Pulsed electromagnetic field changes protein expression in urothelial cells in culture: A pilot study

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Introduction: Pulsed electromagnetic field (PEMF) therapy has been found to be an effective treatment for fracture non-unions, as well as multiple chronic pain conditions including osteoarthritis and fibromyalgia. *In vitro* studies have found that PEMF modulates the inflammatory cytokine profile in immune cells. Recently, it has been suggested that PEMF therapy may be effective for pain management in interstitial cystitis/bladder pain syndrome (IC/BPS), a condition wherein bladder urothelial cell function can be abnormal, resulting in a compromised bladder epithelium. The way in which PEMF may affect urothelial cells (i.e., the mechanism of action) is unknown. Therefore, the objective of the current study is to evaluate changes in inflammatory gene expression in urothelial cells following exposure to PEMF.

Methods: Urothelial cells from a single healthy donor (purchased from Sciencell Research Laboratories; Carlsbad, CA) were cultured in urothelial cell specific growth media under identical conditions (37^{o}C, 5% CO_2) in four individual wells within two 96 well plates. Cells in one plate were exposed to PEMF (33Hz, 0.5mT) for 10 minutes, twice daily on Monday, Wednesday, and Friday for one week. The other (control) plate was incubated under identical conditions in a separate incubator. To control for background, growth media alone (sans cells) was placed in two empty wells within each plate. Immediately following the final treatment, 100 μL of growth media was taken from each well (4 PEMF, 4 non-treated controls, and 4 media-only background controls), frozen at -20°C and sent for analysis with a multiplex protein expression array (measures 48 cytokines and chemokines; Eve Technologies). Cytokine/chemokine baseline (i.e., media alone) expression levels were subtracted from expression levels in supernatant from cultured cells to remove background noise, and then average expression levels were compared between exposed (treatment group) and unexposed (control) cells utilizing student's t-test with a $p<0.05$ being considered significant.

Results: PEMF exposure did not significantly alter baseline cytokine, chemokine, and growth factor levels in growth media alone, except for Eotaxin (1.065 vs 1.905 pg/mL; $p=0.049$). Out of 48 proteins analyzed, 9 were found to be significantly altered via PEMF exposure. Epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF-2) were found to be downregulated by PEMF exposure, while Fms-related tyrosine kinase 3 ligand (FLT-3L), granulocyte colony stimulating factor (G-CSF), growth related oncogene alpha (GROα), Interleukin 15 (IL-15), monocyte chemoattractant protein-1 (MCP-1), monokine induced by gamma (MIG), and platelet derived growth factor-AA (PDGF-AA) were increased by exposure to PEMF ($p<0.05$; Table 1).

Conclusions: This pilot study has revealed that even a relatively brief exposure to PEMF significantly alters the expression of growth factors and cytokines in cultured urothelial cells. Further characterization is needed, specifically in urothelial cells from patients with IC/BPS, to begin to describe the molecular basis for patient benefit/improvement following PEMF treatment.

Table 1. Cytokines, chemokines, and growth factors that are significantly altered by exposure to PEMF

Cytokine/Chemokine/ Growth Factor	**PEMF exposed Mean ± SD (pg/ml)**	**Controls Mean ± SD (pg/ml)**	**Difference; P value**
EGF	4.07 ± 0.38	5.2 ± 0.35	-1.13; p=0.019
FGF-2	1850.5 ± 63.47	2333.46 ± 132.47	-482.96; p=0.012
FLT-3L	2.59 ± 0.56	1.21 ± 0.58	1.38; p=0.041
G-CSF	226.58 ± 70.05	66.60 ± 33.58	159.98; p=0.04
GROα	404.89 ± 89.52	171.91 ± 106.37	232.98; p=0.046
IL-15	2.42 ± 0.67	1.01 ± 0.34	1.41; p=0.048
MCP-1	3.63 ± 0.64	1.71 ± 0.247	1.92; p=0.023
MIG	6.94 ± 2.78	1.56 ± 0.64	5.38; p=0.031
PDGF-AA	826.39 ± 149.37	480.91 ± 111.03	345.48; p=0.036