

Strauch, et al. 2009. Pulsed Electromagnetic Fields Increase Angiogenesis in a Rat Myocardial Ischemia Model. Bioelectromagnetic Society Meetings June, Davos, Switzerland.

Introduction: Myocardial ischemia with consequent loss of functioning cardiac muscle, continues to be one of the leading causes of morbidity and mortality in our society. In response to ischemic injury the myocardium attempts to retain or increase its blood supply. Treatment usually consists of an attempt to enhance the body's natural angiogenic processes. Thus, angiogenic growth factors and pro-angiogenic cytokines have been used in attempts to induce new vessel growth. Unfortunately, this has produced very limited success. Pulsed electromagnetic fields (PEMF), of the type used for recalcitrant fracture repair, were reported to produce a seven-fold increase in endothelial cell tubularization in vitro, as well as four-fold increased angiogenesis in a diabetic mouse model, via increased FGF-2 production [1,2]. In both studies, inhibition of FGF-2 eliminated the PEMF effect. We have also demonstrated, with radio frequency PEMF, increased neovascularization in a transferred arterial loop in a rat groin model sufficient to allow elevation of a free skin flap based on the newly produced vascular bed [3,4]. This study was designed to assess the effect of radio frequency PEMF, configured a priori to modulate the Ca<sup>2+</sup>/CaM (calmodulin) dependent nitric oxide (NO) pathway [5,6], on angiogenesis in an experimental myocardial injury model in the rat.

Materials And Methods: A reproducible thermal myocardial zone of injury was created in the region of the distal aspect of the Left Anterior Descending Artery at the base of the heart of 100 adult male Sprague Dawley rats [7]. Animals were randomly divided into active and sham groups. The PEMF device waveform was a 2 msec burst of 27.12 MHz sinusoidal waves repeating at 5 bursts/sec delivering 0.05 G at the tissue target (Ivivi Health Sciences, Inc., Montvale, NJ). Five freely roaming animals in a standard rat plastic cage, with all metal portions removed, were placed within a single turn 14 x 21 inch coil. Exposure was 30 min twice daily for 3, 7, 14 or 21 days. Sham animals were identically exposed, but received no PEMF signal. A separate group of 20 animals treated for 7 days received L-NAME, a general NOS inhibitor, in their drinking water. Upon sacrifice, myocardial tissue specimens were stained with CD-31 and the number of new blood vessels was counted on histological sections at the interface between normal and necrotic muscle at each time point. Data was analyzed using the Student's t test or ANOVA, as required. Significance was accepted at  $P \leq 0.05$ .

Results: Of the 100 animals which entered the study, 84 were available for analysis. Overall survival rate for this rat myocardial injury model was, thus, approximately 80%. The PEMF effect in this study is best illustrated by examination of normalized (PEMF/sham) mean new vessel counts in the peri-ischemic tissue. Mean new vessel count was not significantly increased by PEMF vs sham treatment at POD 3, but was significantly increased at POD 7 (+ 50%), POD 14 (+ 67%), and POD 21 (+ 99%). These results are summarized in the figure.

The 7 day experiment involving L-NAME, a general NOS inhibitor, was designed to assess whether the PEMF signal had its effect on angiogenesis via the NO pathway. The results show sham and treated animals were equivalent, indicating L-NAME completely blocked the PEMF effect on angiogenesis, and providing strong evidence the PEMF transduction pathway in this study involved Ca/CaM in the NO signaling cascade. These results are summarized in the figure.

Conclusions: Impaired blood flow in ischemic myocardial tissue creates a hypoxic environment, which induces expression of growth factors such as VEGF and FGF-2 [8]. Expression of these growth factors triggers angiogenesis which requires the synthesis of NO, so the manipulation of NO may constitute an effective therapy to increase angiogenesis in cardiac ischemia [9]. In fact, lack of sufficient endogenous NO may be the reason VEGF therapy has had very limited success. There is an intricate relationship between VEGF and endothelial nitric oxide synthase (eNOS) which catalyzes the formation of NO from endothelial cells. This makes the modulation of eNOS activity a potentially useful strategy for modulating angiogenesis. To this end, the PEMF signal utilized in this study was configured a priori, to increase NO production via a Ca/CaM transduction pathway. The signal was configured utilizing the well known binding kinetics of Ca<sup>2+</sup> to CaM in a model which predicted that millisecond range burst durations of a 27.12 MHz sinusoidal wave would increase Ca<sup>2+</sup> binding kinetics at amplitudes in the 0.05 G range [5,6]. Given the results of this study, it is intriguing to speculate that PEMF indeed modulated eNOS activity through an effect on Ca<sup>2+</sup> binding to CaM. Certainly inhibition of the PEMF effect by L-NAME provides strong support. Additionally, the ability to augment angiogenesis with PEMF at the infarct/muscle junction may allow for successful functioning myoblast engraftment, replacing the necrotic muscle.