Electrical Stimulation Promotes Angiogenesis

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Abstract

Introduction: In previous studies, we showed that a modest regimen of electrical stimulation (ES) improves healing, accelerates neovascularization, and enhances angiogenesis in (even severely) ischemic muscle tissue. In this report, we wanted to further understand ES applied for this purpose.

Immediately after excision of the left distal external iliac and femoral arteries, ES (30 contractions per minute [cpm], 2V, single impulses per burst) was applied to rabbit adductor muscle near the site of the excised femoral artery for 24 hr/day over one month. Three other series served as controls: ES without excision; excision without ES or lead implantation; excision with lead implantation but no ES. Histological studies (angiography) and lower-limb-calf blood pressure measurements were performed.

After 30 days of ES alone, 10.5±1.2 contrast medium opacified arteries (COAs) crossed a specific grid segment vs. 7.2±1.5 in controls with ES (p<0.05); 68.2±9.3 COAs crossed a grid section vs. 43.2±6.4 in controls (p<0.05); 27.3±1.2 grids contained COAs vs. 29.3±3.5 in controls (p<0.05); lower-limb-calf blood pressure ratio was 0.81±0.06 vs. 0.31±0.07 in controls (p<0.05) and capillary density was 283.7±24.5 mm² vs. 91.4±20.9 mm² in controls (p<0.001).

Cautious ES enhances and accelerates muscle angiogenesis in severely ischemic tissue.

1. Introduction

In 1999, Kanno et al. [1] reported that low voltage electrical stimulation (ES) of skeletal muscle induced de novo synthesis of vascular endothelial growth factor (VEGF) proteins, promoted local angiogenesis, and restored blood flow in ischemic tissue. Their investigations were based on studies of ES-induced angiogenesis in skeletal muscle [2-4]; however these studies were aimed at producing maximal muscle contraction; the finding of increased capillary density only confirmed improvement in muscle resistance to fatigue but was not itself a study goal. During the same period (1997-1998), we investigated low frequency ES and showed that even in severely ischemic tissues, the healing process can be improved, neovascularization accelerated, and angiogenesis in muscles enhanced [5,6]. In our current study, we wanted to further our understanding of ES as a potential alternative treatment for severe hind limb muscle ischemia using a rabbit model.

1.1. Previous Work

Twenty-four (24) adult New Zealand White rabbits (males, mean weight 4 kg) were used in this study and were randomly assigned to one of four series. In series 1 (control) a small incision was made of the left thigh solely for electrode implantation. In series 2-4, a longitudinal incision was made from the left inguinal ligament to a point just proximal to the patella. The left distal external iliac artery and femoral artery were dissected free (series 2-4), ligated, and excised from the point of the external iliac artery to the bifurcation into the saphenous and popliteal arteries, making blood flow to the hindlimb segment dependent upon flow through the internal iliac artery. One electrode was implanted into the adductor muscle near the site of the excised femoral arteries (series 2,3), and a stimulator (Thera, Medtronic, Minneapolis, MN, USA) was implanted in a separate pocket (series 1 and 2). In series 1 and 2 reported here, we started ES immediately post procedure at 30 cpm, 2.0V, and single impulses 24 hr daily and continued this for one month. To establish the anatomy of the collateral vessels and to
Results

Baseline angiography before surgery and ES showed 5.3±1.3 COAs crossing a specified segment of the grid, which decreased significantly after surgery to 3.2±1.0 (p<0.05). A month later, without treatment or intervention (ES), this increased to 7.2±1.5 (p<0.05 vs. after surgery but p>0.05 vs. baseline)(series 4). Electrode implantation alone (without ES) had no effect on this data (6.9±1.1; p>0.05 vs. baseline and vs. series 4). When ES was applied (series 2), the number of COAs crossing a specified segment increased to 105.±1.2 (p<0.001 vs. after surgery; p<0.05 vs. baseline and series 1, 2, and 4). When ES was applied to normal nonischemic tissue, the number of COAs increased to 6.1±0.8 but this was not statistically significant compared with baseline data (p<0.05).

The same change was seen in grid intersections crossed by COAs: 30.2±6.5 at baseline vs. 19.3±4.85 after surgery (p<0.05). This increased significantly compared with data post procedure (p<0.05) in both control series: no ES with electrode implantation (41.8±4.3) and no ES without electrode implantation (43.2±6.4); but these changes were not significant compared with baseline (p>0.05). In the ES series, grid intersections crossed by COAs were 68.2±9.3 (p<0.001 vs. after surgery; p>0.05 vs. baseline, series 1, 3, and 4).

When ES was applied to the normal nonischemic limb, this increased to 38.1±5.2 (p>0.05 vs. baseline). In all series (even in the control group undergoing femoral excision without ES) angiography revealed progressive linear extension of collateral arteries from the original stem artery to the distal point of the reconstituted parent vessels. However, in series 2 (the experimental group), a great many vessels (especially in series 2) were seen emerging from the pelvis and running through the thigh. One or two of these anastomoses obliquely connected to the distal part of the excised femoral artery to supply the hindlimb with blood.

A Doppler flow signal could not be detected immediately after surgery. At day 30, blood pressure ratio was further improved in all series with arterial excision, but this was statistically significant only in series 2 (excised artery and ES)(0.8±0.06 vs. 0.63±0.08 on day 20). In the series with ES but no excised artery, blood pressure ratio increased to 1.16±0.07 (p<0.05 vs. normal limb). Capillary density for ES-treated ischemic muscle (series 2) was 283.7±24.5/mm² vs. 91.4±20.9/mm² in control series 4 without electrode implantation and ES (p<0.001) and vs. 87.4±31.4/mm² in control series 3 with electrode implantation but no ES. In series 1 (no femoral excision but with ES) capillary density was greater than in normal muscle (201.0±29.3/mm² vs. 183.5±32.2/mm², but this was statistically nonsignificant (p>0.05).

2. Summary and Conclusions

Our results with ES were impressive. The number of COAs crossing a specific grid segment were more than doubled over baseline to 105.55±1.2 (p<0.001); there were 68.2±9.3 grid intersections crossed by COAs vs. 30.2±6.5 at baseline (p<0.01) and with 43.2±6.5 in control (p<0.05); there were 27.3±1.2 out of 30 grids occupied by COAs vs. 18.3±3.8 at baseline (p<0.05) and with 22.3±3.5 in control (p<0.05). Capillary density was 283.7±24.5/mm².

It is interesting to note that one can see ES had a greater effect on the process of angiogenesis than on the process of arteriogenesis; i.e., the number of COAs in the ES series was only 1.5 times that of the control series, but the number of capillaries was three times that in controls (91.4±20.9/mm²; p<0.01). In the control series, ES resulted in both of these processes returning to near normal levels (blood pressure ratio 0.81±0.06 vs. the ratio in control 0.31±0.07; p<0.01). Two additional control series were in our investigation. In series 3, we investigated the effect of electrode implantation without ES on ischemic tissue and found no histologic evidence of an inflammation reaction. All angiographic parameters were practically the same as in series 4 (control without electrode implantation). Also practically the same were capillary density and
lower-limb-calf-blood pressure ratio.

Another control series (series 1) was used to study the effect of ES on normal nonischemic tissue. In this case, ES acted like intensive exercise in causing the muscle to repeatedly contract. A month after ES, all parameters increased over baseline, but these increases were not significant (p<0.05): COAs crossing a specified segment (6.1±0.8 vs. 5.3±1.3); grid intersections crossed by COAs (38.1±5.2 vs. 30.2±6.5; grids occupied by COAs (20.1±4.3 vs. 18.3±3.8); and capillary density (201.0±29.3/mm² vs. 183.5±32.2/mm²). The only statistically significant change (p<0.05) was an increase in blood pressure ratio (115.1±0.07 vs. 0.95±0.06).

Currently, ES therapies are used primarily to treat chronic pain syndromes and soft tissue wounds. Indeed, ES may be used clinically to augment angiogenesis in patients who have ischemic vascular disease. Our study showed that when appropriately applied to ischemic tissues, ES improves their condition by enhancing and accelerating muscle revascularization. In all cases of lower limb ischemia, angiography revealed new vessels arising from the caudal artery in the direction of the distal femoral artery, connecting with to restore distal blood flow. These preliminary results may be interpreted cautiously to suggest use of ES as a new approach to treat subacute limb ischemia, perhaps widening the horizon for patients who have severe localized peripheral artery disease.

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