm², control group 29.72±6.39 mm², p<0.05 at 6 weeks) compared with the other groups. Mechanical testing also showed an excellent result (EA group 16.54±9.92 N, sham group 7.13±3.57 N, control group 6.67±3.12 N, p<0.05) at 6 weeks in the EA group compared with the other groups. There was no difference between the sham and control groups in any evaluation.
Conclusion The use of EA enhanced callus development and bone mineralisation during the bone healing process.

INTRODUCTION
Since Yasuda discovered in 1953 that new bone formation occurs on the cathode electrode side when direct current stimulation is applied to the femurs of rabbits,1 a great number of experiments on callus formation by microelectrical stimulation have been reported. Following this experimental technique, an invasive method of embedding electrodes has been used in clinical practice.2 3 However, the invasive technique required for embedding/removing electrodes makes this technique inconvenient and risky; therefore, in recent years, non-invasive methods such as low-intensity pulsed ultrasound (LIPUS) and pulsed electromagnetic fields have been developed and their effectiveness has been reported.4 5 Yet even with these methods, applying stimulation only to a local fracture site is challenging, and ectopic ossification may occur because of stimulation applied to a wide region of the body. More effective results are obtained when electrical stimulation is applied directly to the fracture site,7 therefore selection of the stimulation site is important in promoting fracture healing. Thus, clearly, establishing a method of bone healing in which stimulation can be applied less invasively to a limited location would allow a wider application of electrical stimulation. The use of acupuncture needles as electrodes eliminates the invasive procedure and allows selective stimulation to deeper regions, making simple, quick and frequent applications possible. The use of needles allows flexibility in selecting a stimulation site. In this study, we investigated the effect of low-frequency electroacupuncture (EA) on the healing process using a rat model of tibia fracture and evaluated the results by radiographic, macroscopic and biomechanical examinations.

This study was conducted with the approval (authorisation No 19–32) of the ethics committee of Meiji University of Integrative Medicine. In previous studies of electrical stimulation applied to fracture models, the relationship between the extent of fracture healing and the location of the embedded electrodes was examined; better results were reported when the cathode was placed in the fracture site.8 In a report studying the effects of EA on peripheral nerve regeneration, direct-current EA with the cathode at a location distal to the injury...
site showed better results, possibly because Ca\(^{2+}\) entry into the cathode may have suppressed die-back.\(^5\) The optimum electrical current stimulation has been reported to be about 20 μA,\(^6\) and LIPUS used in clinical practice has produced effective results with 20 min applications per day.\(^7,8\) In our study, we set our stimulation conditions based on these reports.

**METHODS**

A single-level blocked randomised controlled trial was used in this study. It was not possible to blind the observers. A total of 6 weeks of research period was scheduled, in which EA and sham stimulation were performed daily for 3 weeks starting from the day after surgery, and observations were made over the following 3 weeks.

**Animals**

Thirty Wistar rats (12-week-old male weighing 250–300 g) were used as experimental animals. Under anaesthesia with pentobarbital (50 mg/kg) administered intraperitoneally, a right hind leg of each rat was shaved and sterilised with povidone–iodine solution. An incision of about 3 cm was made at the anterolateral part under the knee in the vertical direction to expose the tibia. After inserting a 22G needle (0.70×38 mm; Terumo, Tokyo, Japan) from under the patella tendon into the intramedullary canal and pulling it out, the tibia was transversely fractured at 17 mm above the lateral malleolus with a ring cutter. At the same time, the fibula that would affect the healing of the fractured tibia was manually fractured for uniform conditions. After cleaning and reducing the fracture, a 21G needle (0.80×38 mm; Terumo) was inserted and fixed at 3 cm into the intramedullary canal to create an intramedullary-nailing model. The wound was closed with a surgical suture (GA03SW; Alfresa Pharma, Osaka, Japan), cleaned with povidone–iodine solution and then soft x-ray examinations (SOFTEXCMB-2; Softex, Kanagawa, Japan) were carried out.

For 3 days after surgery, antibiotics (enrofloxacin; Bayer Medical, Osaka, Japan) were given, and the surgical wound was cleaned with povidone–iodine solution every other day for 3 weeks after surgery. Meanwhile, the rats were allowed to eat, drink and move freely inside the optimally conditioned cage, except during examinations. After surgery, the rats were randomly assigned to three groups.

**Experimental groups**

**Electroacupuncture group (n=10)**

Intermittent direct current EA was performed by using a needle inserted at the fracture site as a cathode and another at a site 15 mm proximal to the fracture site as an anode. For each electrode, a needle (30 mm in length, No 24, stainless steel; Seirin, Shizuoka, Japan) was percutaneously inserted until the tip touched the periosteum. Using an electric stimulator (SEN-3301; Nihon Kohden) and an isolator (SS-104J; Nihon Kohden, Tokyo, Japan), 5 ms square pulses of 50 Hz were delivered at 20 μA for 20 min. The rats were anaesthetised with diethyl ether (Wako Pure Chemical Industries, Osaka, Japan) and their extremities were constrained for EA treatment.

**Sham group (n=10)**

After surgery, the rats were constrained, anaesthetised with diethyl ether and needles were inserted into the same sites with the same depths as the EA group, but no intermittent direct current EA was used.

**Control group (n=10)**

After surgery, no treatment was given.

**Assessment**

**Radiographic evaluation**

Since the tibia and fibula overlapped in the anteroposterior view, the shape of the tibia could not be accurately evaluated, so the lateral view was used for evaluation. On the day the fracture was created and 1, 3, 4 and 6 weeks thereafter, the rats were anaesthetised with pentobarbital (50 mg/kg) administered intraperitoneally and radiographs were taken. Then the developed images were scanned and transferred to a PC. Image analysis software ImageJ\(^9\) was used to measure the total area of the callus and bone of the tibia within the 10×10 mm\(^2\) region of interest around the fracture site.

**Macroscopic evaluation**

Six weeks after surgery, all the rats were killed by administering an overdose of pentobarbital. The right tibia was removed from each rat, and the longitudinal diameter and transverse diameter of the most expanded region of the tibia, which seems to be the subject area of the callus formed around the fracture site, were measured using a digital vernier caliper. After measurements, the tibiae were promptly kept moist with saline and frozen at −20°C for mechanical evaluation, according to the method used by Sedlin and Hirsch.\(^10\)

**Mechanical evaluation**

At 6 weeks after surgery, the strength of the callus was measured as the maximum load at failure using the three-point bending test. (Static material testing machine, EZ Graph; SHIMADZU, Kyoto, Japan was used for testing.) For testing, the stress applied was 0–40 N, the strain was maintained at a rate of 0.5 mm/min and the distance between the supports was 15 mm. Before testing, the intramedullary nail used for fixation was removed carefully with pincers from the insertion site so as not to damage the callus.

**Statistical analysis**

All data are presented as a mean±SD.

For all parameters assessed (longitudinal and transverse diameters of the tibia, total area of the callus and bone of the tibia at 3, 4 and 6 weeks after surgery and the maximum load at failure), data were compared among the EA, sham and control groups using one-way analysis of variance followed by the Bonferroni/Dunn method for multiple comparisons. In addition, the changes over time in the total area of the callus and bone of the tibia of the three
groups were assessed using repeated measures analysis of variance. Statistical analyses were performed using the Statview version 4.5 (SAS Institute, Japan) and data were considered significant when the p value was <0.05.

RESULTS
No adverse events such as infection were detected throughout the course of this study.

Radiographic evaluation
The change over time in the total area of the callus and bone of the tibia was measured for the three groups, and a significant increase was seen in the EA group. In comparison with the control group, the sham group showed a trend towards a slight increase, but statistically there was no significant difference between the two. The total area of the callus and bone of the tibia at 3 weeks after surgery was 29.92±4.55 (mm²) in the EA group, 26.46±5.21 in the sham group and 26.19±2.81 in the control group. At 4 weeks after surgery, the total area was 35.60±4.25 in the EA group, 29.30±6.02 in the sham group and 27.50±4.22 in the control group. At 6 weeks, the total area was 35.66±4.37 in the EA group, 32.60±5.73 in the sham group and 29.72±6.39 in the control group, respectively. Thus, at 3, 4 and 6 weeks after surgery, there was a significant difference (p<0.05) between the EA group and the sham or control group, and a significant increase was seen in the EA group. No significant difference was seen between the sham and control groups (figure 1).

Macroscopic evaluation
Figure 2A shows the longitudinal diameter and figure 2B the transverse diameter of the callus at 6 weeks after surgery based on macroscopic examination. The longitudinal diameter was 5.05±0.70 (mm) in the EA group and a significant difference (p<0.05) was seen compared with 4.24±0.52 in the sham group and 4.27±0.84 in the control group, respectively. Also, in the transverse diameter measurements, there was a significant difference (p<0.01) between the EA group (4.29±0.74) and the sham group (3.57±0.61) or control group (3.88±0.82), with the EA group showing a significant increase in diameter. There was no significant difference between the sham and control groups in any of these measurements.

Mechanical evaluation
At 6 weeks after surgery, the strength of the callus measured in the EA group was 16.54±9.92 (N), which is significantly higher (p<0.001) than 7.13±3.57 in the sham group and 6.67±3.12 in the control group. There was no significant difference between the sham and control groups (figure 3).

DISCUSSION
The application of intermittent direct current EA daily for 3 weeks starting from an early stage of treatment after surgery led to early formation, increased mass and strength of the callus. There was no significant difference between the sham and control groups in any evaluation. Based on these results, it appears that simply inserting (and leaving) acupuncture needles does not produce these effects, and it is very likely that electrical stimulation using acupuncture needles as electrodes had a significant role.
It was reported that the conventional direct-current electrical stimulation method for promoting fracture healing led to a different rapidity of bone healing depending on where the electrodes were placed and better results were obtained when the cathode was placed in the fracture site, as described above. This indicates that the location of the cathode will be an important factor for fracture healing when electric stimulation is employed. EA allows the cathode to be localised to the fracture site. Unlike applying current through the nail for external fixation, with this method the location of the electrodes can easily be changed, offering advantages when the best site for inducing bone healing changes over time. Furthermore, since electrical stimulation can be applied locally, this method can be employed in those cases where a bone is too small for external fixation to be performed or the desirable site for inducing bone formation is small.

The tibiae of the rats used in this study are very small, so we cannot say how effective EA would be in bone forming for larger bones. It might be thought that for larger bones, stimulation would not be enough to cover a desirable range, but this could be solved by increasing the number of acupuncture needles used as electrodes or by changing the insertion site of the needle used as the cathode over the course of treatment. In addition, if insulated acupuncture needles are used, attenuation of the electrical energy generated when stimulation is given from the body surface, as in the case of a surface electrode or LIPUS, can be reduced, thereby achieving more effective electrical stimulation. In this study, since insulated needles were not used, the current may have been attenuated. Even so, bone formation and calcification were promoted; therefore, it is highly possible that EA even with a lower level of electrical energy will still produce an effect.

Studies have shown that changes in the microenvironment around the cathode caused by direct-current electrical stimulation enhance cellular activity. Furthermore, these changes are considered to promote the production and secretion of growth factor and cytokine, which have an important role in the process of fracture healing. In this study, it is believed that EA affected the process of fracture healing through a similar mechanism, leading to the acceleration of bone healing. However, further in-depth studies are needed to confirm this. Additionally, more effective stimulation conditions need to be determined and safety parameters have to be established. We think that the risk of infection due to needle insertion is low, because no such adverse affects occurred during this study. However, when it is used in a clinical setting, attention needs to be given to surgical standards of sterility.

CONCLUSION

Intermittent direct-current EA was performed on a rat tibia fracture model and its effect on bone healing was assessed radiographically, macroscopically and biomechanically. The following points were observed: (1) The EA group showed a significantly better result in all of the evaluations than the sham and control groups. (2) No significant difference was seen between the sham and control groups in any evaluation. (3) Intermittent EA using the fracture site as the cathode promoted bone healing. Unlike the conventional bone healing method with electrical stimulation, acupuncture does not require a surgical procedure; therefore, we propose that EA might be used in clinical practice as an easily applied method of electrical stimulation to a limited region.

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Competing interests None.

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