Nitric Oxide and Shock Waves: Another Brick in the Wall

Author:
E. Amelio

Institution:
Shock Wave Unit, Dept. of Hand Surgery University Hospital Verona, Italy

The clinical observation of immediate vasodilatation and laboratory findings of enhancement of angiogenesis around the ESW-treated area immediately give rise to the hypothesis that ESW may modulate the production of NO. Recently, we have showed that ESW quickly enhance eNOS activity and NO production in human umbilical vein endothelial cells (HUVEC) under either normal or inflammatory conditions. However, we could not show the effect of ESW treatment on iNOS expression. Massive amounts of NO produced by iNOS are potentially harmful. Therefore, evaluation of the ESW effect on iNOS expression is fundamental in further assessing the molecular mechanism of clinically-observed anti-inflammatory action of ESW.
Rat glioma cell line C6, cultured in DMEM supplemented with 10% fetal calf serum, was treated with an electromagnetic lithotripter (MODULITH SLK device Storz Medical AG, Swizerland) as described (FEBS letters 579 2005 6839-6845). Protein extract was subjected to electrophoresis and blotted to a PVDF membrane. Membranes were incubated with anti-eNOS antibody and successively incubated with enhanced chemiluminescent detection reagents. nNOS activity was estimated by measuring the conversion of L-2,3,4,5-[3H]arginine to L-2,3-[3H]citrulline. The production of NO was assayed using the DAF-2DA detection system. NF-kB activation was evaluated by Electrophoretic Mobility Shift Assay. iNOS expression was analyzed by Northern blotting and RT-PCR analysis.
In this study we show that ESW at a low energy density value quickly increase nNOS activity and basal NO production in the rat glioma cell line C6. In addition, the treatment of C6 cells with ESW reverses the decrease of nNOS activity and NO production induced by a mixture of lipopolysaccharides (LPS), interferon-gamma (IFN-gamma) plus tumour necrosis factor-alfa (TNF-alfa). Finally, ESW treatment efficiently suppresses NF-kB activation and NF-kB-dependent gene expression, including iNOS and TNF-alfa.
This report suggests a possible molecular mechanism of the anti-inflammatory action of ESW treatment. Further studies are needed to investigate this mechanism in vivo.