PULSED ACOUSTIC CELLULAR THERAPY SUPPORTS PRO-ANGIOGENIC FACTORS EXPRESSION IN ISCHEMIC MUSCLES

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INTRODUCTION:
PACE (Pulsed Acoustic Cellular Expression) is a novel technology based on the use of pulsed acoustic energy waves (specific shock waves) that has been clinically shown to produce a cellular expression response which may be used in a clinical practice. Electrohydraulic Extracorporeal Shock Waves (ESWT) are generated by high voltage spark discharge under water. This causes an explosive evaporation of water, producing high-energy acoustic waves. By focusing the acoustic waves with a semi-ellipsoid reflector, the waves can be transmitted to a specific tissue site. Extracorporeal Shock Wave Technology (ESWT) was introduced for medical practice approximately 25 years ago for fragmentation of kidney stones. This technique has been successfully employed mostly in orthopedic diseases and several inflammatory tendon diseases. It has been proven that ESWT therapy improves tissue regeneration and neovascularization. Tissue ischemia alters tissue circulations and affects wound healing. The aim of this study is to show whether PACE therapy induces the neovascularization and improves blood supply to the tissues.

MATERIALS AND METHODS:
Cremaster muscles (Figure 2) intravital microcirculation recorded:
1. Non-ischemic controls
2. 5h ischemia control
3. pre-ischemic (5h) PACE treatment
4. post-ischemic (5h) PACE treatment
Assessments:
1. Microcirculatory hemodynamics (capillary perfusion, leukocyte-endothelial interactions)
2. Immunohistochemistry
   - Leukocyte trafficking - adhesion molecules: E-selectin, ICAM-1, VCAM-1
   - Vasculogenesis: VEGF, von Willebrand factor (vWF)
   - Tightly regulated real-time PCR gene expression
     - Pro-angiogenic factors: vascular endothelial growth factor (Vegf), von Willebrand factor (Vwf), and endothelial nitric oxide synthase (eNos)
   - Pro-inflammatory factors: inducible nitric oxide synthase (iNos), chemokines (Ccl2, Cxcl5)
Cremaster muscle flap spread in the tissue bath, prepared for direct in vivo monitoring of microcirculation.

RESULTS:
The aim of this study is to show whether PACE therapy induces the neovascularization and improves blood supply to the tissues.

CONCLUSIONS:
1. Pre-ischemic and post-ischemic PACE treatment down-regulated adhesion molecules expression and this correlated with reduction of sticking leukocytes in microcirculation.
2. Post-ischemic PACE treatment inhibited inflammatory responses by reduced pro-inflammatory genes expression.
3. Post-ischemic PACE treatment had no negative impact on expression of pro-angiogenic genes.
4. Post-ischemic PACE treatment induced expression of pro-angiogenic proteins (VEGF, VWF) which correlated with increased of neovascularization.