A PULSING ELECTROMAGNETIC FIELD PROMOTES THE DIFFERENTIATION OF OSTEOBLASTS (MC3T3) AT LOW CELL DENSITY IN VITRO.

*Button, C; **Spadaro, J A; **Margulies, B S; **Allen, M J; **Wang, Y; **Damron, T A;
*Dept. of Neuroscience and Physiology, **Department of Orthopedic Surgery, SUNY Upstate Medical University, Syracuse, NY
spadaroj@upstate.edu

Introduction:
Pulsing electromagnetic fields (PEMF) have been shown to variously stimulate osteoblast proliferation and differentiation in cell culture, both of which could relate to the mechanism by which such stimuli enhance bone repair in fracture non-unions [1,2,3]. Up-regulated proliferation would tend to increase the number of cells available to make bone while more rapid differentiation would tend to promote mineralization and osteoprotegerin (OPG) expression and reduce coupled osteoclastic resorption. Most observations previously have been made in high density or confluent bone cell cultures where the cells would already be slowly proliferating and more differentiated. The effect of PEMF on lower density, actively dividing osteoblasts, however, does not seem to have been specifically studied.

Objective:
The objective of this work was to investigate the effect of PEMF treatment on osteoblast (OB) proliferation and differentiation in low-density cultures and also on their ability to stimulate osteoclast (OC) formation in osteoblast/pre-osteoclast co-cultures.

Methods:
MC3T3-E1 mouse osteoblast-like cells (OBs) (gift of Dr. Baruch Frankel, USC) were seeded at 1x10^3 cells/cm^2 in 4 well chamber slides to observe proliferation changes and 1x10^4 cells/cm^2 in 12 well plates to study changes in alkaline phosphatase (ALP) and caspase-3. Proliferation changes were measured using cell counting and BrdU labeling (proliferation rate). In the co-culture experiments, MC3T3 OBs were seeded at 2x10^3 cells/cm^2 in 12 well plates 24 hours prior to the addition of 2x10^5 cells/cm^2 of primary human monocytes. The resultant monocyte derived multi-nucleate osteoclasts were counted using TRAP staining (Sigma). PEMF or sham control stimulation was applied for 30 minutes a day at 37°C for 4 days (MC3T3 cells) and for 15 days (MC3/monocyte co-cultures) (the approximate time needed for OC formation to occur). Two PEMF waveforms were used, both similar to those used clinically and composed respectively of short, asymmetric magnetic pulses repeated at 1.5 or 15 Hz, with peak ampitudes of 0.05 and 2 mT, and peak induced EMP bursts of 20 and 100 mV measured in an 8 mm/50 turn pick-up coil [4]. The magnetic field was directed horizontally, parallel to the plane of the plated cells. All studies n=4 per group.

In a follow-on experiment, MC3T3-E1 cells were seeded in 6-well plates at a low density, 1x10^4 cells/cm^2, and also at high density (1x10^5 cells/cm^2). Low density cells were allowed to grow for 24 hours and high density cells were first grown for 5 days to pre-confluency before their single 30 minute PEMF treatment. 24 hours later RANKL, OPG and ALP mRNA expression was measured by RT-PCR vs. GAPDH.

Results:
30 minute PEMF treatment for 4 days decreased growth in MC3T3 cells by 26% and 25% in the 1.5 Hz and 15 Hz groups compared to the control (p<0.001)(Fig 1). The percentage of BrdU labeled cells also markedly decreased in the 1.5 Hz and 15 Hz groups vs. the control (p<0.001)(Fig 2). However, PEMF treatment increased caspase-3 activity over controls by 25% and 38% (p<0.01) as well as ALP activity, by 22% and 32% (p<0.03)(Fig 3) in the 1.5 Hz and 15 Hz groups. PEMF treatment of pre-OC/OB co-cultures for 15 days (30 min./day) decreased OC cell number by 19% and 39% in the 1.5 Hz and 15 Hz groups compared to the control (p<0.01) (Fig 4). RT-PCR did not detect mRNA expression of RANKL or ALP in the low density OB cells but detected mRNA expression of OPG which was increased in the 1.5 Hz and 15 Hz groups compared to control. In high density cultures, mRNA expression of RANKL was also not detected, ALP was unchanged and OPG was slightly decreased in both the 1.5 Hz and 15 Hz cases compared to the control. In general, the 15 Hz waveform appeared to be more influential than the 1.5 Hz in affecting OB function in this study.

Conclusions:
These findings suggest that PEMF treatment slows proliferation and promotes the differentiation of OBs at low OB cell densities which are actively dividing. The decrease in induction of OCs and the increase in caspase-3 and OPG mRNA supports this contention [5]. Therefore, PEMF effects on bone in vivo could include the acceleration of maturation and mineralization with a reduction in resorption, potentially desirable for focal treatment of osteoporosis.


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