Slowing Atherosclerosis Progression or Eliminating It:  
Do Low Frequency Electrical Impulses Help?

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Abstract

In our previous studies, we showed that electrical impulses (EI) could prevent the development of atherosclerosis if started simultaneously with high cholesterol diet (HCD) or in the early stages of atherosclerosis (after 3 weeks of HCD only). In this investigation, we wanted to determine whether low frequency EI either slows atherosclerosis progression or eliminates atherosclerosis in a rabbit model of moderate atherosclerosis (after 8 weeks of HCD).

Series 1 rabbits (control group) were fed HCD for 8 weeks. Series 2 rabbits were fed HCD for 8 weeks and then switched to a normal diet for 8 weeks (no EI). Series 3 rabbits were fed HCD for 8 weeks then switched to a normal diet with simultaneous EI applied near the abdominal aorta for 8 weeks (3V, 30 single impulses per minute, 24 hr/day). After euthanization, the level of atherosclerosis and the percentage of surface area involved in the atherosclerosis process were measured, and score was calculated for disease severity in the aortic arch and in the thoracic and abdominal aortas.

Statistically significant differences were seen in the level of atherosclerosis in the abdominal aorta between series 3 animals (0.4±0.2) and the other two groups: 1.5±0.4 in series 1 (HCD only) and 1.2±0.3 in series 2 (HCD then normal diet). Gross examination of the surface also revealed statistically significant differences (p<0.05) in the percentage of atherosclerosis between the control series (series 1)(30.1±4.1%), series 2 (21.3±3.6%), and series 3 (5.5±5.4%). In addition, the atherosclerosis score was also significantly different: 45.8±3.9 in series 1, 25.2±6.9 in series 2, and 2.2±2.0 in series 3 (p<0.05).

Our study showed that applied near the abdominal aorta, low frequency electrical impulses decrease atherosclerotic deposition in that region.

1. Introduction

Our previous investigations on angiogenesis showed that low frequency and slow rate EI may prevent further muscle deterioration and may stimulate the acceleration of new capillary formation in severely ischemic tissue. Under the influence of low frequency EI, endothelial cells were more able to survive both acute and chronic ischemia and to become the provisional platform for future angiogenesis and vasculogenesis in ischemic tissue. Based on these animal investigations, we hypothesized that the creation of an electrical field by transmission of low frequency EI may affect newly formed atherosclerotic plaque in the endothelium of vessels and may decrease the extent of previous pathologic damage in these structures.

1.1. Previous Work

Twenty-four (24) adult New Zealand White rabbits (males, mean weight 4 kg) were randomly assigned to one of three series (8 in each series). In series 1 (control) HCD (rabbit chow supplemented with 0.5% cholesterol, 3% peanut oil, and 3% coconut oil) was given for 8 weeks. In
series 2, HCD was given then the diet was switched to a normal one for 8 weeks (no EI). In series 3, HCD for 8 weeks was followed by normal diet for 8 weeks plus EI. After the animals were anesthetized, a stimulating lead was implanted into the right psoas major muscle close to the upper portion of the abdominal aorta. The lead was tunneled behind the vertebral column on the left side and connected to a pacemaker that was implanted in a pocket created between the left psoas major muscle and the left abdominal oblique muscle close to the upper portion of the abdominal aorta. A stimulation regimen was then applied (3 V at 30 contractions per minute [cpm] for 24 hr/day).

After the animals were sacrificed, the level of atherosclerosis was ascertained in hemotoxylin-stained tissue sections using our arbitrary scale of 1-4. Gross examination was also used to calculate the percentage of surface area involved in the atherosclerosis process.

### Results

**Serum cholesterol level.** After 8 weeks of HCD (series 1 and 2), serum cholesterol levels increased from 133-375 mg/dL at baseline to 943-1413 mg/dL (p<0.05). After a subsequent 8 weeks of normal diet (series 2), cholesterol levels decreased to 236-561 mg/dL (p>0.05 vs. baseline; p<0.05 vs. series 1). Similar levels (209 mg/dL-499 mg/dL) were seen in series 3 animals where EI was applied with the normal diet (p>0.05 vs. baseline; p>0.05 vs. series 1 and p>0.05 vs. series 2).

**Lower thoracic arch.** After 8 weeks of HCD, atherosclerosis levels in the lower thoracic aorta were 2.0±0.5 in the control animals (series 1). Although the level was lower in series 2 (1.7±0.4) and series 3 (1.2±0.5), the difference was not statistically significant (p>0.05). The percentage of atherosclerosis and the atherosclerosis score, however, were significantly less (p<0.05) in the animals subjected to EI (series 3): (12.3±4.3% vs. 39.0±6.4% (series 1) and 33.1±5.7% (series 2); and 14.4±3.9% vs. 78.2±8.6% (series 1) and 56.1±5.9% (series 3), respectively.

**Abdominal aorta.** Statistically significant differences (p<0.05) were seen in atherosclerosis levels between series 3 animals (0.4±0.2) and the other two groups: 1.5±0.4 in series 1 (HCD only), 1.2±0.3 in series 2 (HCD then normal diet).

Gross examination of the surface revealed statistically significant differences (p<0.05) in the percentage of atherosclerosis between the control series 1 (30.1±4.1%) and series 2 (21.3±3.6%), and between series 2 and series 3 (5.5±5.4%). There were also significant differences in the atherosclerosis score: 45.8±3.9 in series 1, 25.2±6.9 in series 2, and 2.2±2.0 in series 3 (p<0.05).

Histological examination of the abdominal aorta of control animals revealed no endothelial cells. The elastic lamina was distorted, and the distance between the lumina of the aorta and the elastic lamina was considerably increased, causing a moderately fatty plaque. Many fat-laden macrophages were present and their cytoplasm was filled with lipid droplets (foam cells) as the result of lipid digestion. Throughout this foam-cell rich area in the intima of these series 1 rabbits, the smooth muscle cells had migrated and proliferated, lipids had accumulated, and there were deposits in the extracellular matrix.

Atherosclerosis was less evident in series 2 animals. Many monocytes and platelets were seen adhering to endothelial cells. Some monocytes had migrated into the subendothelial layer and a few fat-laden monocytes were found between the endothelium and the elastic lamina. In the series 3 animals, evidence of atherosclerosis was minimal: there were few signs of endothelial cell injury, the elastic lamina was not distorted, fat-laden macrophages were seldom present, and there was no migration of smooth muscle cells.

Gross examination of the surface revealed that the percentage of atherosclerosis in series 3 animals was nearly zero (0.5±0.1%, p>0.05 vs. series 3) as compared with control animals (19.4±3.3%, p<0.05 vs. series 2 and 3). The atherosclerosis score showed a similar trend: 22.8±3.7 (series 1), 5.6±1.4 (series 2), and 0.1±0.9 (series 3).

### 2. Summary and Conclusions

No one has applied EI to prevent atherosclerosis because this concept is still theoretical. Our interest in using EI for this purpose came from our initial studies of low frequency EI in the latissimus dorsi muscle (LDM) used for cardiac bioassist, in which we found that the process of restoring angiogenesis after EI is applied to this ischemic muscle begins with doubling of capillary density.[1] Consequently, we examined the condition of endothelial cells during the phase of acute ischemia and
after being subjected to different regimens of EI. Most endothelial cells in the newly mobilized LDM were damaged by EI applied at 60 cpm. [2,3] Many endothelial cells were found to have a cuboidal shape with discontinuous, disrupted plasma membranes and projections of the degenerated cytoplasm into the capillary lumen. The percentage of area occupied by capillaries decreased from 3.99±0.24% to 2.15±0.70%.

Our investigation with low frequency and low rate EI applied to ischemic skeletal muscle showed positive results. Capillaries occupied 5.01±0.56% of the muscle surface area after 15 cpm EI, which was statistically better than the area occupied in the control muscle (p<0.05). Endothelial cells appeared normal in their ultrastructure without degeneration of intracellular components. On the basis of these findings, we hypothesized that low frequency EI:

1) reduces damage to endothelial cells from both ischemia and atherosclerosis;
2) prevents new atherosclerotic plaque from developing in previously diseased vessels, and
3) perhaps even reduces the extent of previous pathologic damage in these structures.

As we supposed, after discontinuation of HCD, there was a decrease in atherosclerosis level and in the amount of involved surface area. During the 8 weeks of normal diet that followed discontinuation of HCD, a decrease in atherosclerosis was evident in all areas of the aorta from the arch to the lower abdominal portion. None of these decreases were statistically significant, but the fact of the decrease is in itself important. We speculate that prolonging normal diet beyond 8 weeks might have yielded a significant result.

When we examined the effect of low frequency EI, we found a statistically significant decrease in both level of atherosclerosis and in the amount of surface area involved. A possible explanation is that, after only 8 weeks of HCD, the atherosclerotic plaque was not well established and not stable when compared with established, very stable plaque that has developed in human arteries over many years. Thus, perhaps only “young” plaque is susceptible to EI, or EI may be ineffective in treating atherosclerosis in humans (as opposed to our animal model), or EI may be more efficacious when a different regimen is used, perhaps applied for a considerably longer time.

Our goal in this study was to determine the potential for using directly applied EI to eliminate or decrease moderate atherosclerosis in a rabbit model. Our results showed that this is, indeed, possible. Specifically we found that when applied near the abdominal aorta, low frequency electrical impulses decrease atherosclerotic plaque deposition in the treated region.

Acknowledgment

The authors wish to thank Robert Henderson for his editorial assistance in preparing our manuscript for publication.

References