Effects of Pulsing Electromagnetic Fields on the Ligament Healing in Rabbits

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ABSTRACT. Effects of pulsing electromagnetic fields (PEMFs) on ligament healing were investigated using 80 rabbits. All animals received square resection (4 mm × 4 mm) of both patellar ligaments in full thickness at their center. They were divided into 4 groups of 20 rabbits each and stimulated with different electromagnetic intensity of 0 (control), 2, 10, and 50 gauss (G) for 6 hr daily. Pulse frequency and pulse width were 10 Hz and 25 μ sec, respectively. After PEMFs stimulations for 1, 2, 3 and 4 weeks, 5 animals of each group were euthanized and the regenerated tissue at the defective portion was investigated histologically and biomechanically. Histologically, the tissue stimulated by PEMFs showed an earlier increase in capillaries and fibroblasts and more matured, prominent longitudinal orientation of collagen fibers than those of control groups. Among the rabbits stimulated electromagnetically, those stimulated at 50 G revealed the earliest ligament healing. Tensile strength of regenerated ligament tissues of any PEMFs groups increased significantly at 1 and 2 weeks after operation, however, at 3 and 4 weeks after operation, there were no significant differences between groups. Among these values, those of 50 G group were the highest consistently during most of the experimental period. From the above results, PEMFs enhanced the earlier stage of ligament healings and 50 G gauss seemed to be the most effective among the 3 field intensities used. This promoting effect may potentiate the earlier recovery of the function after the ligament injury.—**KEY WORDS:** histology, ligament healing, mechanical property, PEMF, rabbit.

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Since Yasuda discovered the phenomenon of “piezoelectricity” in the bone [18], numerous clinical reports have been published regarding the application of electrical stimulation for enhancing bone healing [3, 4, 10, 14]. Electrical stimulation by direct current has been demonstrated to be effective in promoting the union of the fractured bone [5, 7]. However, electrical stimulation by direct current has a disadvantages to require invasive procedures. On the contrary, utilization of stimulation by pulsing electromagnetic fields (PEMFs) could produce the similar promoting effects on the fractured bone healing without any surgical invasion.

In our previous study [13], PEMFs at 2 gauss (G) promoted the early stage of fracture healing. Blood flow and collagen content at the experimentally created bone defect in dogs stimulated daily for 1 to 4 weeks after operation by PEMFs was much higher than that in non-stimulated dogs.

Compared to a number of studies on electrical stimulation on the fractured bone, there have been a few reports on its effects on the ligaments and tendons [1, 6, 15, 17, 19]. From our preliminary study [8], we deduced that increased blood flow and collagen synthesis by PEMFs stimulation may also play an important role on ligament healing. The

present study was designed to examine the effects of PEMFs on ligament healing in rabbits from histological and biomechanical aspects.

MATERIALS AND METHODS

Eighty male Japanese White rabbits with the body weight ranging from 2.0 to 2.4 kg were utilized in this experiment. Anesthesia was induced with ketamine hydrochloride (35 mg/kg, im.) after pre-medication with xylazine (5 mg/kg, im.). The patellar ligament of both sides were exposed through longitudinal parapatellar incision. Square resection (4 mm × 4 mm) of both patellar ligaments in full thickness at their center was performed (Fig. 1).

All animals were divided into 4 groups of 20 rabbits each. On the following day of operation, electromagnetic stimulation on both stifles was started with different magnetic intensities, 0 (control group), 2, 10, or 50 G, for 6 hr daily using a pair of Helmholtz coil (Fig. 2). Pulse frequency and pulse width were 10 Hz and 25 μ sec, respectively. Animals of control group were comparably restrained 6 hr daily. Both control and experimental animals were allowed free activity in the cage for the

Fig. 1. Anterior view of the defective site of the patellar ligament.

Fig. 2. Diagram of the stimulation method by PEMFs on the defective site.

remaining 18 hr of the day.

Five animals of each group were euthanized weekly at the end of the 1st to 4th week after operation and the regenerated tissue at the defect portion was collected for gross and histological evaluation and biomechanical examination. Samples from the left patellar ligament was histologically examined with Hematoxylin and Eosin and Masson-Trichrome stains. From the right hindlimb, the patellar ligament was dissected connectedly with the tibia and the patella. The bone-ligament-bone complex was wrapped in gauze soaked with saline,

Fig. 3. The special fixation device use for the measurement of the tensile strength.

covered by vinyl bags to prevent dehydration and stored at -80°C until the day of biomechanical measurement [2, 9, 12]. Those specimens were removed from the freezer for 2 hr before the measurement and permitted to thaw at room temperature in their wrappings. After immersion in saline, the patella-ligament-tibia preparations were fixed to an Instron Testing Machine (TOM-5000D, Shinkoh Co., Tokyo) by means of special fixation device (Fig. 3) to measure their tensile strength. The cross head speed used was 10 mm/min and the load-elongation curves were recorded on an x-y plotter. The differences between the mean values in tensile strength were statistically analyzed by Student's *t*-test, and a *P* value less than 0.05 was considered to be significant.

RESULTS

Gross findings: There were no significant differences in gross findings of the defect of the patellar ligament between the rabbits received PEMFs stimulation and the control at 1 week after operation, when the defect appeared dent, hematoma tissue in all groups. At 2 weeks after operation, this finding was not considerably changed in rabbits of control, 2 G and 10 G groups, while in rabbits of 50

Table 1. The histologic feature of the regenerated ligament

Histologic findings	Weeks after operation			
	1W	2W	3W	4W
control group				
clotted blood	+ ^{a)} (100%) ^{b)}	+(60%)	+(60%)	-(100%)
necrotic tissue	+ (100%)	+(60%)	+(40%)	-(100%)
fibroblast	++ (100%)	++(100%)	+(100%)	++(100%)
capillary	++ (80%)	+(80%)	++(100%)	+(100%)
collagen fiber	+ (100%)	+(100%)	++(100%)	+++ (100%)
2 G group				
clotted blood	++ (100%)	-(80%)	-(100%)	-(100%)
necrotic tissue	+ (100%)	+(80%)	-(100%)	-(100%)
fibroblast	+++ (80%)	++(100%)	++(100%)	++(100%)
capillary	++ (80%)	+++ (100%)	++(100%)	++(100%)
collagen fiber	++ (100%)	++(100%)	++(100%)	++(100%)
10 G group				
clotted blood	+ (80%)	-(100%)	-(100%)	-(100%)
necrotic tissue	+ (80%)	-(100%)	-(100%)	-(100%)
fibroblast	++ (100%)	+++ (100%)	++(100%)	+(100%)
capillary	+ (80%)	+++ (100%)	++(100%)	++(100%)
collagen fiber	++ (100%)	++(100%)	+++ (100%)	+++ (100%)
50 G group				
clotted blood	++ (80%)	-(100%)	-(100%)	-(100%)
necrotic tissue	+++ (100%)	+(100%)	-(100%)	-(100%)
fibroblast	++ (100%)	++(100%)	+(100%)	-(100%)
		*(100%)	*(100%)	*(100%)
capillary	+ (80%)	++(100%)	+(100%)	-(100%)
		*(100%)	*(100%)	*(100%)
collagen fiber	+ (100%)	++(100%)	+++ (100%)	+++ (100%)

a) -: Not found, +: Few, ++: Moderate, +++: Rich, *: Degeneration.

b) Percentage of specimens can be observed among each group.

G group, an apparent transition from depressed hematoma tissue to flat grayish-whitish tissue was observed. At 3 weeks after operation, the regenerated tissue of the defect in animals of all groups appeared grayish-whitish and smooth, but the boundary line between the regenerated and the adjacent tissue was distinguishable. At 4 weeks after operation, the defect of the patellar ligament in any rabbits showed more smooth and continuous, however the regenerated tissue still could be distinguished from the adjacent, intact tissue.

Histologic findings : The characteristic histologic features of the regenerated ligament are summarized and shown in Table 1. There were no marked differences in the histologic feature of the defect between control and PEMFs groups at 1 week after operation. The central portion of the defect was filled with clotted blood, necrotic tissue and a few fibroblasts. At the margin of the defect, granulation tissue, slight proliferation of capillaries, many fibroblasts, and thin collagen fibers were observed. At 2 weeks after operation, the central portion of the defect in control group was still filled with

clotted blood, necrotic tissue and fibroblasts (Fig. 4). At the margin of the defect in control group, an increase in fibroblasts, capillaries and thin collagen fibers compared to those at 1 week after operation was observed. Whereas in the tissues of PEMFs groups, most of the clotted blood and necrotic tissue disappeared and the whole defect including the central and marginal portion was replaced by granulation tissue with fibroblasts and collagen fibers with minute longitudinal orientation (Fig. 4). Those changes were the most remarkable in the tissues of 50 G group among those receiving electromagnetic stimulation. Moreover, collagen fibers with higher density were observed in 50 G group than those of the other groups at 2 weeks after operation.

At 3 weeks after operation, the earliest histologic healing of the ligament was observed in the rabbits of 50 G group and those of 2 G and 10 G groups revealed earlier maturity than that of control group. At the defect of 50 G group, the density of collagen fibers distributed longitudinally seemed to be higher compared to those of other groups, in which

Figs. 4a-d. Histologic findings of the regenerated ligaments at 2 weeks after operation. The central portion of the defective site was filled with clotted blood and necrotic tissue in control (a), 2 G (b) and 10 G (c) groups. In 50 G group (d), degeneration of capillaries, fibroblasts and thin collagen fibers were observed. HE. $\times 50$.

accumulation of collagen fiber, degeneration of capillaries and fibroblasts were continued. At 4 weeks after operation, there were less differences within the tissues of control, 2 G and 10 G groups. In the tissues of 50 G group, the number of fibroblasts decreased while the density of collagen fiber increased and their well dispersed distribution was improved.

Tensile strength: Figure 5 shows the tensile strength of the regenerated ligament of the experimental animals. It was evident that the tensile strength of the ligament in the animals of any PEMFs groups was stronger than that in the control group at any week of observation. At 1 week after operation, the tensile strength of control group remained below valuable minimum measurement (0.04 Kgf/mm), while the ligament of PEMFs groups showed 0.25 to 0.32 Kgf/mm of average tensile strength. At 2 weeks after operation, the tensile

Fig. 5. Changes in tensile strength of the regenerated ligaments.

strength of the regenerated ligament in any of PEMFs groups rose suddenly to the values from 1.58 to 1.94 Kgf/mm, while that in control group showed a gradual increase to 0.66 Kgf/mm, which was significantly lower than those in PEMFs groups ($p < 0.05$). However, there were no significant differences in tensile strength of regenerated ligaments at 3 to 4 weeks after operation in all the groups, though those of PEMFs groups showed higher values than that of control group. In addition, tensile strength of 50 G group showed the highest value at 1, 2 and 4 weeks after operation among all the groups.

DISCUSSION

Electromagnetic stimulation on the ligament injury was demonstrated to improve tissue organization histologically and biomechanically. In addition, the higher magnetic intensity was required for the acceleration of the ligament healing than of fracture healing.

The tissue of the defect in 50 G group revealed earlier repairing features than those in other groups at 2 weeks after operation. In this group, vascularity in the defect and an increase in the number of fibroblasts and collagen fibers were evident at the earlier stage of ligament healing. These results may suggest that PEMFs enhances local blood flow and encourages neovascularization at an earlier stage of the repairing process, which induces the earlier ligament healing.

Nishimura *et al.* [13] mentioned that the fracture defect was filled with fibrin network, connective tissue and plenty of fibroblasts at 1 week after PEMFs stimulation. The connective tissue and proliferative neovascularization were seen in the fracture defect at 2 weeks after PEMFs stimulation. The rabbits of 50 G group in this study revealed an earlier increase in fibroblasts and regenerative capillaries, thus, the electromagnetic stimulation may provide the similar effects on both the bone and ligament under their optimal magnetic intensity. At 3 and 4 weeks after operation, an increase in the number of collagen fibers which was well distributed longitudinally were notable in the tissues of PEMFs groups, especially in those of 50 G groups. It is still unclear whether PEMFs directly promotes the maturation of the collagen fibers or the earlier repairing process mentioned above may promote the maturation.

Tensile strength of the ligament receiving PEMFs

therapy at any intensity was significantly higher at 1 and 2 weeks after operation than that of control, however at 3 and 4 weeks, there were no significant differences between the control and PEMFs groups, though the values of PEMFs groups were higher. Histologically, accumulated collagen fibers with minute longitudinal orientation of the tissues in PEMFs groups at 2 weeks after operation were more remarkable than those in control group, which may increase the tensile strength at this repairing stage. At 3 and 4 weeks after operation, differences in the number and thickness of collagen fibers were still obvious between the control and PEMFs groups, however, the degree of the difference reduced. These results might cause in no significant differences in tensile strength among the groups. Nevertheless, the results in this study demonstrate an apparent acceleration of the reversal of the functional strength by electromagnetic stimulation. This is quite important especially in veterinary medicine because the weight bearing on the affected limb at earlier phase of ligament healing can be expected. Early use of the affected ligament might cause further promotion of the healing and earlier recovery of the function without adhesion or contraction of the joint.

In this study, 50 G was the most effective in intensity of PEMFs therapy on injured ligaments by morphological and biomechanical aspects. In our previous study using fresh fracture model of the dog, 2 G was the most effective. Other studies on the use of electromagnetic conditions used in ligament or tendon repair were 250 G, 1 Hz [6] and 150 G, 15 Hz [17]. The most effective conditions of PEMFs on DNA synthesis in cultured fibroblasts are also variable [11, 16]. Liboff [11] reported that the DNA synthesis in human fibroblasts was enhanced when exposed to PEMFs with a wide range of frequency (15 Hz to 4 KHz) and magnetic intensity (0.023 to 5.6 G). Those differences in PEMFs conditions might be due to the difference in sensitivity of the cells used in their experiments, however, precise cause was still unclear. At present, it is quite difficult to estimate the best condition of PEMFs on different biological process. However, it may need to consider the cell types involved and possible varieties in the sensitivity of each biological process to magnetic fields. Unfortunately, 50 G is the maximum intensity of our electromagnetic stimulator, thus further improvement of the stimulator to investigate the effect of higher intensity of the ligament healing is

necessary.

In conclusion, daily stimulation by electromagnetic field at 50 G accelerates the earlier stage of the ligament healing histologically and biomechanically, thus could be used as a non-invasive treatment method for the ligament injury in the veterinary practice.

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