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Original research

Effect of shock waves on macrophages: A possible role in tissue regeneration and remodeling

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HIGHLIGHTS

- We report the effects of Shock Waves (SW) on macrophages activity in vitro.
- SW did not induce activation of resting macrophages.
- Low energy SW dampens the induction of the pro-inflammatory profile in M1 macrophages.
- Low energy SW promotes the acquisition of an anti-inflammatory profile with M2 macrophages.

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ABSTRACT

Introduction: Extracorporeal Shock Wave Therapy (ESWT) is broadly used as a non-surgical therapy in various diseases for its pro-angiogenic and anti-inflammatory effects. However, the molecular mechanisms translating tissue exposure to shock waves (SW) 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 SW on their biology.
Methods: Human monocyte-derived macrophages were polarized to classic (M1) pro-inflammatory macrophages or alternative (M2) anti-inflammatory macrophages and exposed to SW ad different intensities. Expression levels of marker genes of macrophage activation were measured by qPCR at different time points.
Results: SW did not induce activation of resting macrophages at any energy level used. Conversely, when used at low energy SW caused a significant inhibition of some M1 marker genes (CD80, COX2, CCL5) in M1 macrophages and a significant synergistic effect for some M2 marker genes (ALOX15, MRC1, CCL18) in M2 macrophages. SW also affected cytokine and chemokine production, inducing in particular a significant increase in IL-10 and reduction in IL-1β production.
Conclusions: Macrophage exposure to low energy SW dampens the induction of the pro-inflammatory profile characterizing M1 macrophages and promotes the acquisition of an anti-inflammatory profile synergizing with macrophage alternative activation.

1. Introduction

Since its original introduction in medicine for kidney stones treatment (lithotripsy) in the early nineties, extracorporeal shock wave therapy (ESWT) has significantly expanded its fields of clinical applications, first to musculoskeletal diseases and later on to regenerative medicine [1–3]. ESWT are presently applied to a wide range of pathologies of different origins and localization, in

Abbreviations: CCL, CC chemokine ligand; CCR, CC chemokine receptor; CXCL, CXC chemokine ligand; ESWT, extracorporeal shock wave therapy; IL, interleukin; IFN, interferon; LPS, lipopolysaccharide; SW, shock waves.

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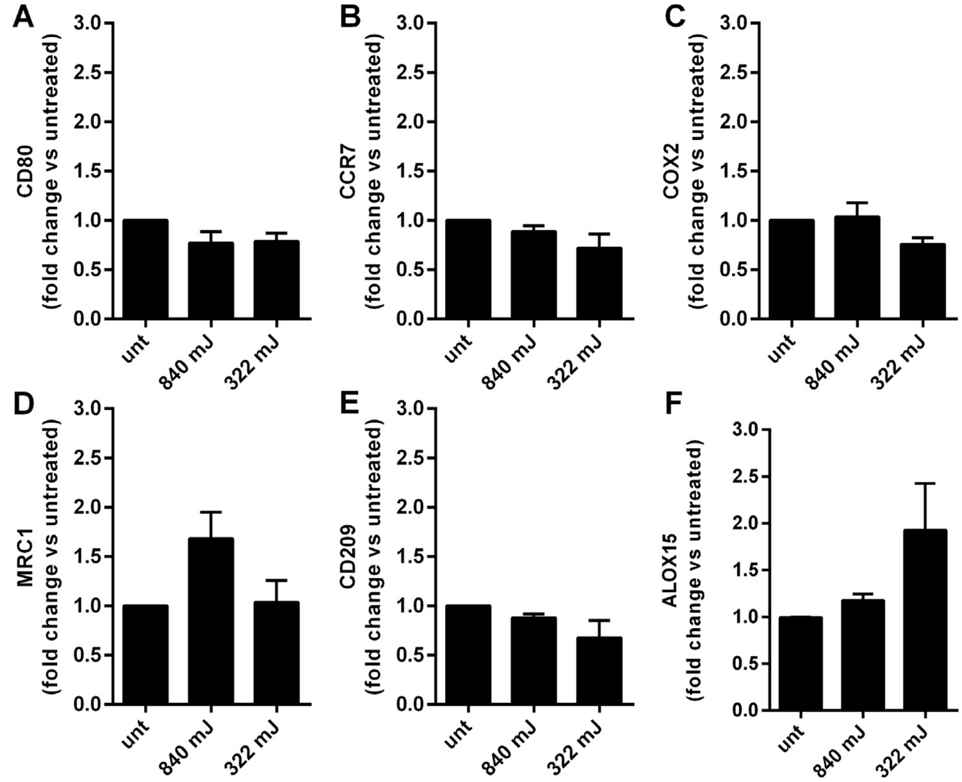

Fig. 1. SW effects on resting macrophages. Resting macrophages were exposed to 322 or 840 mJ SW and after 4 h expression levels of M1 (A–C) and M2 (D–F) markers were analyzed by qPCR. Results were normalized on the housekeeping gene GAPDH and expressed as fold enrichment compared to untreated macrophages (unt). Results are shown as mean ± SEM of 3 independent experiments.

orthopedics (tendinopathies, bone healing disturbances, vascular bone diseases), dermatology/vulnology (wound healing disturbances, ulcers, painful scars) [1], and neurology (spastic hypertonia and related syndromes) [4]. More recently, the positive effects of ESWT on soft tissues and the vascular bed have made it possible its application in clinical practice also for some andrologic disturbances (induratio penis plastic, erectyle disfunctions) [5]. Regenerative and trophic effects have also been demonstrated in ischemic and related heart diseases, although at present ESWT application in this field is still experimental [6,7].

A key point in the ESWT history has been represented by the shift from the mechanical model of lithotripsy to its applications in not-urological fields, where mechanical stimulation is converted in biological reactions in a living tissue. This phenomena is supported by mechanotransduction pathways, which imply the activation of a number of largely unknown cellular events, responsible for the positive effects of ESWT on cell metabolism and cell cycle, which ultimately account for the ductility of the therapy [8]. We can summarize the final effect of ESWT as a general improvement of tissue homeostasis and metabolism, accompanied by improving of the tissue self-healing abilities. Evidence from basic science and clinical studies indicates that this effect involves the ability of shock waves (SW) to support proliferation and differentiation of stem cells, which significantly

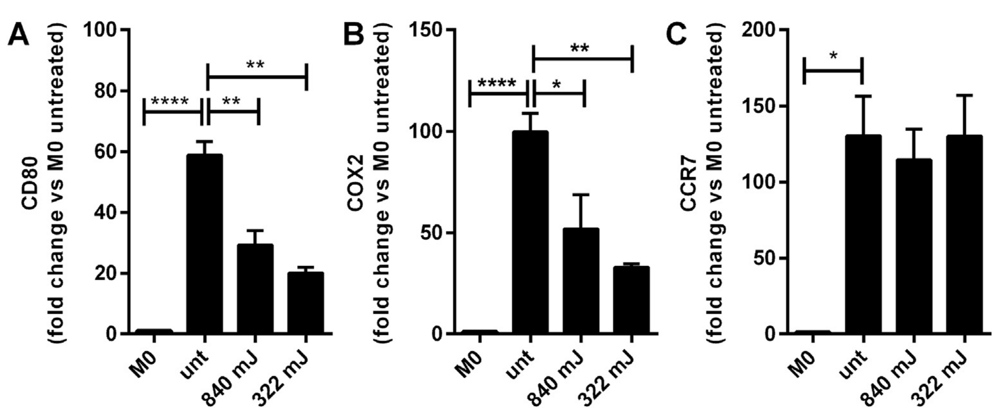

Fig. 2. SW effects on classical activated M1 macrophages. Macrophages were stimulated with LPS plus IFN-γ for 24 h to induce M1 polarization and then exposed to 322 or 840 mJ SW. After 4 h, expression levels of the M1 markers CD80 (A), CCR7 (B), and COX2 (C) were measured by qPCR. Results were normalized on the housekeeping gene GAPDH and expressed as fold enrichment compared to untreated macrophages (unt). Results are shown as mean ± SEM of 3 independent experiments. ****$p < 0.0001$, **$p < 0.01$, and *$p < 0.05$ by Bonferroni's multiple test.