Studying The Relationship Between Pulsed Electromagnetic Field (PEMF) Dosage And In Vitro Functional Response Of Tendon Cells

Deborah Stanco1, Marco Viganò1, Stefania Setti1, Emanuela Galliera3,1, Valerio Sansone4,1, Laura de Girolamo1.

1IRCCS Istituto Ortopedico Galeazzi, Milano, Italy, 2IGEA Clinical Biophysics spa, Carpi, Italy, 3Dipartimento di Scienze Biomediche, Chirurgiche ed Odontoiatriche, Università degli Studi di Milano, Milano, Italy, 4Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Milano, Italy.

Disclosures:
D. Stanco: None. M. Viganò: None. S. Setti: 3A; IGEA Clinical Biophysics spa. E. Galliera: None. V. Sansone: None. L. de Girolamo: 3B; AT Grade.

Introduction: Tendon disorders represent a diagnostic and therapeutic challenge for physicians. Traditional treatments are characterized by a long recovery time and a high occurrence of injury relapses. Despite the growing clinical interest in pulsed electromagnetic fields (PEMFs) few studies on their effect on tendons and ligaments have been conducted. Tendon resident cells (TCs) are a mixed population, made up mostly of tenocytes and tendon stem/progenitor cells, which are responsible for tissue homeostasis. Since studies on the effect of PEMFs on this cell population are conflicting, we evaluated the possible relationship between PEMF dosage and TC response, comparing the effects on tendon cells of different types of PEMF treatment, varying in field intensity, duration and number of exposures. In particular, we compared the in vitro effect of low and high intensity PEMFs on TCs (PEMF-1.5 mT; PEMF-3 mT) and of repeated treatments (R-PEMF-1.5mT).

Methods: TCs were isolated from the waste portion of semitendinosus and gracilis tendons of 6 healthy donors undergoing ACL reconstruction; at P4 they were exposed to different PEMF treatments (intensity: 1.5mT or 3mT; duration: 8 or 12 hours; periodicity: single or 3 treatments with an interval of 48h). Viability and DNA content were assessed by MTT and CyQuant, respectively, immediately at the end of the treatment (0d) and two days later (2d). Live&Dead staining was also assessed in order to detect live (green fluorescence) and dead (red fluorescence) cells immediately after all treatments; the percentage of these cells was defined as PLive = NLive/(NLive+NDead), where NLive is the number of green cells and NDead represents the number of red cells in the same image. At the same time points the expression of SCX, COL1A1 and VEGF were evaluated with RT-Real Time PCR, as well as the release of the cytokines TGFβ, IL6, IL10, IL1β, and TNFα by ELISA.

Results: Both 3mT-PEMF and R-1.5mT-PEMF exposure did not provoked any apoptotic events likely to single 1.5mT PEMF treatment as confirmed by the very low count of dead cells in all samples. Moreover, viability of TCs exposed to 8 hours of all PEMF treatments was very similar to untreated cells at all time points except for R-1.5mT-PEMF treated cells at 2 day that showed a significant decrease of -19% of viability at 2 days respect to untreated cells (p<0.05) and of -15% in comparison with 1.5mT-PEMF treated cells. On the other hand all treatments applied for 12h increased TCs viability respect to untreated cells and, in particular, TCs at 2 days from 3mT-PEMF treatment showed a significant increase of +13% respect to untreated cells (p<0.01). The 8 hours PEMF treatments were able to induced an immediate increases of DNA content in all samples and mostly at day 0 in 1.5mT-PEMF treated cells (+25% versus untreated cells; p<0.05). Otherwise, 12 hours R-1.5mT-PEMF treated cells showed, above all at 2 days, significantly lower DNA levels than untreated one (-25%, p<0.01) contrary to 1.5mT PEMF and 3mT-PEMF cells that showed, at the same time point, DNA content increase of 13% and 19%, respectively, respect to untreated TCs. In regard to gene expression, all PEMF treatments induced SCX expression in a dependent manner by the length of the treatment (8 or 12 hours), but only single 1.5mT-PEMF exposure was able to induced significantly SCX up-regulation in treated cells respect to untreated ones (p<0.05). Even as regards VEGF-A expression, all PEMF treatments were able to induce an increase levels of this transcript, and above all immediately after the single 1.5mT-PEMF treatment (8h: +100%, p<0.05; 12h: +75%, p<0.05). At 2 days VEGF levels decreased in all PEMF treatments expect for 8 hours 3mT PEMF treated cells that showed increase of 6%, respect to 0 day treated cells. In regard to collagen type I expression, only TCs treated with single 1.5mT-PEMF treatment, showed a strongly increase after both 8h (+140%) and 12h (+133%) respect to untreated cells. Indeed, COL1A1 expression of TCs exposed to both R-1.5mT and 3mT-PEMF was only slightly influenced. Interestingly, all PEMF treatment induced a downregulation of collagen type III expression that was statistically significant at Od in TCs treated with 8 hours of 1.5mT-PEMF in respect with untreated cell (-50%, p<0.05). All treatments induced a significant increase of IL6, IL10 and TGFβ release respect to untreated cells (p<0.05), especially R-PEMF-1.5mT that showed higher values in comparison to the single PEMF-1.5mT treatment (p<0.001). Also the release of pro-inflammatory cytokine IL-1β was slightly enhanced in all TCs treated but the TNFα production were not relevantly affected by any treatment.

Discussion: This study demonstrates that the length of exposure, the field intensity and the number of PEMF treatments differently affect tendon cell proliferation, gene expression and the release of pro- and anti-inflammatory cytokines without inducing any cytotoxic events. TC viability and proliferation were enhanced, with different kinetics, by both 1.5mT-PEMF (single or repeated) and 3mT-PEMF treatment. Moreover, the repetition of the 1.5mT-PEMF treatment did not prolong or enhance the...
effect observed after a single exposure to the same electromagnetic fields. Taken together, these results show that the lowest dosage of PEMF in terms of intensity and time is the most suitable treatment to stimulate in vitro TC proliferation and activation. The gene expression analysis performed on tendon-specific markers and VEGF, which are known to participate in the regenerative processes of the tendons, seemed to confirm these findings. The results regarding the effect of highest intensity and repetitive exposure to PEMF on SCX, VEGF and COL1A1 expression shows that only TCs exposed for both 8 and 12 hours to 1.5mT-PEMF elicited a prompt and significant up-regulation of these markers, whereas 3mT and R-1.5mT treatment were not able to induce further increases. It has been reported that tendinopathy increases the proportion of type III collagen, as well as the decrease of type I/type III collagen ratio. We showed a significant decrease in COL3A1 expression in cells treated with 1.5mT-PEMF, whereas lower effects were observed in cells treated with 3mT and R-1.5mT-PEMF. These results suggest that the 1.5mT-PEMF is probably optimal to elicit a response at transcriptional level in TCs and that PEMFs might lead to collagen production in normal rather than pathological proportion. Moreover, the positive effect of single or repeated 1.5mT-PEMF treatment is also confirmed by a significant increase in IL10 and TGFβ release. In conclusion, the lower intensity PEMF treatment was able to give better results in in vitro tendon cells in terms of cell proliferation, upregulation of tendon specific-gene expression and release of anti-inflammatory cytokines and TGFβ. In addition, the repetition of this low intensity treatment leads TCs to significantly increase the production of IL-10, which is strongly linked in the anti-inflammatory pathways. Although these results confirm the effectiveness of PEMF on tendon, a better comprehension of the mechanisms of action by which PEMFs act at cellular level on tendon cells will be necessary to define the best PEMF protocol to be used for tendon pathologies.

Significance: PEMFs treatment has been found to be a valid adjuvant in the treatment of tendon pathologies. A better comprehension of its effects and of its mechanisms of action by which PEMFs act at cellular level on tendon cells may be useful to define the best PEMF protocol to be used for tendon pathologies.

Acknowledgments: Prof. Corsi Romanelli MM; Dr. Marazzi MG;

References:

ORS 2014 Annual Meeting
Poster No: 1356