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ABSTRACTS

Effect of Shock Wave on the Catalytic Activity of Endothelial Nitric Oxide Synthase in Human Umbilical Vein Endothelial Cells.

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Introduction: Recent clinical observation indicates the beneficial effect of shock wave on inflammatory region of soft tissues. Although the molecular mechanism of this effect is poorly understood, shock wave treatment seems to locally trigger immediate vasodilation and successive angiogenesis. Nitric oxide (NO) is a gas synthesised by NO synthase (NOS), which, using L-arginine as a substrate, catalyses the synthesis of NO and citrulline (1). Two types of NOS, constitutive and inducible NOS, regulate the amounts of NO in the body. Constitutively expressed NOS (neuronal and endothelial NOS) produces the low amounts of NO (nM) and its catalytic activity is continuously regulated by a number of compounds such as co-factors and modulators. NO produced by constitutive NOS exerts physiological action such as vasodilation, neurotransmission and angiogenesis. Inducible NOS (iNOS), normally absent, locally produces massive amounts of NO under inflammatory conditions, following its induction by proinflammatory cytokines, such as interferon- γ , tumour necrosis factor- α and interleukin-1 β , and lipopolysaccharides (LPS). Massive amounts of NO act as double-edged sword: on the one hand, they may be involved in killing invading cells such as microbes and parasites and, on the other hand, they may damage tissues. In the present work, we hypothesised that one of the molecular mechanisms of the beneficial effect of shock wave toward inflammatory tissues may be an enhanced production of NO. To envisage this hypothesis, we, using human umbilical vein endothelial cells (HUVEC) as an experimental model, analysed the effect of shock wave on the catalytic activity of endothelial NOS (eNOS).

Materials and Methods: HUVEC, treated with LPS (mg/ml)IFN- γ (U/ml) for 30 min, were suspended in the appropriate vessel for the shock wave treatment with an electromagnetic MODULITH SLK device (Storz Medical AG). The conditions of shock wave treatment were similar to those for the clinical treatment. One thousand shots and 0.030 mJ/mm² have been used. eNOS activity of the membrane fractions of HUVEC was estimated by measuring the transformation of [3H]L-arginine to [3H]L-citrulline.

Results and Discussion: Shock wave-treatment of HUVEC enhanced eNOS activity a several fold over the basal value. LPS/IFN- γ treatment induced a rapid decrease in the catalytic activity of eNOS to 15-25 % of the basal level. Shock wave-treatment reverted this effect, bringing eNOS activity to the basal values. According to our recent report (2), physiological levels of NO (nM) suppress the pro-inflammatory cytokines'-elicited activation of NF- κ B. The situation in which the production of NO is very low (< nM) may facilitate the successive induction of iNOS expression which, unless well-controlled, could damage tissues. Therefore, the maintenance of NO levels at an early phase of inflammatory process may, by suppressing NF- κ B activation, relieve the deleterious situation as a result of massive production of NO by iNOS. Accordingly, shock wave-elicited increase in NO production as observed in HUVEC could account, at least partly, for the clinically observed effect of shock wave treatment such as vasodilation and angiogenesis. As a first step to understand the molecular mechanism of shock wave on eNOS activity, we measured eNOS activity in HUVEC after treatment with both vanadate, potent inhibitor of tyrosine phosphatase, and with genistein, potent inhibitor of tyrosine kinase. Vanadate (1 mM) induced 70% inhibition of eNOS activity, whereas genistein (1 mM) increased slightly eNOS activity (up to 30%). These data indicate that, in HUVEC, endogenous eNOS activity is controlled by tyrosine phosphorylation of the enzyme. Experiments to analyse if shock wave induces the enhancement of eNOS activity by modulating tyrosine phosphorylation are ongoing in our lab. In conclusion, shock wave-elicited rapid activation of eNOS activity in LPS/IFN- γ -treated HUVEC observed in this work could, at least partly, explain the clinically observed vasodilation and successive promotion of angiogenesis in shock wave-treated inflamed tissues.

References 1 Moncada, S. et al. (1997) *Pharmacol. Rev.* 49, 137-142 2. Colasanti, M. and Suzuki, H. (2000) *TIPS*, 21, 249-252.