ΔCarbon Monoxide Releasing Molecule-3 (CORM-3) Diminishes the Oxidative Stress and Leukocyte Migration Across Human Endothelium in an In Vitro Model of Compartment Syndrome

Aurelia Bihari, MS*; Gediminis Cepinskas, DVM, PhD; David Sanders, MD; Abdel-Rahman Lawendy, FRCS;*
London Health Sciences Centre, London, Ontario, Canada

Purpose: Acute limb compartment syndrome (CS), a potentially devastating complication of musculoskeletal trauma, results in muscle necrosis and cell death. Oxidative stress due to ischemia and inflammation both appear to contribute to the microvascular dysfunction and parenchymal injury. Recently, carbon monoxide (CO), liberated from the carbon monoxide releasing molecule-3 (CORM-3), has been shown to protect microvascular perfusion and reduce inflammation in a rat model of CS. The purpose of this study was to replicate the CS conditions in vitro, allowing the study of the mechanism(s) of CO protection on the human microvasculature. The ultimate goal is the development of a rational pharmacologic adjunctive treatment for CS, which would reduce the morbidity and disability in patients.

Methods: Human vascular endothelial cells (HUVEC), grown to confluency, were stimulated for 3 hours with either a cytokine/chemokine cocktail representing the serum levels of inflammatory mediators detected in our experimental model of CS ("CS cocktail"), containing tumor necrosis factor alpha (TNF-a), interleukin (IL)-1b, and GRO (1 ng/mL, 100 pg/mL, and 1 ng/mL, respectively), or human serum (40%) isolated from CS patients. Levels of intracellular oxidative stress, measured by the production of reactive oxygen species (ROS) were assessed by oxidation of dihydrorhodamine 123 (DHR-123). Leukocyte migration (transwell inserts) was assessed by quantifying the number of ^{51}Cr-labeled polymorphonuclear cells (PMNs) moving across the HUVEC monolayer in response to the CS cocktail or CS serum stimulation. All experiments were performed in the presence of CORM-3 (100 mM), or its inactive form iCORM-3.

Results: Stimulation of HUVEC with CS cocktail induced a significant increase in the production of ROS, expressed as fluorescence intensity (FI) per mg protein (1118.6 ± 255.6 in CS cocktail versus 600.8 ± 29.2 in control, $P \leq 0.01$), and increased PMN migration across HUVEC (35.1 ± 4.9% in CS cocktail vs. 10.0 ± 2.0% in control, $P \leq 0.05$). CORM-3 treatment completely prevented CS cocktail-induced ROS production (468.3 ± 37.8 vs. 1169.1±155.8 in iCORM-3 group, $P \leq 0.01$), and PMN migration (12.0 ± 1.5% vs. 35.0 ± .9% in iCORM-3 group, $P < 0.05$). In parallel, experiments employing human CS serum stimulation demonstrated that CORM-3 was also very effective in blocking the CS serum-induced ROS production (644.8 ± 114.5 vs. 1059.6 ± 56.3 in iCORM-3 group, $P \leq 0.01$).

Conclusion: Treatment of human vascular endothelial cells with CORM-3 was able to interfere with the intracellular ROS production, and suppressed leukocyte migration across the endothelial barrier. The data indicate that CORM-3 offers potent antioxidant and anti-inflammatory effects, and thus may have a potential therapeutic application to patients at risk of developing CS.

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See pages 99 - 147 for financial disclosure information.