Carbon Monoxide Releasing Molecule-3 (CORM-3) as a Potential Therapy In Acute Limb Compartment Syndrome.

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Purpose: Acute limb compartment syndrome (CS), a devastating complication of musculoskeletal trauma, results in muscle necrosis and cell death. Fasciotomy, to decompress all affected compartments, remains the only gold standard treatment, but must be performed within the surgical window of 6-8 hours. 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 test the effect of CORM-3 in a preclinical setting, using large animal (porcine) and human (in vitro) models of CS. The ultimate goal is the development of a pharmacologic adjunctive treatment for CS, capable of prolonging the surgical window, thus reducing morbidity and disability in patients.

Methods: Pigs underwent 6 hours of intra-compartment pressure (ICP) elevation by fluid infusion into the anterior compartment of the right hind limb. CORM-3 (or its inactive form, iCORM-3) was administered systemically (2mg/kg, IV) at fasciotomy, and the muscle was allowed to reperfuse for 3 hours. Subsequently, tissue perfusion (orthogonal polarized spectral imaging), cellular injury (ethidium bromide (EB)/bisbenzimide (BB) staining ratio) and apoptosis (FLIVO/BB staining ratio) in the skeletal muscle, as well as systemic PMN activation (L-012 assay) were assessed in all animals. In parallel, human vascular endothelial cells (HUVEC) were stimulated for 6 hours with serum obtained from CS patients. Levels of intracellular oxidative stress (production of reactive oxygen species (ROS)), transendothelial leukocyte migration (the number of 51Cr-labelled polymorphonuclear cells (PMNs) moving across the HUVEC monolayer) and leukocyte adhesion/rolling under conditions of flow (1dyn/cm²) were assessed in the presence of CORM-3 (100μM), or iCORM-3.

Results: Elevation of hind limb ICP for 6 hours in pigs resulted in significant microvascular perfusion deficits (44±1% continuously-perfused capillaries in CS versus 76±4% in sham, p<0.001; 39±3% non-perfused capillaries in CS versus 13±2% in sham, p<0.001), increased tissue injury (EB/BB of 0.31±0.07 in CS versus 0.17±0.03 in sham, p<0.05), apoptosis (FLIVO/BB of 0.26±0.06 in CS versus 0.13±0.03 in sham, p<0.05) and activation of leukocytes in the systemic circulation (14.7 relative luminescence units (RLU)/10⁶ PMNs in CS versus 1.0±0.1 in baseline, p<0.001). Systemic application of CORM-3 (but not iCORM-3) at fasciotomy significantly increased the number of continuously perfused capillaries (68±3%, p<0.001), diminished tissue injury and apoptosis (0.13±0.04 and 0.12±0.03, respectively, p<0.05), and completely blocked the systemic leukocyte activation 3.9±0.3 RLU/10⁶ PMNs, p<0.001). In vitro, CS serum induced a significant increase in the production of ROS within HUVEC, expressed as fluorescence intensity (FI) per mg protein (1118.6±255.6 in CS versus 600.8±29.2 in control, p<0.01), increased PMN activation and migration across HUVEC (35.1±4.9% in CS versus 10.0±2.0% in control, p<0.05). CORM-3 completely prevented CS-induced ROS production, PMN activation and transmigration.

Conclusion: Administration of CORM-3 at fasciotomy offered protection against CS-induced microvascular perfusion deficit, tissue injury and systemic leukocyte activation. The data suggest the potential therapeutic application of CORM-3 to patients at risk of developing CS.