

Shockwave Treatment Promotes the Expression of Alox15 in Pro-Resolving Macrophages

Naths Grazia Sukubo (1), Stefano Respizzi (2), Alberto Mantovani (1),
Maria Cristina D'Agostino (2), Massimo Locati (1)

Institution: Dept. of Medical Biotechnologies and Translational Medicine, University of Milan; (2) Humanitas Research Hospital, Milan, Italy

Device and producing company: OrthoGold 100, MTS Germany

Introduction: Extracorporeal Shock Wave (SW) treatment is broadly used as a non-surgical therapy in various diseases, demonstrating its pro-angiogenic and anti-inflammatory effects. However, the molecular mechanisms translating shock waves in a biological response with potential therapeutic activity are largely unknown. As macrophages take part in both the onset and amplification of the inflammatory response, and well in its resolution, we investigated the effect of SWT on their biology.

Methods: Human monocyte-derived macrophages were polarized to pro-inflammatory classic macrophages (M1) by over-night exposure to LPS+IFN γ or anti-inflammatory alternative macrophages (M2) by overnight exposure to IL-4. Using an Orthogold100 device as electrohydraulic SW source, macrophages were then exposed in a thermostated water bath to SW (400 impulses, 3.5Hz, 0.1-0.03 mJ/mm²) at different time points. RNA was then extracted and expression levels of M1 (CD80, CCL5, CXCL9, CXCL10, COX2) and M2 (CD206, ALOX15, IL-10, TGF β) genes were analyzed by qRT-PCR.

Results: SW had no direct effect on any transcript investigated, when applied to resting macrophages. Though the effect did not reach statistical significance for the elevated variability among different experiments, when applied to inflammatory macrophages SW showed a faint downmodulatory effect on some M1 markers (CCL5, CD80), while others (including COX2) were not affected. Conversely, at low energy level (0.03 mJ/mm²) SW had a significant reproducible and time-dependent synergistic effect with IL-4 for the induction of ALOX15 in M2 macrophages. Interestingly, other M2 genes (including IL-10) were not affected by SW exposure.

Discussion: Synergism with IL-4 in the induction of some M2 genes, suggesting that SW therapeutic potential may be at least in part mediated by their effect on macrophage biology.

Conclusion: Our results demonstrate that SW at low energy level act in In conclusion, we did not detect any synergistic effect of SW on the anti-inflammatory cytokine IL-10, but we identified ALOX15 as a SW-responsive gene in M2 macrophages. ALOX15 has a key role in the resolution of the inflammatory response via production of the pro-resolving eicosanoid lipoxin A4 and of endogenous agonists of the PPAR γ pathway and could therefore be involved in the therapeutic effects observed after exposure to SW.