Nitrosylation of proteins in septic vascular dysfunction

Patrícia de Oliveira Benedet, Ana Maria Favero, Karin Scheschowitsch, Jamil Assreuy

Department of Pharmacology, Universidade Federal de Santa Catarina -UFSC- Florianópolis, Brazil.

Objectives Vascular dysfunction contributes to mortality in sepsis and septic shock. Overproduction of nitric oxide (NO) has been implicated as an important factor in cardiovascular dysfunction of sepsis. Besides soluble guanylate cyclase and potassium channels, one of the main effects of NO is the non-covalent modification of protein cysteine residues (sulphydryls) to form S-nitrosothiols. These compounds may serve as reserve, sink or endogenous NO donors. Therefore, the aim of the present study was to investigate the contribution of S-nitrosylation in sepsis-induced vascular dysfunction.

Methods Wistar female rats were anesthetized and submitted to cecal ligation and puncture (CLP) procedure. Thirty minutes or twelve hours after surgery, animals received DTNB [5,5'-dithio-bis-(2-nitrobenzoic acid; an oxidizing agent of sulphydryl groups) or vehicle. Twenty-four hours after CLP, rats were prepared for invasive blood pressure measurements and the vascular reactivity to phenylephrine was assessed. The effects of DTNB on survival, plasma levels of nitrate plus nitrite (NO\textsubscript{x}) and levels of S-nitrosylated proteins in aorta were also evaluated. All procedures have been approved by our institutional Animal Ethics Committee (protocol number PP 00790/CEUA/UFSC).

Results Twenty-four hours after CLP, rats displayed a pronounced hyporesponsiveness to phenylephrine (10 nmol/kg; 22.6 ± 2.2 mmHg CLP group compared to control group 42.8 ± 0.8 mmHg; p < 0.05, n = 5), which correlated with increased levels of nitrosylated proteins in aorta. Early DTNB injection protected rats against vascular dysfunction. Importantly, even when DTNB was injected 12 hours after CLP, phenylephrine response was completely normalized. DTNB also reduced the mortality of septic rats by 40%. Both findings very well correlated with a reduction in levels of S-nitrosylated proteins. DTNB did not change plasma NO\textsubscript{x} of either control or septic rats.

Conclusions Our results show that NO overproduction increases protein S-nitrosylation that correlated with vascular dysfunction. Previous sulphydryl oxidation with DTNB restored the vasoconstrictor response. A very important finding was that DTNB restores the response to phenylephrine even if used after sepsis onset. This latter finding may open new and important new therapeutic opportunities for sepsis treatment.

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