Nitric oxide mediates anti-inflammatory action of extracorporeal shock waves

Anna R. Ciampa[a], Alessandra Carcereri de Prati[a], Ernesto Amelio[b], Elisabetta Cavalieri[a], Tiziana Persichini[c], Marco Colasanti[c], Giovanni Musci[d], Ernst Marlinghaus[e], Hisanori Suzuki[a,*], Sofia Mariotto[a]

[a] *Dipartimento di Scienze Neurologiche e della Visione, Sezione di Chimica Biologica, Università degli Studi di Verona, Strada Le Grazie, 8, 37134 Verona, Italy*
[b] *Unità Operativa di Chirurgia della Mano, Servizio di Terapia con Onde d'Urto, Policlinico G.B. Rossi, Azienda Ospedaliera di Verona, Verona, Italy*
[c] *Dipartimento di Biologia, Università "ROMA TRE", Roma, Italy*
[d] *Dipartimento di Scienze Microbiologiche Genetiche e Molecolari, Università di Messina, Messina, Italy*
[e] *Applied Research Center, Storz Medical AG, Kreuzlingen, Switzerland*

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Abstract Here, we show that extracorporeal shock waves (ESW), at a low energy density value, quickly increase neuronal nitric oxide synthase (nNOS) activity and basal nitric oxide (NO) production in the rat glioma cell line C6. In addition, the treatment of C6 cells with ESW reverts the decrease of nNOS activity and NO production induced by a mixture of lipopolysaccharides (LPS), interferon-γ (IFN-γ) plus tumour necrosis factor-α (TNF-α). Finally, ESW treatment efficiently downregulates NF-κB activation and NF-κB-dependent gene expression, including inducible NOS and TNF-α. The present report suggests a possible molecular mechanism of the anti-inflammatory action of ESW treatment.

Keywords: Extracorporeal shock waves; Inducible nitric oxide synthase; Inflammation; NF-κB; Nitric oxide

1. Introduction

Nitric oxide (NO) is a highly versatile signaling molecule playing a critical role in the nervous, immune and cardiovascular systems. NO is generated in different cell types by at least three isoforms of NO synthase (NOS) through the conversion of L-arginine and oxygen into L-citrulline. Two enzymes, neuronal NOS (nNOS) and endothelial NOS (eNOS), are constitutively expressed and their enzymatic activity is Ca^{2+}/calmodulin-dependent. These constitutive NOS (cNOS) are responsible for the production of physiological levels of NO involved in events such as vasodilation, angiogenesis, and neurotransmission [1]. The third enzyme is an inducible and Ca^{2+}-independent isoform of NOS (iNOS), virtually expressed in all cell types after stimulation with LPS and/or with different cytokines, such as interferon-γ (IFN-γ), interleukin-1β (IL-1β), or tumour necrosis factor-α (TNF-α). Induction of iNOS expression occurs at the transcriptional level and is mediated by the early activation of some nuclear transcriptional factors, including NF-κB [2]. Massive amounts of NO produced by iNOS under pathological conditions (e.g., inflammatory diseases) are potentially harmful, especially when time-spatial regulation of iNOS expression becomes compromised.

Shock waves (SW), defined as a sequence of single sonic pulses characterized by high peak pressure (100 MPa), fast pressure rise (<10 ns), and short lifecycle (10 μs), are conveyed by an appropriate generator to a specific target area with the energy density in the range of 0.003–0.890 mJ/mm^2. Extracorporeal shock waves (ESW) therapy was first applied in patients in 1980 to break up kidney stones [3]. In the last ten years, this technique has been successfully employed as an anti-inflammatory therapy in a number of orthopedic diseases [4] such as pseudoarthrosis [5], tendinitis calcarea of the shoulder, [6,7] epicondylitis [8], plantar fasciitis [9], and several inflammatory tendon diseases. In particular, ESW treatment is able to induce an increase of neoangiogenesis in tendons [10] and the regeneration of muscle and tendon tissues [11]. More generally, an immediate increase in blood flow around the treated area has been frequently observed.

The clinical observation of an immediate vasodilatation and laboratory findings of an enhancement of angiogenesis around the ESW-treated area immediately give rise to the hypothesis that ESW may modulate the production of NO. In this respect, we have reported that NO is produced non-enzymatically by the treatment of a L-arginine/hydrogen peroxide mixture with ESW, although this NO production requires higher energy potencies (0.89 mJ/mm^2) than those employed clinically [12]. More recently, we have demonstrated that ESW, at a clinically compatible energy density, are able to induce the enhancement of enzymatic NO production in resting cells [13]. Indeed, we have showed that ESW quickly enhance eNOS activity and NO production in human umbilical vein endothelial cells (HUVEC).

*Corresponding author. Fax: +39 045 8027170.
E-mail address: hisanori.suzuki@univr.it (H. Suzuki).

Abbreviations: ESW, extracorporeal shock waves; HUVEC, human umbilical vein endothelial cells; IFN-γ, interferon-γ; IL-1β, interleukin-1β; MIX, mixture of 1 μg/ml LPS, 10 ng/ml IFN-γ plus 10 ng/ml TNF-α; L-NAME, N-nitro-L-arginine methyl ester; NO, nitric oxide; NOS, nitric oxide synthase; cNOS, constitutive NOS; eNOS, endothelial NOS; iNOS, inducible NOS; nNOS, neuronal NOS; TNF-α, tumour necrosis factor-α

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However, it is important to keep in mind that high NO levels produced by iNOS are extensively released during inflammation and that, under these conditions, NF-κB is also activated. Therefore, evaluation of ESW effect on NF-κB activation, iNOS and other NF-κB-dependent gene expression is fundamental in assessing molecular mechanism(s) of the clinically observed anti-inflammatory action of ESW.

In the present study, we examined the effect of ESW on the modulation of nNOS catalytic activity, NO production, NF-κB activation, and iNOS and TNF-α mRNA expression in rat glioma C6 cells, a cell line taken as a cellular model because it expresses both constitutive and inducible NOS.

2. Material and methods

2.1. Reagents

All chemicals used throughout the present study were from Sigma (Milan, Italy), unless otherwise specified.

2.2. Cell cultures

The rat glioma cell line C6 was cultured in Dulbecco's modified Eagle's medium (DMEM; BioWhittaker, Cambrex Bio Science, Belgium) supplemented with 10% v/v fetal bovine serum (FBS; BioWhittaker), 100 UI/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, 40 μg/ml gentamicin, in humidified atmosphere of 95% air, 5% CO_2 at 37 °C.

2.3. ESW treatment conditions

An electromagnetic lithotripter (MODULITH SLK device Storz Medical AG, Switzerland) was used throughout the present study. 3×10^6 cells were cultured in 50 mm Petri dishes in 2 ml medium and treated with ESW directly focusing the centre of the plate under ecographic control. In order to measure NO production, 1.5×10^5 cells were plated on glass coverslips one day before ESW treatment. After ESW treatment, cells were maintained in humidified atmosphere of 95% air plus 5% CO_2 at 37 °C for the time indicated in each experiment.

2.4. Western blotting

C6 cells (3×10^6) were lysed by repeated freezing and thawing in a 50 mM HEPES buffer, pH 7.4, containing 1 mM dithiothreitol, 1 mM EDTA and protease inhibitors. After centrifugation ($16\,500 \times g$ for 30 min at 4 °C) the particulate fractions were washed with the lysis buffer, solubilized with 20 mM CHAPS and centrifuged ($25\,000 \times g$ for 30 min at 4 °C). An aliquot of the cytoplasmatic and membrane fractions (40 μg proteins/lane) were loaded on to 7.5% SDS–polyacrylamide gel. After the electrophoresis, proteins were blotted to a PVDF membrane (Immobilon P, Millipore S.p.A., Rome, Italy) and Western blot analysis was performed using an anti-eNOS monoclonal antibody (BD Transduction Laboratories, Franklin Lakes, NJ, USA). Protein concentration in samples was determined by the method of Bradford [14].

2.5. nNOS assay

NOS activity was estimated by measuring the conversion of L-2,3,4,5-[^{3}H]arginine to L-2,3-[^{3}H]citrulline, according to the method described by Colasanti et al. [15].

2.6. DAF-2DA method

The production of NO was assayed using the DAF-2DA detection system, as previously described [13]. Briefly, 10 μM 4,5-diaminofluorescein diacetate (DAF-2DA; Alexis-Corp., San Diego, CA, USA) was added to the cells cultured in serum free medium and incubated at 37 °C for 10 min. After washings with PBS plus 1.2 mM $CaCl_2$, the cells were fixed with 3% w/v paraformaldehyde plus 4% w/v sucrose, and cellular fluorescence was imaged using confocal laser scanning microscope (Axioplan 2, LSM 510, Carl Zeiss, Göttingen, Germany) equipped with argon (488 nm) excitation beams. The laser intensity, the shutter aperture, and the exposure/integration settings were kept constant to allow quantitative comparisons of relative fluorescence intensity of cells between treated groups. Images were digitally acquired and processed for fluorescence determination at the single-cell level, using the public domain NIH Image 1.61 program (developed at the U.S. National Institutes of Health and available on Internet at http://rsb.info.nih.gov/nih-image/).

2.7. Electrophoretic mobility shift assay

Nuclear extracts of C6 cells were prepared according to Osborn et al. [16] and EMSA analysis was performed as described elsewhere [17].

2.8. RNA preparation and Northern blot analysis

Total cellular RNA was isolated using Trizol reagent (Invitrogen-Life Technologies Corp., Carlsbad, CA). Samples of 40 μg of total RNA were separated by electrophoresis on 1% agarose denaturing gel in MOPS buffer and then blotted onto a Hybond N membrane (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). In order to control the amount of RNA in each lane, the gel was stained with ethidium bromide before and after blotting. RNA was hybridized with the cDNA specific for rat iNOS [15] previously labeled by means of a DECAprime II DNA Labeling Kit (1.0–2.0× 109 cpm/μg; Ambion, Austin, TX, USA). The intensity of hybridization was visualized by autoradiography and mRNA expression was quantified using the public domain NIH Image 1.61 program (developed at the U.S. National Institutes of Health and available on Internet at http://rsb.info.nih.gov/nih-image/).

2.9. RT-PCR

Total cellular RNA was purified as described above and reverse transcribed into cDNA, using MMLV reverse transcriptase and oligo $dT_{(12–18)}$ as primers. cDNA was amplified for the iNOS gene (450 bp) or TNF-α gene (200 bp) using specific primers (iNOS forward: 5′-TCCTTGCATCCTCATCGGGCC-3′, iNOS reverse: 5′-TCGTGATAGCGCTTCTGGCTCT-3′; TNFα forward: GAGCTGAGAGATAACCAGCTGGTG-3′, TNFα reverse 5′-CAGATAGATGGGCTCATACCAGGG-3′). The mRNA for the constitutive GAPDH (forward: 5′-CCATGGAGAAGGCTGGGG-3′ reverse: 5′-CAAAGTTGTCATGGATGACC-3′) was examined as the reference cellular transcript. Estimates of the relative iNOS or TNF-α mRNA amounts were obtained dividing the area of the iNOS or TNF-α band, respectively, by the area of the corresponding GAPDH band (Bio-Rad Multi-Analyst™/PC Version 1.1).

2.10. Statistical analysis

Statistical analysis of the data for single comparisons was performed by Student's *t* test.

3. Results and discussion

We have recently reported that exposure of HUVEC to ESW leads to an increase in eNOS activity and NO formation [13]. The results illustrated in Fig. 1 confirm and extend these findings by showing that ESW rapidly increase NO production by enhancing catalytic activity of nNOS in rat C6 glioma cells. In particular, ESW treatment of C6 cells at energy densities of 0.03 mJ/mm^2 (Fig. 1A) and 0.11 mJ/mm^2 (Fig. 1B) enhanced cytosolic NOS activity in a shot-number-dependent manner (500–1500 shots), the maximum value (about 80% over the control value) being reached at 500 shots for both energy densities. In order to better examine the energy density dependency of ESW-elicited enhancement of cytosolic NOS activity, C6 cells were treated with ESW (500 shots) at energy densities varying from 0.003 to 0.11 mJ/mm^2. As shown in Fig. 1C, the maximum effect was achieved already at 0.03 mJ/mm^2. Therefore, unless otherwise described, ESW treatment was performed throughout this study at 0.03 mJ/

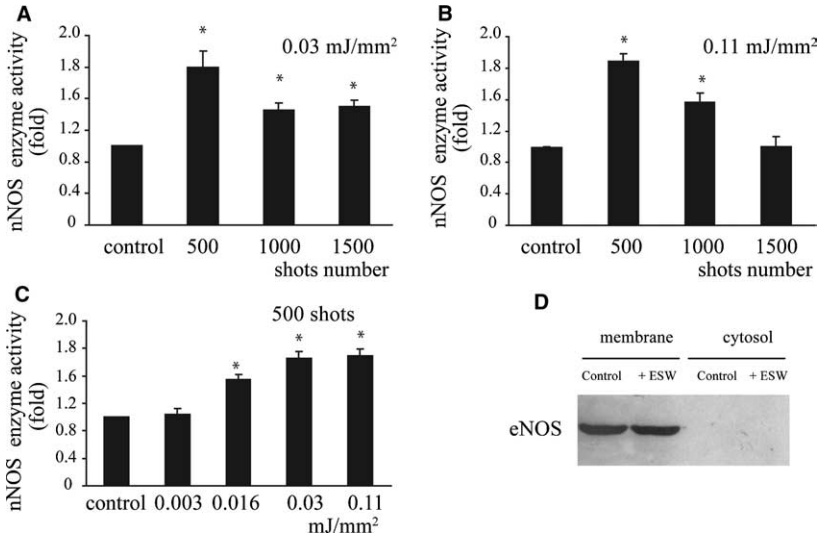

Fig. 1. (A) Modulation of nNOS activity by different ESW shots at 0.03 mJ/mm^2 energy level. C6 cells were treated at energy level 0.03 mJ/mm^2 with a range of 500–1500 ESW shots, and nNOS activity was measured. (B) Modulation of nNOS activity by different ESW shots at 0.11 mJ/mm^2 energy level. C6 cells were treated at energy level 0.11 mJ/mm^2 with a range of 500–1500 ESW shots, and nNOS activity was measured. (C) Modulation of nNOS activity by different energy levels of ESW treatment. C6 cells were treated at energy levels between 0.003 and 0.11 mJ/mm^2 with 500 shots, and nNOS activity was measured. (D) Localization of eNOS after ESW treatment. C6 cells were treated at the energy level of 0.03 mJ/mm^2 with 500 shots. Then, proteins in the membrane and cytosolic fractions were analyzed by Western blot, using an anti-eNOS antibody. For A, B and C, data are shown as a fold increase (means ± S.D., $n = 6$); $^*P < 0.005$ versus non-treated cells.

mm^2 with 500 shots, i.e., under conditions close to those employed in clinical anti-inflammatory treatments.

Note that NOS activity was measured after enzyme purification from the cytosolic fraction where normally nNOS resides. However, C6 cells also express eNOS, the latter being localized on the membrane fraction in the inactive form [18]. In order to exclude a possible translocation of eNOS after ESW treatment and its presence in the cytosolic fraction, cytoplasmatic and membrane fractions of cells were western blotted using an anti-eNOS antibody. As shown in Fig. 1D, eNOS, although present in the membrane fraction, was essentially absent in the cytosolic fraction of either control or ESW-treated cells, indicating that in any case cytosolic NOS activity exclusively arises from nNOS.

To verify whether ESW-elicited enhancement of nNOS activity resulted in an increase of the NO synthesis in ESW-treated C6 cells, the intracellular NO production was measured using the DAF-2DA detection system. When the cells were treated with ESW, DAF-2T fluorescence was significantly enhanced above the background level in C6 cells (Fig. 2). As expected, this fluorescence response was prevented in cells treated with 1 mM N-nitro-L-arginine methyl ester (L-NAME) for 30 min before ESW treatment (Fig. 2), thus, indicating that the increase of DAF-2T fluorescence was consequent to the activation of the L-arginine-NO pathway.

The ESW-induced enhancement of constitutive NOS-dependent NO levels is worth of interest when we take into account the recent notion that physiological, low levels of NO, similar to those produced by the basal activity of nNOS and/or eNOS, prevent induction of iNOS mRNA expression (but also of other NF-κB-dependent genes, including TNF-α and cyclooxygenase-2) through the suppression of NF-κB activation [17,19–23]. As a consequence, iNOS gene expression takes place after LPS/cytokine stimulation, provided that the cNOS-generated NO is reduced below a threshold value in a short time [15,17]. In this respect, we have recently reported that iNOS inducers (e.g., LPS and IFNγ) elicit a rapid inactivation of nNOS and a decrease of basal NO levels [24], an

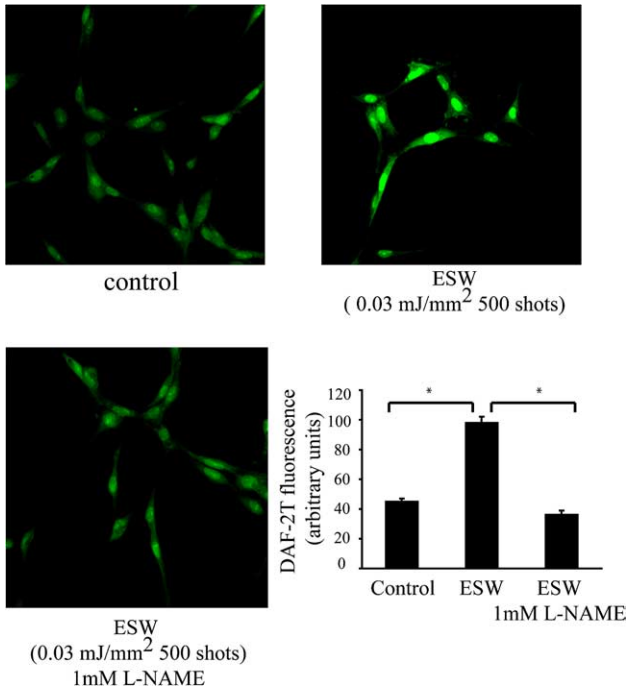

Fig. 2. Modulation of NO production by ESW treatment. Confocal images of C6 cells treated with ESW in the presence and absence of 1 mM L-NAME. Control represents non-treated C6 cells. Magnification: 40×. DAF-2T fluorescence is expressed as arbitrary units; digital scale ranging from 0 to 255, minimum to maximum fluorescence intensity, respectively. The mean fluorescence was calculated from 20 to 40 cells/observation field; two observations field/treatment condition/experiment. Bars represent the means ± S.D. from four to six separate experiments. $^*P < 0.005$.

event mediated by arachidonic acid-dependent tyrosine phosphorylation of nNOS [15,25].

On the basis of these considerations, we next verified the effect of ESW on nNOS activity and on intracellular NO production in the presence of iNOS inducers. As expected, a mixture of 1 μg/ml LPS, 10 ng/ml IFN-γ plus 10 ng/ml TNF-α (MIX) rapidly and gradually decreased nNOS activity in C6 cells, reaching an undetectable value after 1 h of treatment (Fig. 3A). Note that at these concentrations, MIX promoted maximal activation of NF-κB and iNOS expression (see below). As shown in Fig. 3A, treatment of MIX-stimulated C6 cells with ESW (0.03 mJ/mm^2, 500 shots) fully reversed the suppressive effect of MIX on nNOS activity at any time point examined (i.e., 30 min and 1 h). Further analyses using the DAF-2DA detection system confirmed the above results and revealed that ESW treatment brought DAF-2T fluorescence back to the control value, thus counteracting the MIX-triggered drop in NO production (Fig. 3B). This persisting effect strongly suggests that this may take place when ESW are therapeutically employed, the kinetic data being consistent with the clinically observed long-lasting results of ESW treatment. Thus, the clinically observed beneficial effects of ESW fit, at least in part, to their ability of keeping NO amount at the basal level, despite the presence of pro-inflammatory cytokines.

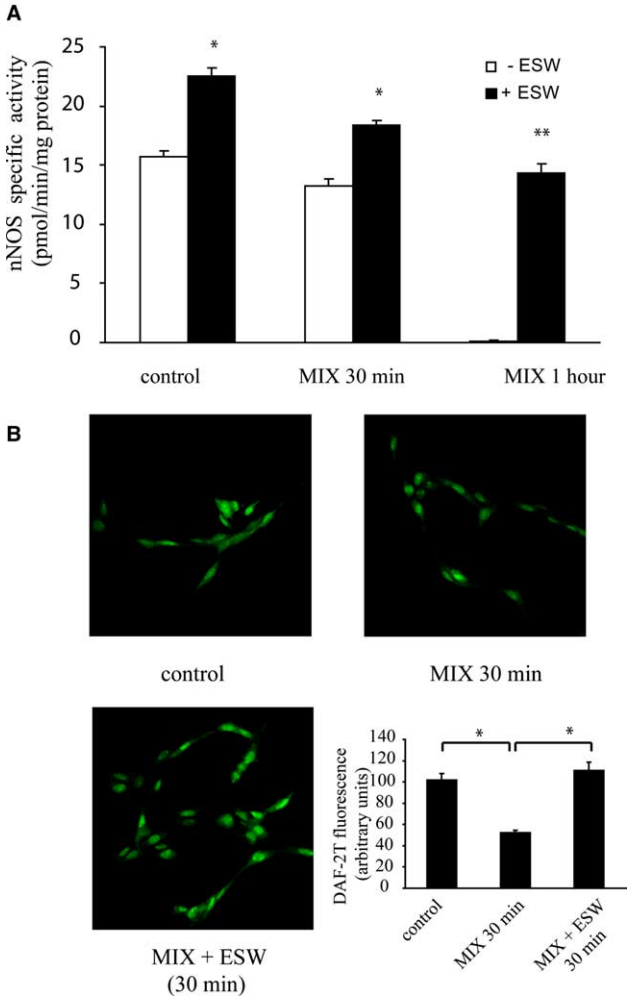

Fig. 3. Effect of ESW on nNOS activity and NO production in MIX-treated C6 cells. (A) nNOS activity was measured in homogenates of C6 cells treated with MIX or with MIX plus ESW (0.03 mJ/mm^2, 500 shots) for 30 min and 1 h. Data are shown as specific activity (means ± S.D., $n = 10$); $^{*}P < 0.05$, $^{**}P < 0.01$ versus not ESW-treated cells. (B) Confocal images of C6 cells treated with MIX with or without ESW treatment (0.03 mJ/mm^2, 500 shots) and then incubated for 30 min. Controls represent non-treated cells. Magnification: 40×. DAF-2T fluorescence of cells is expressed as arbitrary units; for further details see legend of Fig. 2. $^{*}P < 0.05$.

Because it is well established that suppression of cNOS activity represents an early, necessary event for cytokine-induced NF-κB activation and iNOS expression, the effect of ESW in these responses was investigated next.

In order to address whether ESW could interfere with the MIX-elicited NF-κB activation, the DNA-binding activity of NF-κB was measured by using EMSA assay. As expected, exposure of C6 cells to MIX for 30–60 min caused rapid activation of NF-κB (Fig. 4A, lanes 3 and 5, respectively). It is important to recall that under these conditions, MIX was able to quickly inhibit nNOS activity in C6 cells. Interestingly, when C6 cells were incubated with MIX in the presence of concomitant ESW treatment, a downregulation of NF-κB activation was observed. The maximal effect was reached after a 60-min treatment (Fig. 4A, lane 6), although a partial reduction of DNA binding was also found after a 30-min treatment (Fig. 4A, lane 4). Treatment of cells with ESW alone did not affect NF-κB activation (Fig. 4A, lane 2).

The inhibitory effect of ESW on MIX-elicited NF-κB activation was mimicked by treating C6 cells with the NO donor NOR-3 (400 μM) for 30 min (Fig. 4B, lane 5), whereas it was completely reversed when C6 cells were pre-incubated with 1 mM L-NAME for 30 min (Fig. 4B, lane 4). Finally, treatment of MIX-stimulated cells with 1 mM L-NAME for 30 min increased NF-κB activation (Fig. 4B, lane 3) with respect to MIX treatment (Fig. 4B, lane 2).

These results indicate that ESW, being able to rapidly enhance nNOS activity, efficiently reduced MIX-elicited NF-κB activation, confirming the notion that physiologically produced NO levels keep NF-κB activation suppressed [17,26,27]. In order to prove that the observed downregulation of NF-κB activation by ESW ended up in transcriptional depression of NF-κB-dependent genes, iNOS mRNA expression levels were analyzed. In this respect, C6 cells were treated with ESW (0.03 mJ/mm^2 with 500 and 1000 shots) at the same time points of MIX administration, and then kept in the incubator for 3–4 h. By using Northern blot analysis, we confirmed that MIX treatment induced iNOS mRNA expression, the peak being observed after 4 h of treatment (Fig. 5, lane 7). Interestingly, ESW downregulated MIX-induced iNOS gene expression and the maximum effect was reached with 500 shots (Fig. 5, lane 9). An identical outcome was obtained by using RT-PCR analysis (Fig. 6).

Since NF-κB activation is a key event in the induction of a number of inflammatory cytokines, the effect of ESW treatment on TNF-α gene expression was also analyzed. By using RT-PCR, we found that the treatment with ESW (in particular when used at 500 shots) strongly inhibited TNF-α mRNA expression induced by MIX for 4 h, although a complete inhibition was never attained (Fig. 6).

Altogether, these results identify ESW therapy as a possible useful tool for downregulating NF-κB and NF-κB-dependent genes (e.g., iNOS and TNF-α), leading to a drastic reduction in the whole inflammatory process. On the other hand, potentially beneficial effects of ESW due to their capacity of enhanc-

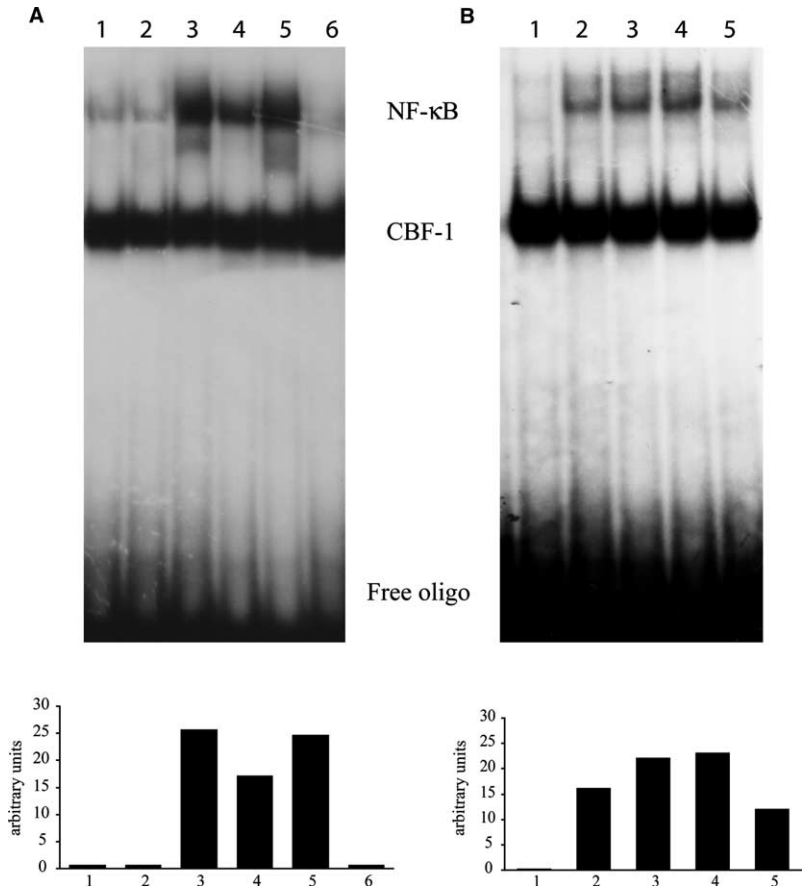

Fig. 4. Effect of ESW (0.03 mJ/mm^2, 500 shots) on MIX-elicited NF-κB activation in C6 cells. (A) lane 1: control cells; lane 2: cells treated with ESW; lane 3: cells treated with MIX for 30 min; lane 4: cells treated with MIX plus ESW and incubated for 30 min; lane 5: cells treated with MIX for 1 h; lane 6: cells treated with MIX plus ESW and incubated for 1 h. (B) Lane 1: control cells; lane 2: cells treated with MIX for 30 min; lane 3: cells pre-incubated with 1 mM L-NAME for 30 min and then treated with MIX; lane 4: cells pre-incubated with 1 mM L-NAME for 30 min and then treated with MIX plus ESW; lane 5: cells pre-incubated with 400 μM NOR-3 for 30 min and then treated with MIX for 30 min. NF-κB activation is expressed as arbitrary units. The constitutively expressed transcription factor CBF-1 is used as an internal standard. The experiment was repeated three times, and the sheet and the histogram show a representative result.

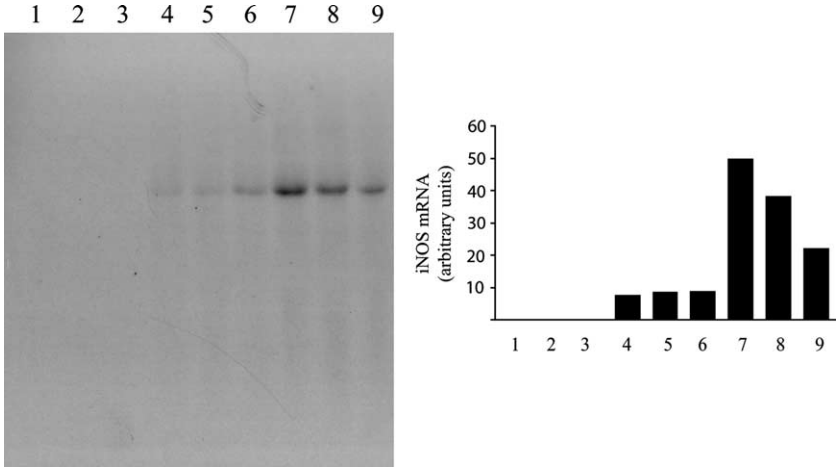

Fig. 5. Effect of ESW on iNOS mRNA expression. Northern blot analysis of iNOS mRNA. Lane 1: control cells; lane 2: cells treated with ESW, 0.03 mJ/mm^2, 1000 shots; lane 3: cells treated with ESW, 0.03 mJ/mm^2, 500 shots; lane 4: cells treated with MIX for 3 h; lane 5: cells treated with MIX plus ESW (0.03 mJ/mm^2, 1000 shots) for 3 h; lane 6: cells treated with MIX plus ESW (0.03 mJ/mm^2, 500 shots) for 3 h; lane 7: cells treated with MIX for 4 h; lane 8: cells treated with MIX plus ESW (0.03 mJ/mm^2, 1000 shots) for 4 h; lane 9: cells treated with MIX plus ESW (0.03 mJ/mm^2, 500 shots) for 4 h. iNOS mRNA expression is expressed as arbitrary units. The experiment was repeated three times, and the blot and the histogram show a representative result.

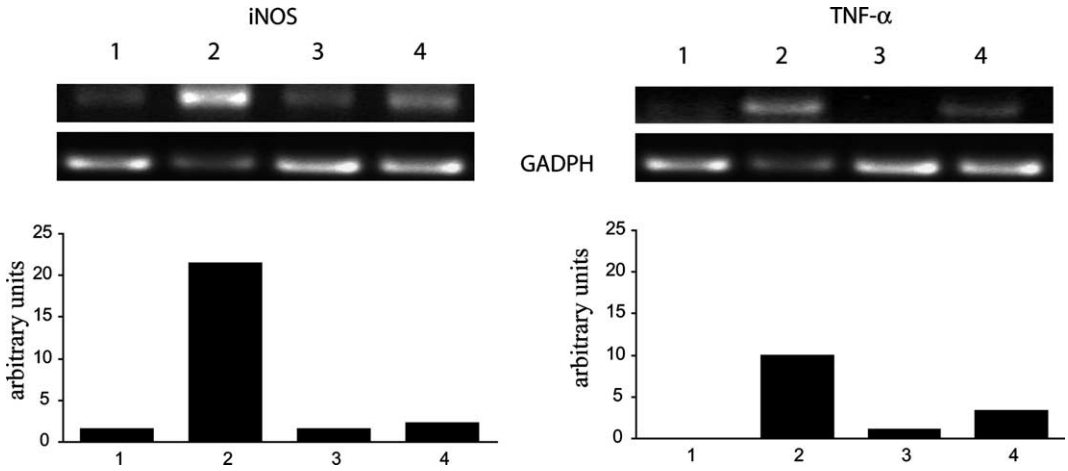

Fig. 6. Effect of ESW on NF-κB dependent gene expression. RT-PCR analysis of iNOS and TNF-α mRNA expression. Lane 1: control cells; lane 2: cells treated with MIX for 4 h; lane 3: cells treated with MIX plus ESW (0.03 mJ/mm^2, 500 shots) for 4 h; lane 4: cells treated with MIX plus ESW (0.03 mJ/mm^2, 1000 shots) for 4 h. GAPDH mRNA was examined as a reference cellular transcript. The experiment was repeated three times, and the gel and the histogram show a representative result.

ing eNOS activity in endothelial cells have been recently reported [13]. Furthermore, the same report indicates that ESW treatment is capable not only to prevent but also down-regulate NF-κB activation, in line with clinical observations of a positive anti-inflammatory action of ESW treatment in most patients with ongoing inflammatory events.

In conclusion, the results obtained in C6 cells provide evidence that clinically observed ESW anti-inflammatory action may be exerted, at least in part, by counteracting the cytokine-induced drop in constitutive NOS activity. Maintenance of the proper amounts of NO may contribute to contrast the cytokine-elicited NF-κB activation and the successive induction of NF-κB-dependent genes, including iNOS and TNF-α. However, further studies are needed to investigate this mechanism in vivo.

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References

[1] Moncada, S., Palmer, R.M. and Higgs, E.A. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. 43, 109–142.
[2] Ganster, R.W., Taylor, B.S., Shao, L. and Geller, D.A. (2001) Complex regulation of human inducible nitric oxide synthase gene transcription by Stat 1 and NF-kappaB. Proc. Natl. Acad. Sci. USA 98, 8638–8643.
[3] Chaussy, C., Brendel, W. and Schmiedt, E. (1980) Extracorporeally induced destruction of kidney stone by shock waves. Lancet II, 1265–1268.
[4] Siebert, W. and Buch, M. (1997) Extracorporeal Shock Waves in Orthopaedics, Springer-Verlag, Berlin, Heidelberg, New York.
[5] Valchanov, V.D. and Michailov, P. (1991) High energy shock waves in the treatment of delayed and nonunion of fractures. Int. Orthop. 15, 181–184.
[6] Rompe, D., Zoellner, J. and Nafe, B. (2001) Shock wave therapy versus conventional surgery in the treatment of calcifying tendinitis of the shoulder. Clin. Orthop. 387, 72–82.
[7] Loew, M., Deacke, W., Kusnierczak, D., Rahmanzadeh, M. and Ewerbeck, V. (1999) Shock-wave therapy is effective for chronic calcifying tendinitis of the shoulder. J. Bone Joint Surg. 81B, 863–867.
[8] Rompe, J.D., Hopf, C., Kullmer, K., Heine, J. and Burger, R. (1996) Analgesic effect of ESWT on chronic tennis elbow. J. Bone Joint Surg. 78B, 233–237.
[9] Rompe, D., Decking, J., Schoellner, C. and Nafe, B. (2003) Shock wave application for chronic plantar fasciitis in running athletes: a prospective, randomized, placebo-controlled trial. Am. J. Sports Med. 31, 268–275.
[10] Wang, C.J., Huang, H.Y. and Pai, C.H. (2002) Shock wave-enhanced neovascularization at the tendon–bone junction: an experiment in dogs. J. Foot Ankle Surg. 41, 16–22.
[11] Wang, C.J., Wang, F.S., Yang, K.D., Weng, L.H., Hsu, C.C. and Huang, C.S., et al. (2003) Shock wave therapy induces neovascularization at the tendon–bone junction. A study in the rabbits. J. Orthop. Res. 21, 984–998.
[12] Gotte, G., Amelio, E., Russo, S., Marlinghaus, E., Musci, G. and Suzuki, H. (2002) Short-time non-enzymatic nitric oxide synthesis from L-arginine and hydrogen peroxide induced by shock waves treatment. FEBS Lett. 520, 153–155.
[13] Mariotto, S., Cavalieri, E., Amelio, E., Ciampa, A.R., de Prati, A. Carcereri and Marlinghaus, E., et al. (2005) Extracorporeal shock waves: from lithotripsy to anti-inflammatory action by NO production. Nitric Oxide 12, 89–96.
[14] Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
[15] Colasanti, M., Persichini, T., Cavalieri, E., Fabrizi, C., Mariotto, S., Menegazzi, M., Lauro, G.M. and Suzuki, H. (1999) Rapid inactivation of NOS-I by lipopolysaccharide plus interferon-gamma-induced tyrosine phosphorylation. J. Biol. Chem. 274, 9915–9917.
[16] Osborn, L., Kunkel, S. and Nabel, G.J. (1989) Tumor necrosis factor alpha and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor kappa B. Proc. Natl. Acad. Sci. USA 86, 2336–2340.
[17] Colasanti, M., Persichini, T., Menegazzi, M., Mariotto, S., Giordano, E. and Caldarera, C.M., et al. (1995) Induction of nitric oxide synthase mRNA expression. Suppression by exogenous nitric oxide. J. Biol. Chem. 270, 26731–26733.
[18] Venturini, G., Colasanti, M., Persichini, T., Fioravanti, E., Ascenzi, P. and Palomba, L., et al. (2002) Beta-amyloid inhibits NOS activity by subtracting NADPH availability. FASEB J. 16, 1970–1972.
[19] Chang, K., Lee, S.J., Cheong, I., Billiar, T.R., Chung, H.T. and Han, J.A., et al. (2004) Nitric oxide suppresses inducible nitric oxide synthase expression by inhibiting post-translational modification of IkappaB. Exp. Mol. Med. 36, 311–324.

[20] Lee, J.I. and Burckart, G.J. (1998) Nuclear factor kappa B: important transcription factor and therapeutic target. J. Clin. Pharmacol. 38, 981–993.
[21] Zingarelli, B., Sheehan, M. and Wong, H.R. (2003) Nuclear factor-kappaB as a therapeutic target in critical care medicine. Crit. Care Med. 31, S105–S111.
[22] Togashi, H., Sasaki, M., Frohman, E., Taira, E., Ratan, R.R. and Dawson, T.M., et al. (1997) Neuronal (type I) nitric oxide synthase regulates nuclear factor kappaB activity and immunologic (type II) nitric oxide synthase expression. Proc. Natl. Acad. Sci. USA 94, 2676–2680.
[23] Colasanti, M. and Suzuki, H. (2000) The dual personality of NO. Trend Pharmacol. Sci. 21, 249–252.
[24] Colasanti, M., Cavalieri, E., Persichini, T., Mollace, V., Mariotto, S. and Suzuki, H., et al. (1997) Bacterial lipopolysaccharide plus interferon-gamma elicit a very fast inhibition of a Ca^{2+}-dependent nitric-oxide synthase activity in human astrocytoma cells. J. Biol. Chem. 272, 7582–7585.
[25] Palomba, L., Persichini, T., Mazzone, V., Colasanti, M. and Cantoni, O. (2004) Inhibition of nitric-oxide synthase-I (NOS-I)-dependent nitric oxide production by lipopolysaccharide plus interferon-gamma is mediated by arachidonic acid. Effects on NFkappaB activation and late inducible NOS expression. J. Biol. Chem. 279, 29895–29901.
[26] Mariotto, S., Menegazzi, M. and Suzuki, H. (2004) Biochemical aspects of nitric oxide. Curr. Pharm. Des. 10, 1627–1645.
[27] Colasanti, M. and Persichini, T. (2000) Nitric oxide: an inhibitor of NF-kappaB/rel system in glial cells. Brain Res. Bull. 52, 155–161.