Extracorporeal Shock Wave Therapy in Inflammatory Diseases: Molecular Mechanism that Triggers Anti-Inflammatory Action

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Abstract: Shock waves (SW), defined as a sequence of single sonic pulses characterised by high peak pressure (100 MPa), a fast rise in pressure (< 10 ns) and a short lifecycle (10 μs), are conveyed by an appropriate generator to a specific target area at an energy density ranging from 0.03 to 0.11 mJ/mm^2. Extracorporeal SW (ESW) therapy was first used on patients in 1980 to break up kidney stones. During the last ten years, this technique has been successfully employed in orthopaedic diseases such as pseudoarthosis, tendinitis, calcarea of the shoulder, epicondylitis, plantar fasciitis and several inflammatory tendon diseases. In particular, treatment of the tendon and muscle tissues was found to induce a long-time tissue regeneration effect in addition to having a more immediate anthalgic and anti-inflammatory outcome. In keeping with this, an increase in neoangiogenesis in the tendons of dogs was observed after 4-8 weeks of ESW treatment. Furthermore, clinical observations indicate an immediate increase in blood flow around the treated area. Nevertheless, the biochemical mechanisms underlying these effects have yet to be fully elucidated.

In the present review, we briefly detail the physical properties of ESW and clinical cases treated with this therapy. We then go on to describe the possible molecular mechanism that triggers the anti-inflammatory action of ESW, focusing on the possibility that ESW may modulate endogenous nitric oxide (NO) production either under normal or inflammatory conditions. Data on the rapid enhancement of endothelial NO synthase (eNOS) activity in ESW-treated cells suggest that increased NO levels and the subsequent suppression of NF-κB activation may account, at least in part, for the clinically beneficial action on tissue inflammation.

PHYSICAL PROPERTIES OF SHOCKWAVES

SW are longitudinal acoustic waves that propagate in water-like soft tissue in very much the same way as ultrasound does. However, in contrast to ultrasound, SW are single pulses with a duration of around one μsecond, a peak pressure amplitude of up to one hundred MPa and an energy flux density in excess of 2 mJ/mm^2.

Besides the "primary" use of ESW for kidney and urinary stone lithotripsy that started in 1980, it was observed, about a decade ago, that ESW at lower energy levels (0.03 to 0.11 mJ/mm^2) had an immediate anthalgic and anti-inflammatory effect [1-5]. Even at higher intensities and repetition rates than those used in lithotripsy, ESW do not cause significant macroscopic tissue heating [6]. Since ESW are typically focused on a spot that is only a few millimetres in diameter, the mechanical force is highly localised and generates shear stress.

Lithotripters use electrohydraulic, piezoelectric or electromagnetic generators to produce shock waves. The generator consists of a source that generates the acoustic wave and a focusing device. Focusing is achieved either by the geometric form of the source itself (a piezoelectric dish), by an acoustic lens (flat coil generator) or by ellipsoid reflectors (spark gap generator) and parabolic reflectors (cylindrical coil generator). The size of the therapy head is determined by the aperture and the treatment depth needed to treat obese patients. Newer lithotripters typically have larger apertures to reduce the energy flux density of the shock waves delivered to the skin so that less anaesthesia is necessary, or, in some cases, none at all. For soft tissue treatment, less focal depth is required than that used in a typical lithotripter, so the therapy head can be made smaller.

The acoustic pressure field of a lithotripter is measured in a water bath with a hydrophone. According to the International Electrotechnical Commission (IEC 61846), several parameters are determined as a function of the localisation of the reflector, including the peak-positive and peak-negative pressure, the rise time and the duration of the wave, the size of the focus in terms of full width at half the maximum pressure distribution in lateral and axial orientation (Table **1**). A typical pressure signal is shown in Fig. (**1**).

The mechanical forces of SW on acoustic interfaces like the stone urine boundary are strong enough to disintegrate a kidney stone. Transient haematuria is a well-known side effect of lithotripsy when the stone is in the kidney. Damage to the kidney ranges from damage on the cellular level to haemorrhaging due to vascular lesions most likely caused by cavitation [7]. In addition to direct mechanical forces, cavitation also contributes to stone disintegration.

Cavitation due to a single SW can be visualised by shadow or Schlieren photography in partially degassed water, Fig. (**2**). When the cavitation bubbles collapse, a secondary SW is emitted, shown as circles in the figure. The effect of several cavitation events is more difficult to measure quantitatively since cavitation is a stochastic process. Putting a thin aluminium foil in the focus and applying sev-

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Table 1. Peak Positive Pressure is the Maximum of the Signal in Fig. (1)

		Setting Energy-Level		
		Minimum 10	**Medium 60**	**Maximum 90**
Peak positive pressure	MPa	18	74	120
-6dB focal extend fx (-6dB)	mm	6.5	3	3
-6dB focal extend fy (-6dB)	mm	6.5	3	3
-6dB focal extend fz (-6dB)	mm	75	47	57
5 MPa focal extend fx (5MPa)	mm	13	34	34
5 MPa focal extend fz (5MPa)	mm	120	170	230
Positive energy flux density	mJ/mm^2	0.16	0.7	1.5
Energy flux density	mJ/mm^2	0.2	1	2
Positive energy of the 6 dB focus (E+)	mJ/mm^2	3.9	5	9.2
Energy of the 6 dB focus (E)	mJ	5.8	8.2	13.5
Positive energy of the 5 MPa focus (E+)	mJ	8.5	80	150
Energy of the 5 MPa focus (E)	mJ	16	160	270
Positive energy of the 5 mm focal area (E+)	mJ	2.2	10.7	21
Energy of the 5 mm focal area (E)	mJ	3.7	18.5	33

An orthogonal coordinate system x-y-z with the origin in the focus is used. Z is the axis symmetry and propagation of the SW, x-y is the lateral plane. f is the focal extend in the corresponding direction.

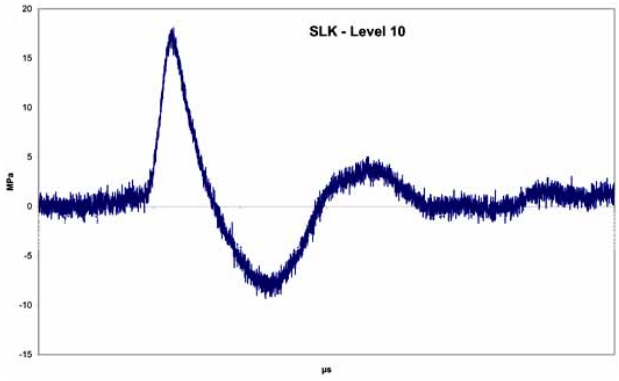

Fig. (1). Pressure pulse signal of the Modulith SLK at energy level 10 measured with a fibre optic hydrophone in the focus. Pressure is measured in MPa as a function of time, and the scale is 100 nsec.

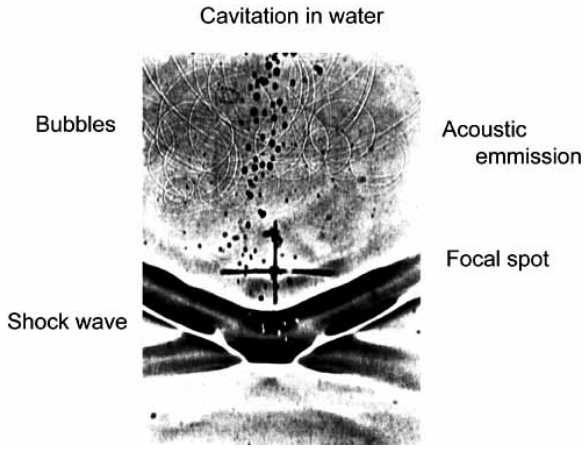

Fig. (2). A shadow image of the focal area (cross) is shown. The SW moves from top to bottom, and flash time is 20 nsec. Behind the shock front, cavitation bubbles are visible (black spots). Some of the bubbles have already collapsed, emitting secondary SW (circles).

eral hundred SW causes pitting and erosion of the foil, as shown in Fig. (**3**). The diameter of the erosion area is several times larger than the classic focus size f determined by measuring pressure distribution (see Table **1**). Cavitation in soft tissue has been measured *in vivo* with a focusing hydrophone (S. Russo, E. Marlinghaus, E. Amelio Unpublished data).

CLINICAL APPLICATION OF EXTRACORPOREAL SHOCK WAVES

As previously mentioned, ESW therapy (ESWT) was first applied in the first 1980 to break up kidney stones in patients [8]. Thereafter, this method rapidly became the first choice of treatment for urinary calculi. The application fields for ESWT now include gallbladder, pancreatic and salivary stones.

In 1987, Karpmann was the first to use ESWT on the musculoskeletal system [9]. In 1991, Valchanou applied ESWT in the treatment of delayed and non-union of fractures for the first time [1]. In 1992, Dahmen tried using shock waves to disintegrate calcareous deposits in a tendonitis of the shoulder [10]. Subsequently, ESWT was also observed to have a good clinical effect on pain. This led to ESWT being used as pain therapy in chronic enthesopathies.

Currently, ESWT has further applications in modern medicine. ESWT is frequently used in orthopaedic diseases [11] to induce bone healing in cases of non-union, delayed union and bone necrosis [12, 13]. It is also applied in musculoskeletal pathologies such as lateral elbow pain [14], upper

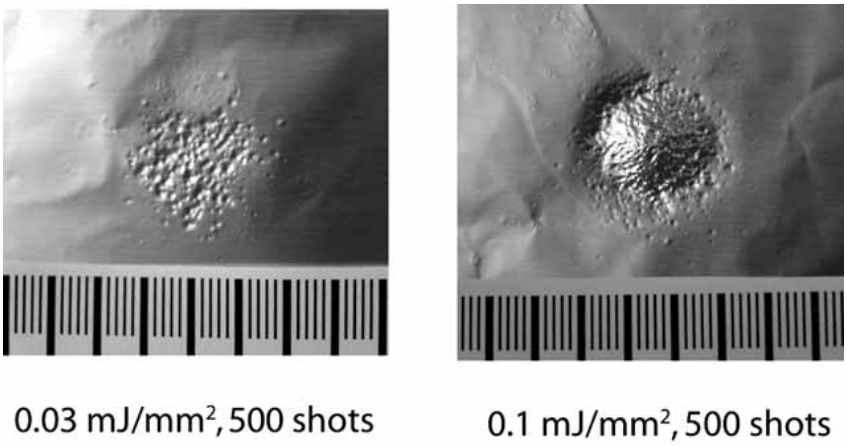

Fig. (3). Image of a thin aluminium foil in the focal area. The scale between the small bars is 2mm. Pitting by cavitation bubbles collapsing on or near the foil extends for over more than 1 cm.

limb hypertonia in patients who have had strokes [15], plantar fasciitis [16] and symptomatic heel spur [17].

Most recently, ESW treatment of end-stage coronary artery disease in ischaemic hearts has succeeded in significantly increasing perfusion in those regions with reduced blood flow [18, 19], suggesting that extracorporeal cardiac shock wave therapy (ECSWT) is an effective and non-invasive therapeutic strategy for myocardial ischaemia.

In 2008, K.F. Novak *et al.* [20] demonstrated that low energy levels of ESWT have an antibacterial effect on certain oral bacteria. In the same year, Sathishkumar *et al.* [21] suggested that ESWT could have promoted the regeneration of alveolar bone in a rodent model of periodontitis.

Accordingly, ESWT has been revealed as a safe and highly versatile therapeutic tool for enhancing tissue regeneration. It is optimistically supposed that the application field for ESWT will grow significantly in the near future.

EXPERIMENTAL APPLICATION OF ESW

ESW have been applied in *in vitro* systems at a significantly lower energy flux density (0.03 to 0.11 mJ/mm^2) than that used in lithotripsy in order to study the changes in molecular events inside cells. Change of acoustic impedance from liquid to cell membrane or vessel wall is considerably lower than that from fluid to stone, bone or lung [22] , so the direct mechanical forces of SW to cells are lower by at least one or two orders of magnitude. Indeed, animal or human studies have shown neither macroscopic tissue damage in the focus of the ESW in the myocardium nor a rise in the amounts of creatine kinase or troponin at these energy levels [19, 23].

An energy flux density of 0.1 mJ/mm^2 was assumed to be the threshold for the formation of stress fibres in the endothelial cells of human umbilical veins exposed to SW *in vitro* [23]. However, in the same model, Seidl [24] found lower thresholds (0.3 mL/mm^2) for the detachment of endothelial cells from the vessel wall.

The effects of ESW on cell integrity have been discussed in detail by Lokhandwalla and Sturtevant [25]. In an *in vitro* model of red blood cells exposed to a focused SW field of 40 MPa peak focal pressure, they calculate the mechanical force due to the fluid flow caused by the primary SW and the radial flow due to collapsing and expanding cavitation bubbles. Depending on the local and temporal gradient of the SW field and on the distance of the cell to an oscillating cavitation bubble, they calculate the maximum mechanical tension in the cell membrane as being in the order of 0.1 N/m.

Since the cells can be positioned anywhere in the highly anisotropic SW field and be between several micrometers and several hundred micrometers distant from a collapsing cavitation bubble, the tension at the cell membrane can vary from nearly 0 to 0.1 N/m. Thus, the effect of SW on the cell membrane ranges from no damage at all to mechanotransduction or the transient formation of pores or even lyses.

When SW hit tissue or cells in suspension, they induce cavitation and exercise shear stress on the cell membranes [25]. It therefore seems justifiable to assume that the biological effects of SW are based on either shear stress [26] or mechanotransduction [27].

ESW MOLECULAR MECHANISM OF ANTI-INFLAMMATORY ACTION

Although the biochemical mechanisms underlying the anti-inflammatory effect of ESW treatment are not fully understood, some reports present data indicating the critical role played by NO in this therapeutic effect.

NO Homeostasis

In any part of the body, there is a complex array of constitutively expressed nitric oxide synthase (cNOS), including neuronal and endothelial NOS (nNOS and eNOS, respectively). These enzymes guarantee the production of "physiological" or "tonic" amounts of NO, playing a critical role in a number of fundamental events such as vascular tone regulation, retrograde neurotransmission, long-terminal potentiation, angiogenesis and immune response [28]. "Physiological" amounts of NO, however, continuously fluctuate, depending on changes in the content of the enzyme substrates, L-arginine and oxygen, and of enzyme co-factors such as

NADPH, FAD, FMN and tetrahydrobiopterine as well as Ca^{2+}/calmodulin. Furthermore, changes in the serine and tyrosine phosphorylation states of enzymes and in the interaction with other proteins modulate NO production [29, 30]. Physical insults such as shear stress contribute to the temporally enhanced production of eNOS-derived NO in the blood vessel [31, 32]. Nevertheless, under physiological conditions, "tonic" amounts of NO may not exceed either the upper or lower limit, thus maintaining the general situation in which NO is allowed to exert its physiological action. This situation is known as "NO homeostasis".

Cross-Talk Between Constitutive NOS and Inducible NOS Under Inflammatory Conditions

Under pathophysiological conditions such as inflammation, another isoform of NOS, inducible NOS (iNOS), enters into the picture. Pro-inflammatory cytokines such as interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α) and interleukin 1-β (IL 1-β), together with other molecules such as lipopolysaccharides (LPS) [28], which are deeply involved in the inflammation process, induce the local and temporal expression of this enzyme. Time-spatially correct production of massive amounts of NO (presumably in the order of μM , [33]) by iNOS during its relatively brief period of expression (usually up to a few days) normally exerts beneficial action on the body, since invading organisms or damaged cells may quickly be eliminated by the cytotoxic action caused by the massive production of NO. Under certain circumstances, however, massive amounts of NO reacting with superoxide (O_2^-) could trigger the production of a highly toxic peroxynitrite, thereby inducing severe inflammatory damage to tissues and organs, culminating in ulcer formation [28].

Starting from 1995, we [34-37] reported a series of data which indicate that cNOS and iNOS may functionally cross-talk during the early phase of inflammatory response, at least in some *in vitro* cell culture systems such as astrocytes and endothelial cells. NO, at low physiological concentrations (presumably <50 nM [33])**,** is a powerful suppressor of the activation of NF-κB, a main nuclear factor modulating the induction of the expression of a number of inflammatory genes [38]. The conditions in which the NO content drops far below the physiological level may favour the local activation of NF-κB due to the loss of its suppressive action. This subsequently triggers the induction of the expression of inflammatory genes, including iNOS. In accordance with this view, the potential involvement of arachidonic acid (AA) in the inhibition of cNOS activity in the very early phase of inflammatory response has recently been proposed [39, 40]. It has, therefore, been postulated that any treatment counteracting the drop in NO at inflammatory sites may represent a new strategy for preventing or treating tissue damage induced by deregulated inflammatory processes.

Non-Enzymatic Production of NO by ESW

The principal clinical observation after ESW treatment was the immediate increase in blood flow around the treated area. This observation led to the assumption that the effect of ESW may directly involve the production of NO, one of the most strong endogenous vasodilatators known up to date.

There are a number of reports describing the non-enzymatic production of NO [41-44]. They include both the non-enzymatic *in vivo* formation of NO due to the reaction of dietary/salivary nitrites with gastric acid [9] and the *in vitro* synthesis of nitrites in a solution containing L-arginine (10-20 mM) and hydrogen peroxide (10-50 mM) [43]. Gotte *et al.* [45] recently showed that ESW treatment, under conditions mimicking physiopathological situations (i.e. 10 mM L-arginine and 1 mM H_2O_2), induces non-enzymatic production of physiologically relevant amounts of NO (108 ± 16 nM, 1500 shots at the highest energy level (0.89 mJ/mm^2) applied in 12.5 min.), depending on both the number of shots and the applied energy levels. These data represented the first step to understand the biochemical events that underline the clinically observed anti-inflammatory effect.

Enhancement of cNOS-Derived NO Production by ESW

More recently, it has been reported that ESW, at an energy density corresponding to that employed in the clinical treatment of soft tissue inflammation, rapidly enhance eNOS catalytic activity in human umbilical vein endothelial cells (HUVEC) (Table **2**). In accordance with these results, ESW induce an NO intracellular accumulation which is significantly reduced when the cells are pre-incubated with L-NAME, a strong NOS inhibitor [46], (Table **3**). eNOS activity is modulated by post-translational modification such as tyrosine and serine phosphorylation, indicating that overall NO production is regulated by the equilibrium between the enzyme with differently phosphorylated states. Although the effect of tyrosine phosphorylation is still a matter of debate [47, 48], ESW quickly increase eNOS activity by shifting the balance to a less tyrosine-phosphorylated form [46].

As already stated in a preceding section, we recently proposed that any treatment counteracting the decrease in the amounts of NO should modulate not only NF-κB activation, but also successive whole inflammatory events [49]. When

Table 2. eNOS Activity in HUVEC

	eNOS activity (fold)	
Treatment	**Without LPS/cytokines**	**With LPS/cytokines**
0 shots	1	0.3
500 shots, 0.03 mJ/mm^2	2.8	n.d.
1,000 shots, 0.03 mJ/mm^2	3.2	1.2
1,500 shots, 0.03 mJ/mm^2	2.7	n.d

The cells, in normal or inflammatory condition, were treated at an energy level of 0.03 mJ/mm^2 with varying different numbers of ESW shots . n.d.: not determined.

Table 3. NO Accumulation in HUVEC

	NO accumulation (fold)	
Treatment	**Without LPS/cytokines**	**With LPS/cytokines**
0 shots	1	0.32
1,000 shots, 0.03 mJ/mm^2	3.59	1.1
1,000 shots, 0.03 mJ/mm^2 +L-NAME	1.3	n.d.

The cells, in normal or inflammatory condition, were treated at an energy level of 0.03 mJ/mm^2 and 1,000 shots. n.d.: not determined.

HUVEC cells were treated with LPS/cytokines, mimicking inflammatory conditions, there was a rapid drop in either the NOS activity (Table **2**) or in NO (Table **3**) content. ESW were able to counteract these events, leading to an efficient down-regulation of NF-κB activation [36]. Therefore the clinically observed anti-inflammatory action of ESW can be presumed to imply ESW-elicited rapid up-regulation of eNOS activity and successive increases in NO output, inhibiting NF-κB activation.

Furthermore, ESW treatment down-regulates NF-κB both before and after its activation. Since most of the patients treated with ESW suffer from ongoing inflammatory events, the finding that ESW treatment is capable not only of preventing but also of down-regulating NF-κB activation seems to better match the clinical observations on ESW having an anti-inflammatory effect.

It is important to note that eNOS activity is modulated not only by the enzyme phosphorylation, but also by the interaction with other proteins such as calmodulin [50], caveolin [48] and HSP90 [51]. In this context, a recent report postulates that physical forces such as shear stress may induce a drastic change in the cytoscheletal structure of endothelial cells [52] as well as enhanced eNOS activity due to phosphorylation [53, 54]. Since ESW at low energy levels may elicit a force similar to that produced by shear stress [55], the possibility that ESW treatment may induce some changes in the interaction of eNOS with other proteins, thus triggering the activation of eNOS activity, makes for a fascinating working hypothesis.

Similar results were obtained in rat glioma C6 cells [37] that, unlike HUVEC, express both constitutive and inducible NOS. In this cell system, the down-regulating effect of ESW was observed not only on NF-κB activation but also on iNOS expression and on other NF-κB-dependent inflammatory gene expression. This is a further indication that the clinically observed anti-inflammatory action of ESW may, at least in part, be mediated by an ESW-induced increase in NO production.

To sum up, ESW treatment could be considered as a potentially useful tool for down-regulating NF-κB and NF-κB-dependent inflammatory genes (i.e. iNOS, TNF-α, ICAM, VCAM and COX-2), leading to the modulation of the whole inflammatory process. Further studies are needed to investigate this mechanism *in vivo*.

CONCLUSIVE REMARK

The clinical application of ESW, originally applied exclusively to destroy kidney stones, has unexpectedly been extended to include treatment of a number of inflammation-correlated pathologies. As pointed out in this brief review, one of the possible molecular mechanisms of action behind the anti-inflammatory effect of ESW seems to be their capacity to keep local NO contents at a physiological level in the early phase of inflammatory response, enhancing either a non-enzymatic or enzymatic production of NO. The marked effect of ESW on angiogenesis that has been observed clinically is also consistent with the widely reported angiogenic action of NO. Keeping NO contents at a physiological level during the initial period of inflammatory response has been postulated as a promising strategy in the treatment of pathologies deeply correlated to inflammation. ESW, therefore, may in future be applied in an increasing number of inflammatory diseases for which there is presently no effective therapy.

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