Effects of Pulsed Magnetic Energy on a Microsurgically Transferred Vessel

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This article reports the findings of a study that attempted to elucidate whether pulsed magnetic energy stimulates neovascularization in vivo, using a microsurgically created arterial loop model in a prospective randomized trial of 108 rats (n = 12/group). Pulsed magnetic energies of 0.1 and 2.0 gauss were applied immediately postoperatively and for 4, 8, and 12 weeks, respectively, with a statistically significant increase in neovascularization among the treated animals compared with control rats. The study provides a starting point for future study and evaluation of the stimulation of angiogenesis with the use of pulsed magnetic energy and suggests a possible use of this modality to increase the quality of revascularized tissue. (*Plast. Reconstr. Surg.* 105: 1371, 2000.)

Extensive research has been conducted on the use of pulsed magnetic energy and its effect on soft-tissue injury and bone healing. In a clinical study conducted by Pennington and colleagues,[1] the authors noted a reduction in periarticular edema in acutely sprained ankles when nonthermal pulsed magnetic energy was applied to the injury. Other researchers have examined the use of this modality and its effects on resistant fracture nonunions and have found a significant reduction in the time required for bony union.[2] In a rabbit spinal-fusion model, Glazer et al.[3] observed that the use of electromagnetic field therapy increased bone rigidity and achieved increased load (to stress).

It has been suggested that endothelial cell-derived growth factors or mitogens might promote this osteogenesis.[4] These data led Yen-Patton et al.[5] to speculate that pulsed magnetic energy-induced osteogenesis could result from stimulation of endothelial cell neovascularization at the site of fracture nonunions. This group found that in vitro human umbilical vein endothelial cells exposed to an electromagnetic field began the initiation of vascularization within hours of treatment, as contrasted to the findings of earlier researchers, which showed that untreated cell cultures took approximately 1 to 2 months to accomplish this.[5,6] According to Pilla,[7] for an electrical "electromagnetic field bioeffect" to occur, the electromagnetic signal not only should satisfy the dielectric properties of the target cells but must be detectable above normal thermal noise. Theoretically, the field should be capable of inducing selective changes in the microenvironment around and within the cell.[8]

The purpose of this study was to clarify whether electromagnetic field energy could stimulate neovascularization in an in vivo model. Two different energy levels were tested to determine if any differences could be observed in the outcomes of neovascularization.

Materials and Methods

Sprague-Dawley male rats (n = 108), weighing approximately 300 g each, were equally divided into nine groups. All animals were anesthetized with a mixture of ketamine/acepromazine/Stadol at 0.1 cc/g. Using sterile surgical techniques, each animal had a 12- to 14-cm segment of tail artery harvested using microsurgical technique. The artery was flushed with 60 U/ml of heparinized saline to remove any blood or emboli. These tail vessels, with an average diameter of 0.4 to 0.5 mm, were then sutured to the transected proximal and distal segments of the right femoral artery using two end-to-end anastomoses (Fig. 1), creating a femoral arterial loop. The resulting

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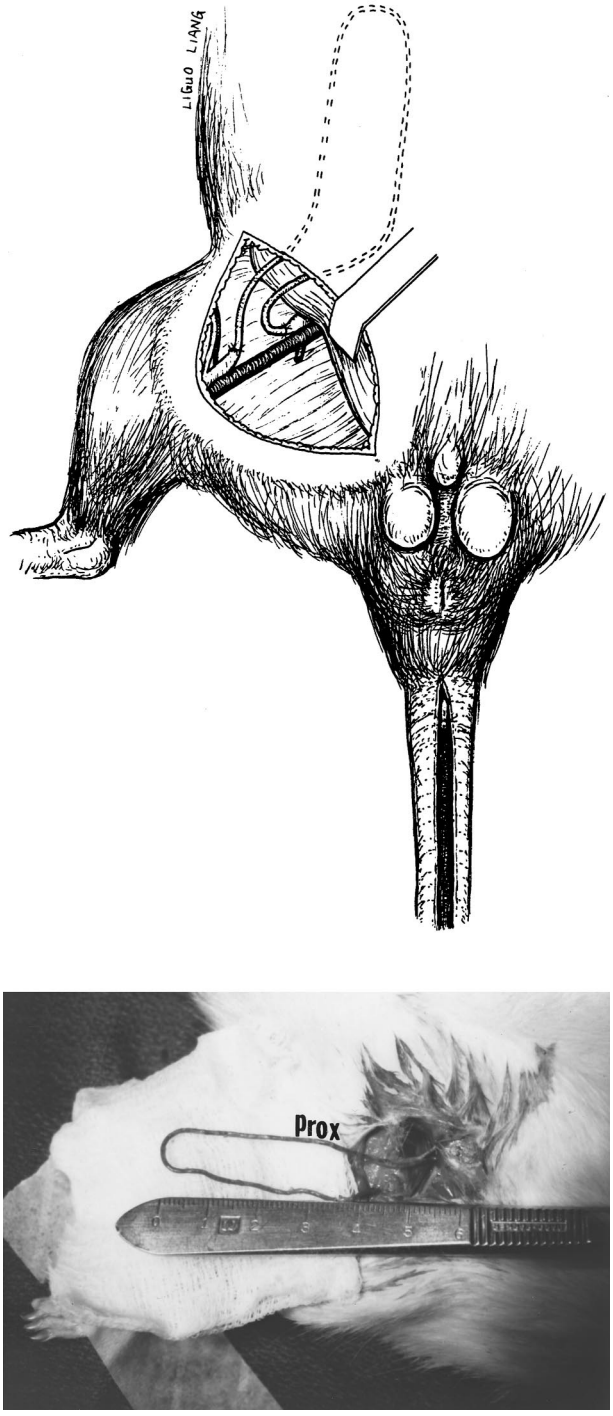

FIG. 1. (*Above*) Drawing depicting arterial loop from harvested tail artery. (*Below*) Femoral artery/tail artery loop after proximal and distal anastomoses; proximal graft portion is indicated.

loop was then placed in a subcutaneous pocket created over the animal's abdominal wall/groin musculature, and the groin incision was closed with 4-0 Ethilon. Each animal was then randomly placed into one of nine groups (n = 12/group): groups 1 to 3 (controls), these rats received no electromagnetic field treatments and were killed at 4, 8, and 12 weeks; groups 4 to 6, 30-min treatments twice a day using 0.1-gauss electromagnetic fields for 4, 8, and 12 weeks (animals were killed at 4, 8, and 12 weeks, respectively); and groups 7 to 9, 30-min treatments twice a day using 2.0-gauss electromagnetic fields for 4, 8, and 12 weeks (animals were killed at 4, 8, and 12 weeks, respectively).

Pulsed electromagnetic energy was applied to the treated groups using an MRT *sof*Pulse (Electropharmacology, Inc., Pompano, Fla.). Animals in the experimental groups were treated for 30 minutes twice a day at either 0.1 or 2.0 gauss, using short pulses (2 to 20 msec) 27.12 MHz. Animals were positioned on top of the applicator head and confined to ensure that treatment was properly applied.

The rats were reanesthetized with ketamine/acepromazine/Stadol intraperitoneally and 100 U/kg of heparin intravenously. Using the previous groin incision, the femoral artery was identified and checked for patency. The femoral/tail artery loop was then isolated proximally and distally from the anastomoses sites, and the vessel was clamped off. Animals were then killed. The loop was injected with saline followed by 0.5 to 1.0 cc of colored latex through a 25-gauge cannula and clamped. The overlying abdominal skin was carefully resected, and the arterial loop was exposed. Neovascularization was quantified by measuring the surface area covered by new blood-vessel formation delineated by the intraluminal latex. All results were analyzed using the SPSS statistical analysis package.

RESULTS

Table I demonstrates the extent of surface area neovascularization expressed in cm^2, and Figure 2 graphically illustrates the extent of neovascularization in the arterial loop expressed in cm^2. Clinically, all rats treated with either energy level experienced no ill effects. Before being killed, the animals were weighed and noted to have gained 85 to 200 g over the length of the experiment. None of these ani-

TABLE I

Neovascularization Expressed in cm^2 ± SD

	4 Weeks	8 Weeks	12 Weeks
Controls (n = 12)	0.0	0.7 ± 0.82	1.75 ± 0.95
0.1 gauss (n = 12)	1.42 ± 0.80	3.57 ± 1.26	5.95 ± 3.25
2.0 gauss (n = 12)	1.42 ± 0.80	3.77 ± 1.82	6.20 ± 3.95

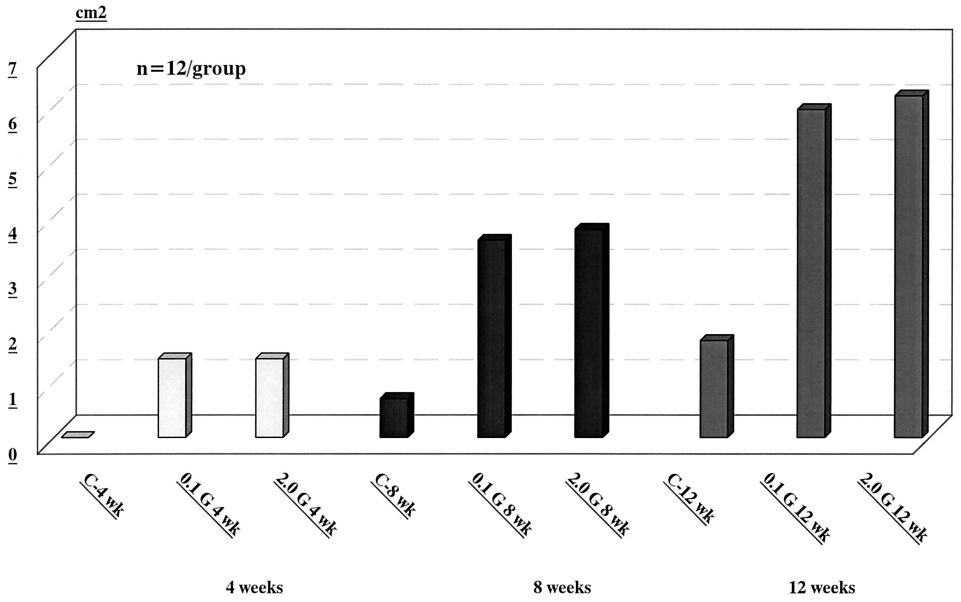

FIG. 2. Neovascularization of the arterial loop (expressed in centimeters).

FIG. 3. Arterial loop control at 4 weeks. Note lack of neovascularization.

mals suffered any burns, nor did any animal die during the course of treatment. Immediately after pulsed electromagnetic energy treatment, animals had an approximately 10 percent increase in water consumption.

The most noticeable difference in neovascularization between treated versus untreated rats occurred at week 4. At that time, no new vessel formation was found among controls (Fig. 3); however, each of the treated groups had similar statistically significant evidence of neovascularization at 0 cm^2 versus 1.42 $\pm$ 0.80 cm^2 ($p < 0.001$). These areas appeared as a latex blush segmentally distributed along the sides of the arterial loop. At 8 weeks, controls began to demonstrate neovascularization measured at 0.7 $\pm$ 0.82 cm^2. Both treated groups at 8 weeks again had approximately equal statistically significant ($p < 0.001$) outcroppings of blood vessels of 3.57 $\pm$ 1.82 cm^2 for the 0.1-gauss group and of 3.77 $\pm$ 1.82 cm^2 for the 2.0-gauss group (Fig. 4, *above*). At 12 weeks, animals in the control group displayed 1.75 $\pm$ 0.95 cm^2 of neovascularization, whereas the 0.1-gauss group demonstrated 5.95 $\pm$ 3.25 cm^2, and the 2.0-gauss group showed 6.20 $\pm$ 3.95 cm^2 of arborizing vessels (Fig. 4, *below*). Again, both treated groups displayed comparable statistically significant findings ($p < 0.001$) over controls. However, no statistically significant differences in neovascularization were found between the two gauss levels tested at any of the sacrifice dates.

DISCUSSION

Researchers have studied the effects of pulsed magnetic energy in vitro using human umbilical vein endothelial cells.[5] It was demonstrated that these cells formed a "sprouting" pattern after the electromagnetic field was applied to areas of denuded endothelium. When complete disruption of the endothelial monolayer was achieved, some of these cells reorganized into three-dimensional vessel-like structures within 5 to 8 hours after application.

The exact mechanism by which the electromagnetic field interacts with cells is not yet known. However, many cell types have been shown to respond in various ways to this modality, including neurons, muscle cells, and fibroblasts.[9] Some researchers in wound healing

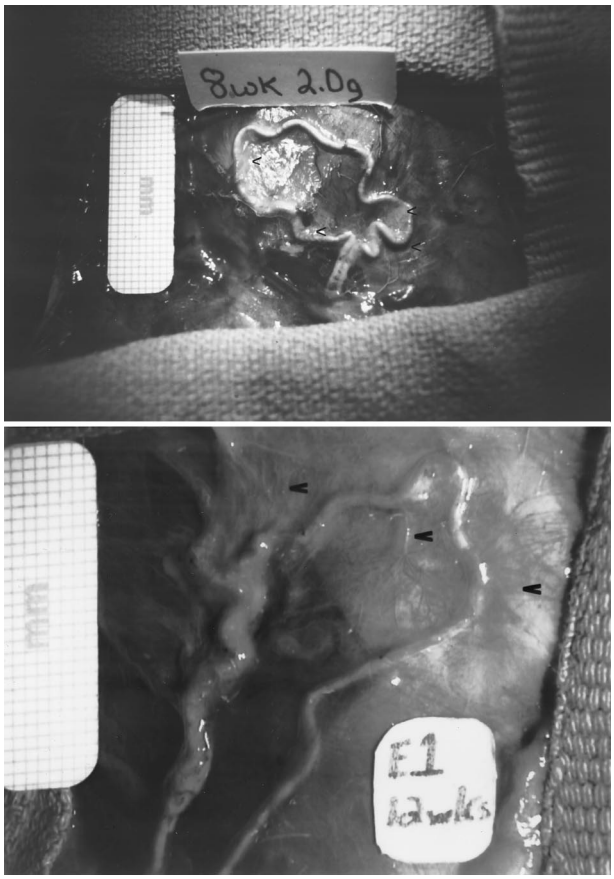

FIG. 4. (*Above*) 2.0-gauss-treated rat (8 weeks) with significant neovascularization (*arrows*). (*Below*) 2.0 gauss (12 weeks) with markedly demonstrable arborization from the arterial loop (*arrows*).

have theorized about biologic repair systems that are triggered by changes and differences in the bioelectric potential created across wounds; thus, these fields may encourage or support such changes in the bioelectric phenomenon.[10–13] More specifically, electromagnetic fields may change the cellular plasma membrane potential, encouraging calcium flux that could stimulate a cellular response.[5]

Our experimental findings suggest that electromagnetic field stimulation of an isolated arterial loop increases the amount of quantifiable neovascularization in an in vivo rat model. This was demonstrated in each of our treated groups at each of the sacrifice dates. No differences were found between the results of the two gauss levels tested; this may have been a result of our energy levels being too close in range. Further studies need to be carried out to define how rapidly differences can be found between treated versus untreated animals. This information might be used for an increase in the quality of revascularized tissue. For example, if trials were to reveal significant increases in neovascularization within days of beginning treatment, the modality could serve to aid in some revascularization and replantation procedures. Although chronic wound and bone healing do not require the immediate augmentation of circulation, they could certainly be subsequently supported by increased angiogenesis.

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