Is arginine/asymetric dimethylarginine ratio depletion an indicator of insufficient resuscitation in a porcine model of hemorrhage-reperfusion?

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Background. *Hemorrhagic shock leads to a complex cascade of metabolic and hormonal processes that may result in hypoperfusion, end organ damage, and death even when blood pressure is restored. Studies have shown that morbidity and mortality could be attributable to a diminished availability of endothelial-derived nitric oxide (eNO). It is unclear whether adequate levels of citrulline (CIT) and arginine (ARG)—the precursors of eNO synthesis—are available to sustain the eNO needed to maintain adequate perfusion in severe shock. An indirect measure of eNO is the ratio between the levels of ARG and its inhibitor asymmetric dimethylarginine (ARG/ADMA). The purpose of the study was to identify the temporal impact of the ARG/ADMA ratio, ARG, CIT, and ADMA in response to hemorrhage and crystalloid fluid resuscitation by the use of a porcine model of severe hemorrhagic shock.*

Methods. *Hemorrhagic shock was induced in Yorkshire cross pigs by mimicking a bleeding pattern of rapid uncontrolled hemorrhage to achieve a shed volume of 30 mL/kg, a 50% decrease in mean arterial pressure, and an oxygen debt of >60 mL/kg. Normal saline, up to 2 times the shed blood volume, was started 1 hour after the start of hemorrhage with the goal of restoring mean arterial pressure to >50 mm Hg. Hemodynamics, blood gas measurements, and plasma samples were obtained at baseline, 1 hour after the start of hemorrhage, and 1 hour after resuscitation. Amino acids were measured by liquid chromatography coupled to mass spectrometry.*

Results. *During hemorrhage, a distinct subset of pigs was better able to tolerate ischemia than the rest. These pigs required less resuscitation, had evidence of better organ perfusion, and exhibited less of an increase in interleukin-6 (IL-6) after resuscitation. Compared with their less-tolerant counterparts, this group had a greater increase in CIT above baseline (analysis of variance, $P < .05$) with hemorrhage. ARG levels were similar and remained stable with hemorrhage, which indicated the similar availability of substrate for eNO synthesis but differences in the quantity produced in response to the blood volume loss. With crystalloid fluid resuscitation, ARG levels and ARG/ADMA decreased (analysis of variance, $P < .05$), whereas CIT remained increased in the group less able to tolerate hemorrhage. ARG/ADMA decreased proportional to greater oxygen debt during hemorrhage and greater IL-6 levels with fluid resuscitation.*

Conclusion. *Our results suggest that a sufficient decrease in MAP during hemorrhagic shock is associated with a subsequent increase in IL-6, persisting impairment of end organ perfusion, and evidence of ongoing eNO deficit and an increase in ADMA despite resuscitation. The ARG/ADMA ratio reflects both of these parameters and corresponds to the increase in IL-6 and persistent ischemia after resuscitation. We propose that the mechanism of IL-6 increase in trauma derives from eNO deficiency, and the ARG/ADMA ratio more accurately depicts the pathologic mechanism responsible for increased morbidity and mortality in trauma. (Surgery 2014;156:861-70.)*

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SEVERE HEMORRHAGE SECONDARY TO TRAUMA accounts for approximately 40% of trauma-related deaths. Systolic blood pressure <90 mm Hg in the field or on initial hospital presentation is associated with eventual organ failure and septic complication.[1] Resuscitation of severe hemorrhagic shock encompasses volume repletion through crystalloid or colloid (including blood) fluid replacement and exogenous supplementation of vasopressors for maintenance of perfusion pressure to vital organs. It is likely that shunting intravascular volume to vital organs competes with the function of the local endothelial nitric oxide (eNO) to maintain tissue microperfusion.

Pervasive endothelial dysfunction after severe hemorrhage and resuscitation has been described.[2] If systemic ischemia develops, endothelial nitric oxide synthase (eNOS)-mediated production of eNO may become impaired as a result of depletion of L-arginine (ARG; the substrate for eNOS) and/or accumulation of asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA), inhibitors of eNOS activity.

The purpose of this study was to ascertain more precisely how the levels of amino acids L-citrulline (CIT), the byproduct of eNO synthesis, ARG, ADMA, and SDMA are affected by severe ischemia and how they may relate to the systemic inflammatory response associated with ischemia-reperfusion injury that occurs with hemorrhage and subsequent resuscitation. We present the effects of severe hemorrhagic shock and crystalloid resuscitation in a porcine model on the circulating levels of these amino acids. The goal of this research was to gain a better understanding of the pathophysiology induced by ischemia and resuscitation and to identify the use of clinical markers of nitric oxide synthesis as potential indicators for the severity of microvascular ischemic damage or adequacy of resuscitation to guide continued therapy. These data temporally associate the endogenous responses of CIT, ARG, ADMA, SDMA, and interleukin-6 (IL-6) with hemorrhagic shock and fluid resuscitation.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee at Tripler Army Medical Center. Investigators complied with the policies as prescribed in the National Research Council's Guide for the Care and Use of Laboratory Animals and the USDA Animal Welfare Act. Animals were handled in accordance with the National Institute of Health Guidelines in facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care International.

Animal preparation. Seventeen Yorkshire cross *Sus scrofa* (31 ± 2 kg body weight) were sedated with acepromazine 1.1 mg/kg intramuscular (IM), ketamine 22–33 mg/kg IM, midazolam 0.5 mg/kg IM, and glycopyrrolate 0.003 mg/kg IM. Surgical anesthesia was maintained by IV titration of ketamine 8–33 mg/kg/hr, midazolam 0.5–1.5 mg/kg/hr, and sufentanil 1–5 μg/kg/hr with a continuous normal saline infusion of 0.1 mL/kg/min as a vehicle for sedation medications and to maintain euhydration for the remainder of the experiment. The pigs were intubated and mechanically ventilated (fraction of inspired oxygen 21%, 0.4 inspiratory time, positive end expiratory pressure of 5–6 mm Hg, peak inspiratory pressure of 14–16 mm Hg) to maintain tidal volume at 8–10 mL/kg and at a respiratory rate to maintain end tidal CO_2 at 35–40 mm Hg.

The following vascular catheters were placed by cut-down: (1) left femoral artery (4–6 Fr triple lumen) catheter for continuous blood pressure monitoring and access to blood sampling. (2) Left femoral vein (4–6 Fr triple lumen) catheter for access venous blood and continuous infusion of normal saline and administration of medications. (3) Right atrial (Swan-Ganz) catheter via the internal or external jugular vein to monitor central venous pressure and pulmonary artery pressure with extension into pulmonary artery verified by characteristic pressure wave form. (4) Left and right cephalad (4–6 Fr triple lumen) catheter via the carotid artery and internal or external jugular vein, respectively for cephalic blood sampling. (5) Left ventricular (8–12 Fr single lumen) catheter via the carotid artery for measuring ventricular pressure, blood samples reflective of the pulmonary circulation, and administration of microspheres. (6) A suprapubic bladder catheter was placed through a small midline laparotomy and secured with a purse string suture for collection of urine and assessment of adequate volume replacement. Normal body temperature was maintained by use of a heated surgery table or warm air circulator as necessary. Animals were allowed a 1- to 2-hour equilibration period after catheter placement before the start of experimental procedures or measurements were taken.[3]

Experimental protocol. After baseline measurements were obtained, hemorrhagic shock was induced by mimicking a bleeding pattern of rapid uncontrolled hemorrhage (3 mL/kg/min for 7 minutes) followed by a continued slower loss of blood (1 mL/kg/min), as described by Frankel et al.[4]