Pathologic and histologic results of electrical impulses in a rabbit model of atherosclerosis: 24-hour versus 8-hour regimen

Valeri S. Chekanov, MD, PhD,[a] Mohammad E. Mortada, MD,[a] Guennady V. Tchekanov, MD,[a] Michelle A. Maternowski, MS,[a] Rueben Eisenstein, MD,[a] Natalia Pello, MD,[b] David Krum, MS,[a] and **Masood Akhtar, MD,[a]** *Milwaukee, Wis*

Objective: **Low frequency electrical impulses (EIs) reduce new atherosclerotic plaque formation in previously diseased arteries and may reverse the extent of previous pathologic damage in these structures.**
Methods: **A pacemaker was implanted on the left side of rabbit abdominal aortas, and an electrode was placed close to the other side of the aorta in the psoas major muscle. For the induction of atherosclerosis, the rabbits were placed on a high cholesterol diet (HCD) for 11 weeks. No EIs were applied to the control series I. In the experimental series, the rabbits were fed an HCD for 3 weeks, after which EIs were applied simultaneously with an HCD for 8 additional weeks (3V, 30 contractions per minute). Experimental series II had 24-hour/day EIs, and series III had 8-hour/day EIs.**
Results: **The closer to the area where the EIs were applied, the more local severity increased (atherosclerosis level and surface area). In the control series, the severity of atherosclerosis in the lower aorta assessed with an arbitrary grading system was 1.75 ± 0.5 (versus 1.5 ± 0.57 with 8-hour/day EIs and 0.5 ± 0.3 with 24-hour/day EIs). The involved surface area was 32.5% ± 9.5% (versus 1.0% ± 0.8% with 8-hour/day EIs and 0.75% ± 0.95% with 24-hour/day EIs).**
Conclusion: **Both 24-hour/day and 8-hour/day EIs applied close to the abdominal aorta decreased the severity of atherosclerosis in rabbits placed on a HCD, but 24-hour/day EIs decreased the severity more extensively. (J Vasc Surg 2002;35:554-62.)**

A large number of diseases are related to atherosclerosis and contribute to high rates of morbidity and mortality.[1-3] To manage any complication caused by atherosclerosis, scientists and researchers are trying to create the appropriate conventional treatment method. Current methods include dilatation of narrowed arteries with balloon angioplasty or stenting, coronary atherectomy, and bypass grafting of arteries (coronary or peripheral). However, some patients are poor candidates for these treatments because they have severe diffuse disease with severe ischemia of the target organs or some comorbidity. The search continues for a method to inhibit the development of advanced atherosclerosis.

Theoretically, any treatment of atherosclerosis that inhibits intimal thickening with the inhibition of collagen synthesis will reduce the luminal narrowing of the vessel.[4] Calcium channel blockers,[5] derived dextrans,[6] tranilast,[7] protamine,[8] halofuginone,[9] and L-minostine[10] have been studied and are shown to inhibit collagen synthesis and thus to decrease the level of atherosclerosis. Our preliminary investigations showed that the application of low frequency electrical impulses (EIs) and the subsequent creation of an electrical field in rabbits with the early stages of atherosclerosis may inhibit intimal thickening, affect newly formed atherosclerotic plaque in the endothelium of vessels, and decrease the extent of previous pathologic damage in these structures.[11,12]

After 8 weeks of EIs, the levels of atherosclerosis in the upper abdominal aorta (AA) at 30 contractions per minute (cpm) were 0.5 ± 0.1 (3V) versus 2.5 ± 0.4 (2V) and 0.9 ± 0.2 (4V). At 60 cpm, the levels were 0.8 ± 0.2 (3V) versus 2.6 ± 0.5 (2V) and 1.3 ± 0.3 (4V). And at 120 cpm, the levels were 2.9 ± 0.3 (3V) versus 2.8 ± 0.4 (2V) and 3.2 ± 0.5 (4V) and 2.8 ± 0.5 (control).

Similarly, the combination of 30 cpm and 3V yielded the best results in terms of percentage of area involved in the atherosclerosis process. At 30 cpm, these percentages were 5.2% ± 3.6% (3V) versus 29.3% ± 10.1% (2V) and 17.2% ± 4.3% (4V). At 60 cpm, the percentages were 31.3% ± 7.6% (3V) versus 32.3% ± 2.9% (2V) and 34.7% ± 5.9% (4V). And at 120 cpm, the percentages were 36.0% ± 13.0% (3V) versus 39.3% ± 11.1% (2V) and 30.6% ± 5.7% (4V) and 32.5% ± 15% (control). The best results were obtained at 3V with a rate of 30 cpm, the combination we used in the following investigation.

The aim of this study was the determination of the optimal length of time that EIs should be applied daily to

From Heart Care Associates, Milwaukee Heart Institute at Sinai Samaritan Medical Center,[a] and the Pathology Department, St Luke's Medical Center.[b]

Competition of interest: Dr Chekanov holds a patent (#6.201.991) entitled "Method of prevention and treatment of atherosclerosis and article of manufacture thereof."

Reprint requests: Valeri S. Chekanov, MD, PhD, 945 N. 12th St, Rm W419, PO Box 342, Milwaukee, WI 53201-0342 (e-mail: ahcvch@execpc.com).

0741-5214/2002/$35.00 + 0 **24/1/121756**
doi:10.1067/mva.2002.121756

obtain the best results. We therefore compared 24-hour/day EIs and 8-hour/day EIs in a rabbit model of diet-induced atherosclerosis.

MATERIALS AND METHODS

The animal studies in this investigation were approved by our institution's Animal Care Committee, which is in compliance with all federal laws and with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research. All the animal studies were conducted in a full American Association for Accreditation of Laboratory Animal Care–accredited research facility. Our investigation used three series of animals: series I (control, 11 weeks of a high cholesterol diet [HCD] but no EIs; n = 4 animals), series II (similar diet and with EIs 24hour/day; n = 4 animals), and series III (similar diet with EIs 8-hour/day; n = 4 animals).

High cholesterol diet

Important similarities between atherosclerosis in a rabbit aorta and in a human aorta reinforce the advantages of this model. In rabbits fed a cholesterol-rich diet, a similar pattern of plaque distribution, especially in the young,[13-21] to the disease distribution pattern in rabbits and humans is similar in the aorta but dissimilar in some peripheral arteries. In adult human arteries, lesions more frequently form upstream of branch points than they do downstream.[22-24]

All the rabbits were put on a 2% cholesterol diet and fed the same amount of food at the same times. The plasma cholesterol levels were determined in all animals with the Abbott VP Blood Chemistry Analyzer (Abbott Laboratories, Inc, Abbott Park, Ill) at the conclusion of the experiment.

Operative technique

Animal preparation. Before surgery, the rabbits underwent anesthesia with a cocktail of ketamine hydrochloride (25 mg/kg intramuscularly), acepromazine maleate (1 mg/kg intramuscularly), and glycopyrrolate (0.22 mg/kg intramuscularly). Once they were sedated, the rabbits were placed on a semi-open, nonrebreathing ventilation system with halothane gas (0.75% to 2%) mixed with 2 to 3 L O_2 via mask and then given buprenorphine hydrochloride (0.05 mg/kg intramuscularly) for pain (two thirds of the dose after the induction of anesthesia and one third after recovery from surgery). Additional pain medication was given as needed (buprenorphine hydrochloride 0.02 to 0.05 mg/kg twice a day). Antibiotic therapy included enrofloxacin (5 to 10 mg/kg intramuscularly twice a day for 14 days) to treat pasteurella pneumonia, if necessary, and chloramphenicol sodium succinate (30 mg/kg intramuscularly once per day for 5 to 7 days) for prophylactic postoperative treatment of infection. The incision sites were checked at least once daily for any abscess formation.

Stimulator and lead implantation. The surgery was performed with strict sterile technique. After the induction of anesthesia, the rabbits had the stimulating lead implanted into the right psoas major muscle close to the upper part of the AA. The lead for the myostimulator was tunneled behind the vertebral column on the left side and connected to the pacemaker (Terra, Medtronic, Inc, Minneapolis, Minn), which was implanted in a pocket between the left psoas major muscle and the abdominal oblique muscle, close to the upper part of the AA (Fig 1). The incision was closed in layers with tie sutures. The same surgery was performed on the animals in all three series, but EIs were used only in series II and III.

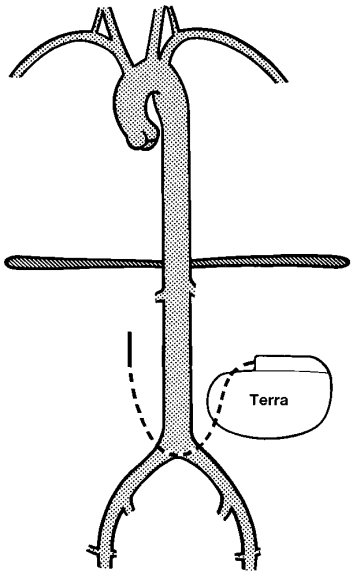

Fig 1. Drawing shows electrode implanted in psoas major muscle and stimulator lead placed opposite near abdominal aorta.

The pacemaker and lead were implanted in such a manner that electrical current flowing from the pacing lead to the generator crossed the AA. Pacing impulses of 30 cpm at 3V were programmed with a Medtronic programmer.

Previous investigations[11,12] have shown that early atherosclerosis appears in the AA, the area of particular interest in these studies, within 3 weeks of beginning an HCD. In pilot studies, atherosclerosis in the coronary arteries was advanced and many rabbits died with myocardial infarction after week 11. On the basis of these observations, the study design was to feed experimental animals with an HCD for 3 weeks and then apply EIs for an additional 8 weeks while maintaining this diet, for a total period of 11 weeks.

Pathology investigations

Evaluation of atherosclerosis. After death, the heart aortic arch and the entire aorta plus the iliac arteries were removed. These tissues were fixed in formalin and stained with Sudan IV dye, and the extent of stainable lipid on the surface then was assessed. Atherosclerosis was assessed

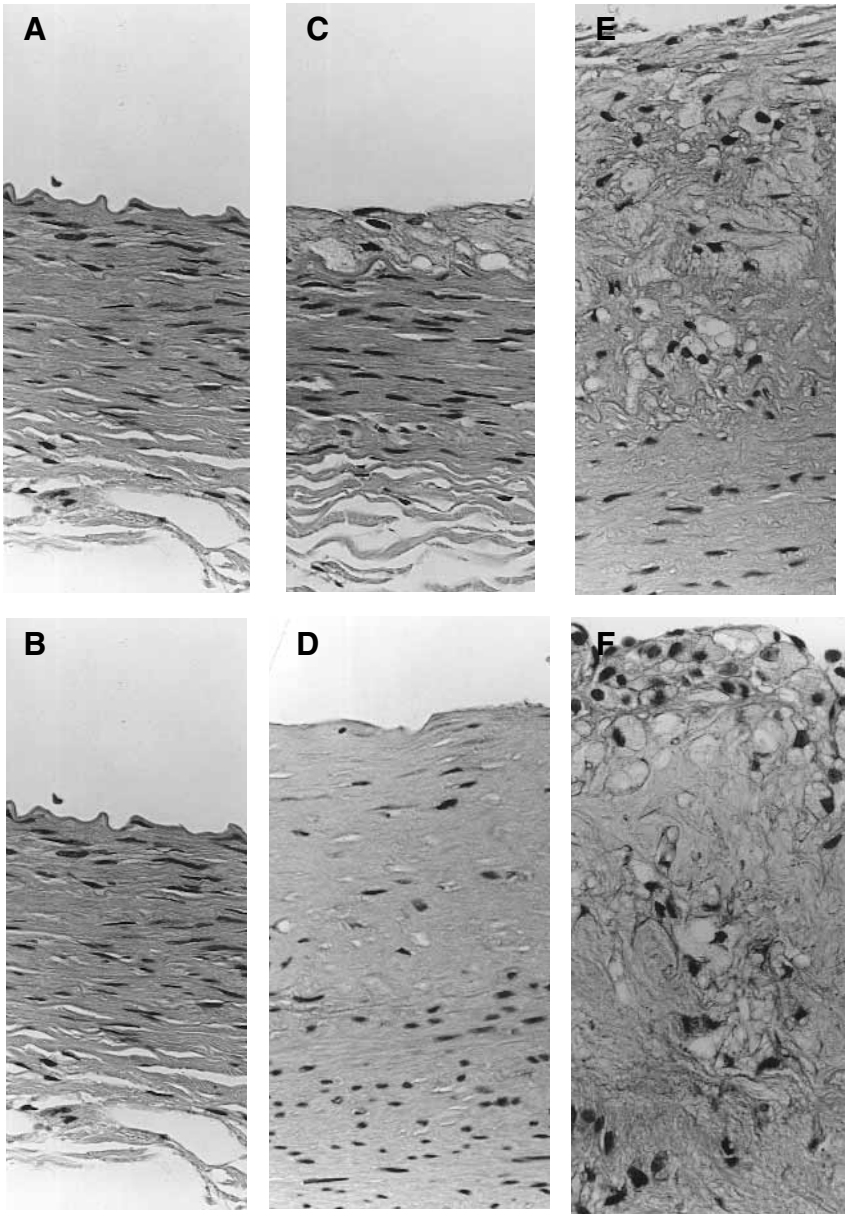

Fig 2. Histopathologic section of aorta. **A,** Healthy, without atherosclerosis. **B,** Trace of atherosclerosis. **C,** Level I atherosclerosis. **D,** Level II atherosclerosis. **E,** Level III atherosclerosis. **F,** Level IV atherosclerosis. *Scale bar* represents 25 mm.

with the following arbitrary scale of 1 to 4 in hematoxylin-stained sections (Fig 2): trace, minimal thickening of the endothelium; grade 1, plaque containing foam cells less than half as thick as the media (Fig 2, *C*); grade 2, plaque half as thick as the media or more with accumulation of intracellular lipid (lesions as advanced as this often contained areas of fibrosis, but its presence did not affect grading; Fig 2, *D*); grade 3, plaque at least as thick as the media; and grade 4, plaque thicker than the media with evidence of cellular degeneration, fibrosis, or both.

For the calculation of the percentage of surface area involved in the atherosclerotic process, gross examination was used. The entire aorta was dissected and removed (Fig 3), and then the aorta was divided into the following four sections: the upper thoracic aorta (from the aortic arch to the mid point of the thoracic aorta), the lower thoracic aorta (from the mid point of the thoracic aorta to the level of the diaphragm), the upper AA (from the level of the diaphragm to the mid point of the AA), and the lower AA (from the mid point of the AA to the bifurcation of the aorta).

Gross color photographs of Sudan-stained sections of the aorta were overlaid with a transparent grid that contained 100 squares. Because the overlay was larger than

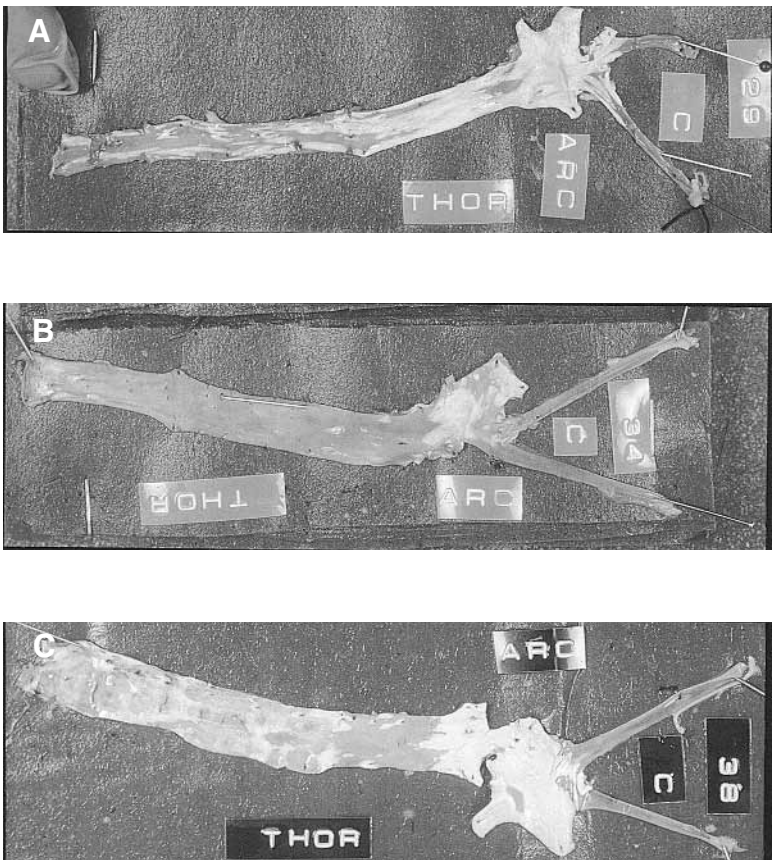

Fig 3. Autopsy photographs of thoracic aorta after: **A,** 11 weeks of high cholesterol diet (HCD) only; **B,** 11 weeks of HCD plus 24-hour/day electrical impulses; and **C,** 11 weeks of HCD plus 8-hour/day electrical impulses.

the examined section of the aorta, only the squares on the overlay that fell within the specimen were counted. Next, the number of squares that contained aortic lesions was counted. The number of squares with lesions then was calculated as a percentage of the total number of overlay squares contained within the specimen. All the microscopic evaluations were done without the evaluator knowing the treatment for any of the rabbits.

In a previous study,[11,12] we found that a stimulation regimen of 3V impulses at 30 cpm has the best results in the reduction of atherosclerosis as compared with other voltages (2V, 4V) or impulse rates (60 cpm, 20 cpm). Therefore, we used this regimen in this study. Stimulation at 3V produced slight muscle contractions but did not disturb the animals.

Statistical analysis

The results are expressed as the mean ± the standard error of the mean. All the analyses were performed by one of the authors (MAM) with appropriate software (Statview 5.0, SAS Institute, Inc, Cary, NC). All the continuous variables (in control and 24-hour and 8-hour EI groups) were studied with analysis of variance. If a resultant fraction was found to be significant (ie, established at $P < .05$), a Scheffé test was used to specify pairwise differences.

RESULTS

No animals lost weight during the course of the study. The condition and overall activity level of the electrically stimulated animals was no different from those in the control series.

Upper thoracic aorta (Figs 4 and 5). The grades of atherosclerosis were higher in the control series (3.0 ± 1.1) and in the rabbits subjected to 8-hour EIs (3.0 ± 0.8) as compared with those subjected to 24-hour EIs (1.75 ± 0.9). The difference between the three series was statistically nonsignificant ($P > .05$). However, in two of the four rabbits subjected to 24-hour/day EIs, the atherosclerosis level in the upper thoracic aorta was only 1+. Gross examination results revealed that the percentage of atherosclerotic surface was significantly less ($P < .05$) in the series subjected to 24-hour/day EIs, but not to 8-hour/day EIs, as compared with the control series: 67.5 ± 33.04 (control series), 30.0 ± 23.09 (8-hour EIs), and 12.5 ± 11.9 (24-hour EIs).

Lower thoracic aorta (Figs 4 and 5). After 11 weeks of an HCD, the atherosclerosis grades in the lower

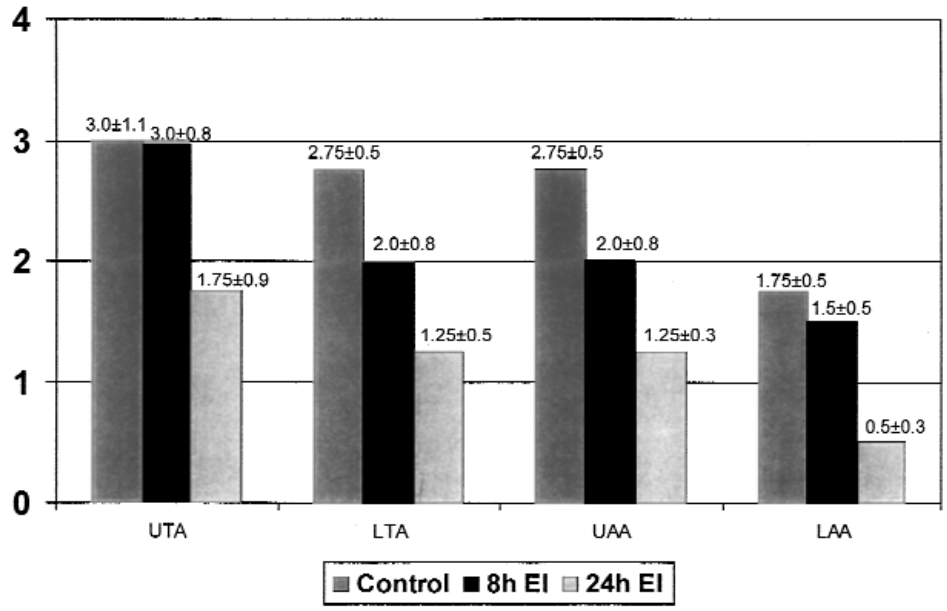

Fig 4. Graph shows aortic thickness grade in upper thoracic aorta *(UTA)*, lower thoracic aorta *(LTA)*, upper abdominal aorta *(UAA)*, and lower abdominal aorta *(LAA)*. *EI,* Electrical impulses.

thoracic aorta were 2.75 ± 0.5 in the control series. Although the grades were lower in the 8-hour/day EIs series (2.0 ± 0.8), the difference in the grades was not statistically significant ($P > .05$). The grade was lower in the 24-hour/day EIs series (1.25 ± 0.5) than in the control series and in the 8-hour EIs series, but this difference was statistically significant ($P < .05$) only when contrasted with the control series.

Gross examination results showed that the percentage of atherosclerotic surface was 47.5% ± 17.0% in the control series but only 18.0% ± 23.1% in the 8-hour/day EIs series. Although there was a difference between the control and 8-hour/day EIs series, this difference was not statistically significant. The percentage was 2.75% ± 2.6% in animals in the 24-hour/day EIs series, which was considerably less than in either the control or 8-hour/day EIs series ($P < .001$).

Upper abdominal aorta (Figs 4 and 5). The atherosclerosis grades in the upper AA were practically the same as in the lower thoracic aorta (2.75 ± 0.5 in the control series, 2.0 ± 0.8 in the 8-hour/day EIs series, and 1.25 ± 0.35 in the 24-hour/day EIs series), which was not significantly less ($P > .05$) as compared with the 8-hour/day EIs series but was significantly less ($P < .05$) as compared with controls. Gross examination results showed that the difference in the percentage of involved surface area was statistically significant ($P < .001$): 32.5% ± 15.0% (control series) versus 5.25% ± 3.6% (24-hour/day EIs). Results from the 8-hour/day EIs were between those of the control and 24-hour/day EIs series: (11.5% ± 8.1%; $P > .05$ versus control and 24-hour EIs).

Lower abdominal aorta (Figs 4 to 7). Greater differences in atherosclerosis grades were found in the lower AA (ie, in the area where the pacemaker and electrode were implanted). The grades were 1.75 ± 0.5 in the control series versus 0.5 ± 0.3 in the 24-hour/day EI series ($P < .05$). In contrast, the atherosclerosis grades in three rabbits in the control series were 2+, but in three rabbits in the 24-hour/day EIs series, there were no signs of atherosclerosis (grade 0). Eight hours of EIs daily did not change the atherosclerosis grades in the lower AA as compared with the control series (1.5 ± 0.5; $P > .05$).

Histologic examination results of the control rabbits revealed that the elastic lamina was distorted and that the distance between the lumina of the aorta and the elastic lamina was considerably increased, which caused a moderately fatty plaque. Many fat-laden macrophages were present, and their cytoplasm were filled with lipid droplets (foam cells) as the result of lipid digestion. Throughout these foam cell–rich areas in the intima, the smooth muscle cells had migrated and proliferated, lipids had accumulated, and there were lipid deposits in the extracellular matrix. However, in rabbits with 24-hour/day EIs, there was no endothelial cell injury in the AA. The elastic lamina was undisturbed. In the rabbits with 8-hour/day EIs, some monocytes had migrated into the subendothelial layer and occasionally a few fat-laden monocytes were found between the endothelium and the elastic lamina.

Gross examination results revealed that the percentage of involved surface area was 32.5% ± 9.5% in the control series and only 0.75% ± 0.9% in the 24-hour/day EIs series ($P < .001$). Initial findings of involved surface area

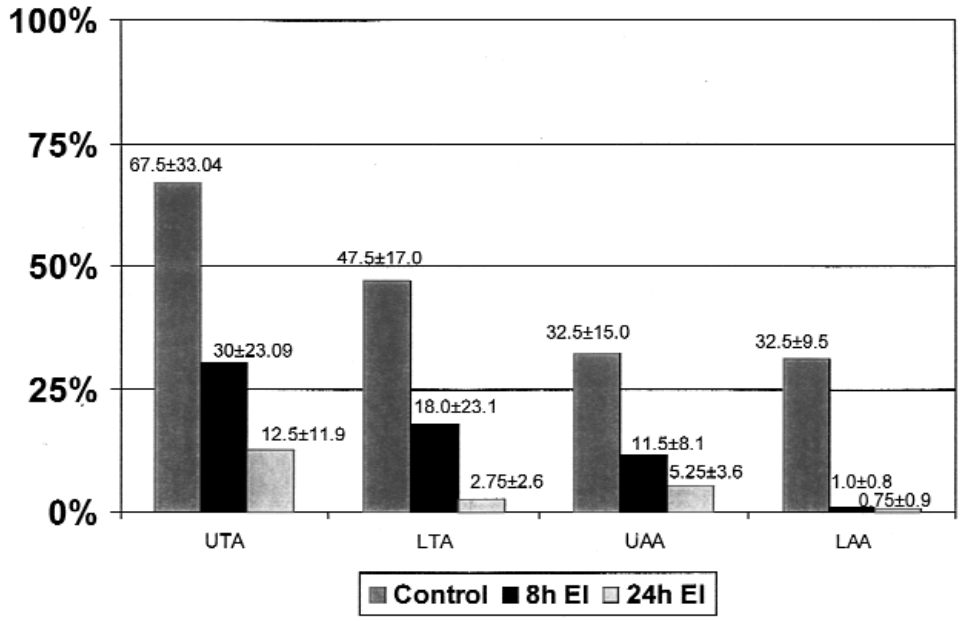

Fig 5. Graph shows percentage of involved surface area in upper thoracic aorta *(UTA)*, lower thoracic aorta *(LTA)*, upper abdominal aorta *(UAA)*, and lower abdominal aorta *(LAA)*. *EI,* Electrical impulses.

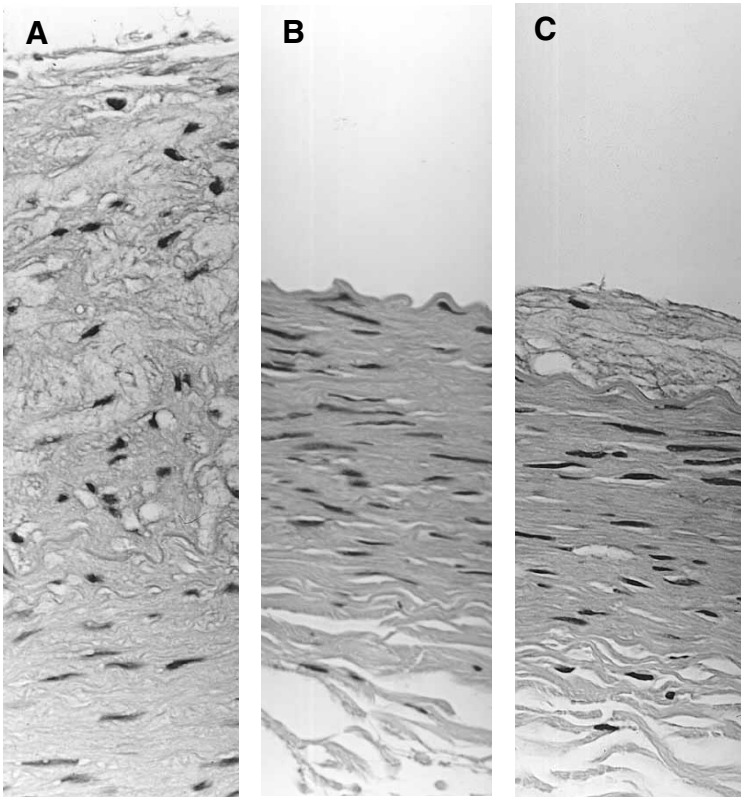

Fig 6. Histopathologic section of lower abdominal aorta. **A,** Level II to III atherosclerosis with high cholesterol diet (HCD) but no electrical impulses (EIs). **B,** Disease free with HCD plus 24-hour/day EIs. **C,** Level I atherosclerosis with HCD plus 8-hour/day EIs.

Fig 7. Autopsy photographs of abdominal aorta after *(upper)* 8-hour/day electrical impulses (EIs), *(middle)* 24-hour/day EIs, and *(lower)* no EIs.

from the 8-hour/day EIs series were similar to those of the 24-hour/day EIs series (1.0% ± 0.8%; $P < .001$ versus control, but $P > .05$ versus 24-hour EIs). Moreover, in three of eight rabbits in the two EIs series, the lower AA had no atherosclerotic areas as compared with 40% of involved surface area in two rabbits in the control series.

Our data showed that, in both the control group and the groups subjected to EIs, there was a decrease in atherosclerotic burden in the more distal aortic regions. The atherosclerotic level in the lower AA of the control group was 1.75 ± 0.5 versus 3.0 ± 1.1 in the upper thoracic aorta. However, this difference was not statistically significant ($P > .05$). In the 24-hour/day EIs group, the level of atherosclerosis in the lower AA was only 0.5 ± 0.3, which was significantly less than that of the control group (1.75 ± 0.5; $P < .05$) and the upper thoracic aorta of the electrically stimulated animals (1.75 ± 0.9; $P < .05$). Thus, in keeping with the data of many other investigators, there was centrifugal spread of the process down the aorta.

Serum cholesterol level. After 11 weeks of an HCD, the serum cholesterol levels in the control animals increased from 292 ± 81 mg/dL to 1736 ± 158 mg/dL ($P < .001$). In the 24-hour/day EIs group, the serum cholesterol levels increased to 1562 ± 114 mg/dL ($P < .001$ versus baseline, but $P > .05$ versus control). In the 8-hour/day EIs group, the serum cholesterol levels were 1786 ± 137 mg/dL ($P < .001$ versus baseline, but $P > .05$ versus control and 24-hour/day EIs).

DISCUSSION

Our interest in EIs for the prevention of atherosclerosis came from our initial studies of low frequency EIs in ischemic muscle, in which we found that the process of angiogenesis starts with doubling capillary density in the target muscle after EIs are applied.[25,26] However, no such effect is possible without the beneficial influence of EIs on the morphology of the endothelial cells and on vascular endothelial growth factor (VEGF). It is well known that VEGF is a prototypic angiogenic growth factor that is associated with angiogenesis in vivo and causes endothelial cell proliferation in vitro.[27,28] Annex et al[29] strongly supported the hypothesis that some regimen of EI may play a positive role in severely ischemic tissue, which shows that chronic motor nerve stimulation increases VEGF at the protein level. Hang et al[30] showed similar findings with the increase of VEGF protein in hypoxia, especially in conjunction with EIs.

In our previous investigations, we examined endothelial cells during acute ischemia and when subjected to different regimens of EIs. Most endothelial cells were damaged when EIs were applied at 60 cpm.[25,26] On the basis of these findings, we hypothesized that low frequency EIs: 1, reduce damage to endothelial cells from both ischemia and atherosclerosis; 2, prevent new atherosclerotic plaque from developing in previously diseased vessels; and 3, perhaps even reduce the extent of previous pathologic damage in these structures. Our evaluation of the atherosclerotic process includes both local severity of atherosclerosis and the percentage of the surface area involved in the affected vessel.

We found that the percentage of atherosclerotic surface was lower in all four rabbits in the 8-hour/day series than in the control series in the lower AA. The level of atherosclerosis was not significantly different from the control series. When we compared the 8-hour/day EIs series with the 24-hour/day EIs series, we found that the atherosclerosis levels were less in the 24-hour/day EIs series. This difference was not statistically significant ($P > .05$) in the thoracic aorta, but it was statistically significant ($P < .05$) in the lower AA near the area where the EIs were applied.

As noted previously, in general, atherosclerosis tends to decrease when moving downward from the thoracic aortic to the AA. The level of atherosclerosis decreased from 3.0 ± 1.1 (upper thoracic aorta) to 1.75 ± 0.5 (lower AA) in the control series, from 1.75 ± 0.9 to 0.5 ± 0.3 in the 24-hour/day EIs series, and from 3.0 ± 0.8 to 1.5 ± 0.5 in the 8-hour/day EIs series.

Our data showed that EIs have a greater influence on the development of atherosclerosis in the more distal aorta. The difference was statistically significant, even in the upper thoracic aorta: 12.5% ± 11.9% (24-hour/day EIs) versus 67.5% ± 33.0% (control; $P < .05$). In the lower AA, the difference was evident: 0.75% ± 0.9% (24-hour/day EIs) versus 32.5% ± 9.5% (control; $P < .001$). The involved surface area in the lower AA with 8-hour/day EIs was small: only 1.0% ± 0.8% ($P < .001$) versus the control series. However, the level of atherosclerosis with 8-hour/day EIs was close to that in the control series: 1.5 ± 0.5 versus 1.75 ± 0.5 ($P > .05$).

Among the interesting results, after 11 weeks of an HCD, the AA in the control animals had 32.5% ± 9.5% of

involved surface area and a plaque thickness level of 1.75 ± 0.5 (ie, advanced atherosclerosis). When EIs were applied to the AA for 8 hours/day for 11 weeks, the involved area diminished from 32.5% to 1%, but the plaque thickness level (in this case, 1% of the area) remained the same as in the control animals. These findings changed dramatically when 24-hour/day EIs were applied; both atherosclerotic surface area (0.75% ± 0.9% versus 32.5% ± 9.5%) and atherosclerosis level (0.5 ± 0.3 versus 1.75 ± 0.5) considerably decreased as compared with the control animals. In other words, 8-hour/day EIs decreased only the involved surface area, whereas 24-hour/day EIs decreased both surface area involvement and atherosclerosis level.

All of our experimental data showed that EIs applied around the aorta can prevent, and even decrease, fatty deposits, despite a continued HCD, by yet unclear mechanisms. The relationship between atherosclerosis and angiogenesis is now being scrutinized.[31] The results are controversial. Some treatment scenarios favor proangiogenesis, others favor antiangiogenesis. Moulton et al[32] showed that neovascularization in the lesion is proatherosclerotic.

Recently, several investigations showed the possibility of regressing established atherosclerosis in rabbits with protein-bound polysaccharide,[33] with vaccine-induced antibodies,[34] with policosanol,[35] with mycophenolate mofetil,[36] and with L-arginine.[37] Because the application of EIs is still a new approach, additional studies are needed to add EI application to this list and confirm its efficacy.

One possible explanation of the mechanism for the prevention of atherosclerotic plaque formation may be in the change in iron ion concentration in the AA with the influence of EIs. It is now known that redox-active iron can initiate lipid peroxidation, an important early event in the development of atherosclerosis.[38,39] In an animal model of atherosclerosis,[40] it was shown that the progression of atherosclerosis and low density lipoprotein oxidation are closely related to vascular iron deposition.

Any method that decreases the availability of iron for the production of reactive oxygen species[41] will be useful for atherosclerosis prevention.[42] Halliwell[41] showed that in higher concentrations (>0.05 mmol/L), deferoxamine, an iron chelator, may scavenge reactive oxygen species.

Iron is known to stimulate low density lipoprotein and membrane lipid peroxidation,[38,39,43] and its transition metal ions are present in atherosclerotic lesions in sufficient quantities to catalyze this reaction.[39] Duffy et al[42] reported that non-protein bound iron may directly inactivate nitric oxide[43] and iron may be mobilized from ferritin by superoxide,[44] which is increased in atherosclerosis.[45] Therefore, if deferoxamine decreases the atherosclerotic lesion with iron chelation,[41,42] low frequency EIs, by creating an electrical field around the target vessels (in this case, the AA), may also decrease atherosclerotic lesions or even prevent their development by removing the iron ions from the target vessels. This is only a preliminary hypothesis because we did not measure serum iron levels in this study.

This study did provide evidence that EI application may prove to be a useful tool for atherosclerosis prevention and treatment in those at risk for, or with, aortic atherosclerosis or peripheral vascular disease. It can be used regardless of degree of vessel patency or the number of atherosclerotic vessels, and it does not traumatize the inner wall of the vessel. EIs may be applied with the implantation of electrodes intramuscularly, topically, or intravenously close to target vessels.

CONCLUSION

Our study has yielded new data about the possible future use of EIs in atherosclerosis prevention and reversal in rabbits. In a previous investigation, we found that the best variant of EIs for the prevention of atherosclerosis in a rabbit aorta was 3V and 30 cpm. This study shows that continuous EIs (24 hours/day) yield better results than do 8-hour/day EIs and that the optimal variant now becomes 24-hour/day EIs at 3V and 30 cpm.

Contemplated further may be attempts to answer the following questions:

1. Can these results be approximated with EIs (or an electrical field) in animals and later in patients who have atherosclerosis of varying severity in other arteries (eg, in the coronary arteries, the carotid arteries, and the iliac and femoral arteries, or in the AA)?

2. Can EI application be integrated with other methods of accelerating angiogenesis in patients who have atherosclerosis included in blood flow and deterioration in angiogenesis and vasculogenesis?

3. Can we determine the actual mechanism of how EI application affects the formation of atherosclerosis (ie, the influence of EIs primarily on contracting muscle, on smooth muscle cell migration, on the stability of the endothelial cell layer, or, as we suspect, on the adhesion of leukocytes and platelets to the endothelium)?

We thank Brian Miller and Brian Schurrer for their technical assistance in preparing the figures for this manuscript and Debra Waller, Barbara Danek, and Rob Henderson for their editorial assistance.

REFERENCES

1. Murray CJL, Lopes AD. The global burden of disease. Boston: Harvard School of Public Health/Harvard University Press; 1996. p. 1-34.
2. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801-9.
3. Van der Wal AC, Becker AE. Atherosclerosis plaque rupture: pathologic basis of plaque stability and instability. Cardiovasc Res 1999;41: 334-44.
4. Rekhter MD. Collagen synthesis in atherosclerosis: too much and not enough. Cardiovasc Res 1999;41:376-84.
5. Roth M, Eickelberg O, Kjohler E, Erne P, Block LH. Ca channel blockers modulate metabolism of collagens within the extracellular matrix. Proc Natl Acad Sci U S A 1996;93:5478-82.
6. Bannazoug Y, Logeart D, Labat-Robert L, Jezenfonvicz J, Kern P. Derivatized dextrans modulate collagen synthesis in aortic smooth muscle cells. Biochem Pharmacol 1995;49:847-53.
7. Fukuyama J, Mizayawa K, Hamano S, Ujie A. Inhibitory effects of tranilast on proliferation, migration, and collagen synthesis of human vascular smooth muscle cells. Can J Physiol Pharmacol 1989;74:80-4.

8. Perr HA, Drucker DE, Cochran DL, Diegelmann RF, Lindblad WJ, Graham MF. Protamine selectively inhibits collagen synthesis by human intestinal smooth muscle cells and other mesenchymal cells. J Cell Physiol 1989;140:463-70.
9. Nagler A, Miao HQ, Aingorn H, Pines M, Genina O, Vladavsky I. Inhibition of collagen synthesis, smooth muscle cell proliferation, and injury-induced intimal hyperplasia by halofuginone. Arterioscler Thromb Vasc Biol 1997;17:194-202.
10. McCaffrey TA, Pomerantz KB, Sanborn TA, Spokojny AM, Du B, Park MH, et al. Specific inhibition of elF-5A and collagen hydroxylation by a single agent. Antiproliferative and fibrosuppressive effects on smooth muscle cells from human coronary arteries. J Clin Invest 1995;95:446-55.
11. Chekanov V, Tchekanov G, Maternowski M, Mortada ME, Eisenstein R. Use of electrical stimulation to prevent atherosclerosis in the abdominal aorta: experimental study [abstract]. J Am Coll Cardiol 2000;35(suppl A):1022.
12. Chekanov V, Maternowski M, Tchekanov G, Mortada ME, Eisenstein R. A new alternative for the use of electrical stimulation: atherosclerosis prevention [abstract]. J Basic Applied Myology 2000;10:62-3.
13. Anitschkow N. Experimental atherosclerosis in animals. In: Cowdry EV, ed. Atherosclerosis. New York: Macmillan; 1993. p. 271-322.
14. Cornhill JF, Roach MK. A quantitative study of the localization of atherosclerotic lesions in the rabbit aorta. Atherosclerosis 1976;23: 489-501.
15. Roach MR, Cornhill JF, Fletcher J. A quantitative study of the development of sudanophile lesions in the aorta of rabbits fed a low-cholesterol diet for up to six months. Atherosclerosis 1978;29: 259-64.
16. Zeindler CM, Kratky RG, Roach MR. Quantities measurements of early atherosclerotic lesions on rabbit aortae from vascular casts. Atherosclerosis 1989;75:245-55.
17. Kratky RG, Ivey J, Rogers KA, Daley S, Roach MR. The distribution of fibro-fatty atherosclerotic lesions in the aortae of casein and cholesterol fed rabbits. Atherosclerosis 1993;99:121-31.
18. Daley SJ, Herderick EE, Cornhill JF, Rogers KA. Cholesterol-fed and casein fed rabbit models of atherosclerosis, part 1: differing lesion area and volume despite equal plasma cholesterol levels. Arterioscler Thromb 1994;14:95-104.
19. Ivey J, Roach MR, Kratky RGH. A new probability mapping method to describe the development of atherosclerotic lesions in cholesterol fed rabbits. Atherosclerosis 1995;115:73-84.
20. Forster BA, Javed Q, Leake DS, Weinberg PD. High-resolution mapping of the frequency of lipid deposits in thoracic aortae from cholesterol-fed and heritable hyperlipidemic rabbits. Atherosclerosis 1996;120:249-53.
21. Sinsinger H, Silberbauer K, Auerswald W. Quantitative investigation of sudenophilic lesions around the aortic ostia of human fetuses, newborns and children. Blood Vessels 1980;17:44-52.
22. Caro CG, Fitz-Gerald JM, Schroter RM. Atheroma and arterial wall shear: observation correlation and proposal of a shear dependent mass transfer mechanism for atherodenesis. Proc R Soc Lond B 1971;177:109-59.
23. Svindland A, Walloe L. Distribution pattern for sudanophilic plaques in the descending thoracic and proximal abdominal aorta. Atherosclerosis 1985;57:219-22.
24. Cornhill JF, Herderick EE, Stary HC. Topography of human aortic sudanophilic lesions. In: Leipsch DW, ed. Blood flow in large arteries: application to atherogeneisis and clinical medicine. Basel, Swizerland; S Karger; 1990. p. 13-9.
25. Chekanov V, Maternowski M, Eisenstein R, Hernandez I, Nikolaychik VV. Angiogenesis in the latissimus dorsi muscle using different regimens of electrical stimulation and pharmaceutical support. ASAIO J 2000;46:305-12.
26. Chekanov VS, Rieder MA, Tchekanov GV, Smith LM, Eisenstein R, Schmidt DH. Comparison of different regimens of electrical stimulation applied to nonmobilized and newly mobilized latissimus dorsi muscle. J Card Surg 1997;12:343-53.
27. Ferrara N. Vascular endothelial growth factor. Trend Cardiovasc Med 1993;3:244-50.
28. Folkman J. Clinical application of research on angiogenesis. N Engl J Med 1995;337:1757-63.
29. Annex B, Torgan CE, Lin P, Taylor DA, Thompson MA, Peters KG, et al. Induction and maintenance of increased VEGF protein by chronic motor nerve stimulation in skeletal muscle. Am J Physiol 1998;274:H860-7.
30. Hang J, Kong L, Gu JW, Adair TH. VEGF gene expression is upregulated in electrically stimulated rat skeletal muscle. Am J Physiol 1995;269:H1827-31.
31. Silverstein RL, Nachman RL. Angiogenesis and atherosclerosis. The mandate broadens. Circulation 1999;100:783-5.
32. Moulton KS, Heller E, Konerding MA, Flynn E, Palinski W, Folkman J. Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apoliprotein E-deficient mice. Circulation 1999;99:1726-32.
33. Zhou M, Chen Y, Ougang Q, Liu SX, Pang ZJ. Reduction of the oxidative injury to the rabbits with atherosclerosis by protein bound polysaccharide from coriolus vesicolor. Am J Clin Med 2000;28: 239-49.
34. Ritterhaus CW, Miller DP, Thomas LJ, Witzenbichler B, Yang J, Magner M, et al. Vaccine-induced antibodies inhibit CETP activity in vivo and reduce aortic lesions in a rabbit model of atherosclerosis. Arterioscler Thromb Vasc Biol 2000;20:2106-12.
35. Arruzabala ML, Noa M, Menendez R, Mas R, Carbajal D, Valdes S, et al. Protective effect of policosanol on atherosclerotic lesions in rabbits with exogenous hypercholesterolemia. Braz J Med Biol Res 2000;33:835-40.
36. Greenstein SM, Sun S, Calderon TM, Kim DY, Schreiber TC, Schechner RS, et al. Mycophenolate mofetil treatment reduces atherosclerosis in the cholesterol fed rabbit. J Surg Res 2000;91:123-9.
37. Wang B, Ho HK, Lin PS, Schwarzacher SP, Pollman MJ, Gibbons GH, et al. Regression of atherosclerosis: role of nitric oxide and apoptosis. Circulation 1999;99:1236-41.
38. Diaz M, Frei B, Vita J, et al. Antioxidants and atherosclerotic heart disease. N Engl J Med 1997;337:408-16.
39. Smith C, Mitchinson M, Arnoma O, et al. Stimulation of lipid peroxidation and hyroxyl-radical generation by the contents of human atherosclerotic lesions. Biochem J 1992;286:901-5.
40. Lee T, Shiao M, Pan C, et al. Iron-deficient diet reduces atherosclerotic lesions in apo E-deficient mice. Circulation 1999;99:1222-9.
41. Halliwell B. Use of sesferrioxamine as a "probe" for iron-dependent formation of hydroxyl radicals. Biochem Pharmacol 1985;34:229-33.
42. Duffy S, Biegelsen E, Holbrook M, et al. Iron chelation improves endothelial function in patients with coronary artery disease. Circulation 2001;103:2799-804.
43. Cooper E. Nitric oxide and iron proteins. Biochem Biophys Aeta 1999;1411:290-309.
44. Biemond P, Swaak A, Beindorff C, et al. Superoxide-dependent and independent mechanisms of iron mobilization from ferritin by xanthine oxidase. Biochem J 1986;239:169-73.
45. Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med 1999;340:115-26.

Submitted Apr 30, 2001; accepted Oct 31, 2001.