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NHE1 inhibition improves tissue perfusion and resuscitation outcome after severe hemorrhage

Dongmei Wu, MD, PhD,[a,c,*] *Kristina Russano, BS,*[a] *Irene Kouz, MD,*[a] *and William M. Abraham, PhD*[b]

[a] *Division of Neonatology, Department of Research, Mount Sinai Medical Center, Miami Beach, Florida*
[b] *Division of Pulmonary and Critical Care Medicine, Mount Sinai Medical Center, Miami Beach, Florida*
[c] *The WCU Program, Department of BIN Fusion Technology, Chonbuk National University, Korea*

ARTICLE INFO

Article history:
Received 4 March 2012
Received in revised form
5 July 2012
Accepted 11 July 2012
Available online 31 July 2012

Keywords:
Na^+/H^+ exchanger
Hemorrhagic shock
Resuscitation
Neurological outcomes
Oxygen transport

ABSTRACT

Introduction: This study tested the hypothesis that blockade of the pH-regulatory protein, Na^+/H^+ exchanger (NHE1) during prolonged hemorrhagic shock can protect against whole-body ischemia-reperfusion injury, resulting in improved neurological outcomes.
Methods: We used a total of 24 male pigs in this study. We excluded two animals: one because of cardiac arrest after the initial hemorrhage, and the second because of a catheter malfunction for color microspheres. In Series 1, anesthetized pigs underwent an initial hemorrhage of 40 mL/kg for 30 min, and then were given either 3 mg/kg of NHE1 selective inhibitor BIIB513 ($n = 6$) or vehicle ($n = 6$). At 1 h after treatment, all animals received fluid resuscitation. We assessed survival and neurologic outcomes 72 h postresuscitation. In Series 2, we measured organ blood flow in a separate group of control ($n = 5$) and BIIB513-treated pigs ($n = 5$) undergoing the same experimental paradigm.
Results: Five of six control animals failed to be weaned from mechanical ventilation. We killed another control animal the next day because of severe complications. In contrast, all six animals treated with BIIB513 were weaned off the ventilator, and all but one survived the 72-h experimental period with normal neurological outcome. Results showed that NHE1 inhibition with BIIB513 improved blood flow to the brain, heart, and kidney, and prevented the development of metabolic acidosis in the 1-h hypovolemic period. In addition, BIIB513 facilitated the hemodynamic response to fluid resuscitation, increased mixed venous blood oxygen saturation and oxygen delivery, and reduced proinflammatory cytokine release and multiorgan injury compared with vehicle controls.
Conclusions: In this study, NHE1 inhibition with BIIB513 improved vital organ blood flow, prevented the development of metabolic acidosis during prolonged hypovolemia, and facilitated the hemodynamic response to fluid resuscitation, resulting in increased survival and normal neurological outcomes.

1. Introduction

Hemorrhagic shock resulting from traumatic injury remains a major cause of mortality and disability in both military and civilian patients. As yet, an unmet research challenge is to determine optimal treatments to prevent delayed multiple-organ failure after hemostasis and all-out resuscitation [1,2]. Identifying effective pharmacologic agents to ameliorate

* *Corresponding author*. Department of Research, Mount Sinai Medical Center, 4300 Alton Road, Miami Beach, FL 33140. Tel.: 305-674-2199; fax: 305-674-2198.
E-mail address: dongmeiwu@bellsouth.net (D. Wu).
0022-4804/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2012.07.026

ischemia-reperfusion injury, thereby protecting the patient during stasis and reperfusion, could enhance survival for battlefield and civilian trauma patients [3,4].

Activity of the Na+/H+ exchanger (NHE1) is an important determinant of the magnitude of cellular and, thus, tissue injury [5]. Na+/H+ exchanger activation can be induced by intracellular acidosis through a proton-dependent regulatory pathway and endogenous inflammatory mediators and/or oxidant stress [6]. We recently reported that administration of NHE1 selective inhibitor BIIB513 before fluid resuscitation facilitates the hemodynamic recovery, resulting in a reduced tissue inflammatory response [7]. These data are consistent with previous studies showing that NHE1 inhibition reduces tissue damage in the brain, heart, lung, liver, kidney, and vascular tissues in regional ischemia-reperfusion injury [5–11]. The present study tested the hypothesis that the inclusion of Na+/H+ exchanger inhibitor BIIB513 during a prolonged hypovolemia period can optimize the extracellular and intracellular milieus during prolonged hypovolemia and resuscitation, resulting in improved survival with normal neurologic outcomes.

2. Materials and methods

2.1. Animal preparation

The Mount Sinai Medical Center Animal Care and Use Committee approved all animal studies, and all studies complied with the Animal Welfare Act. We used a total of 24 male pigs in this study. We excluded two animals: one because of cardiac arrest after the initial hemorrhage of 40 mL/kg (Series 1), and the second because of a catheter malfunction for color microspheres (Series 2).

We anesthetized male Yorkshire pigs (26.7 ± 4.9 kg) with ketamine, 10 mg/kg, intramuscularly, and maintained them in a surgical plane of anesthesia with intravenous propofol. The animals were ventilated with room air with a tidal volume of 10 mL/kg. The respiratory rate was adjusted to ensure a P_aCO_2 from 35 to 45 mm Hg. We cannulated the left external jugular vein for the administration of fluids and drugs. We placed another catheter into the right femoral artery for the measurement of arterial blood pressure (AP) and blood sampling. We inserted a 5.5-F, balloon-tipped, flow-directed thermodilution pulmonary arterial catheter (Opticom; Abbot Laboratories, Chicago, IL) via the right jugular vein and floated it into the pulmonary artery under direct pressure monitoring for measurements of pulmonary arterial pressure (PAP), right atrial pressure (RAP), core body temperature, and cardiac output (CO). We continuously recorded all hemodynamic parameters with a Powerlab data acquisition system (AD Instruments Inc, Colorado Springs, CO). We measured arterial and central venous blood gases at various intervals during the experiments using a blood gas analyzer (Rapidlab 855; Bayer Corporation, New York, NY). The cardiac output was determined by thermodilution in triplicate using ice-cold saline. We maintained body temperature between 37°C and 39°C by means of a heating pad.

2.2. Experimental protocol (Series 1)

After completing the surgical procedure, we allowed animals to stabilize for 30 min. We induced severe hemorrhage in pigs by bleeding through the femoral artery catheter (40 mL/kg blood removed within 30 min). After completing the initial hemorrhage of 40 mL/kg for 30 min, we randomly assigned 12 pigs to receive either 3 mg/kg BIIB513 (in 25 mL Hextend [Hospira, Inc, Lake Forest, IL]; $n = 6$) or vehicle (Hextend; $n = 6$). Staff technicians were blinded to the treatment. At 1 h after treatment, we reversed the hypovolemia by giving animals fluid resuscitation with a Hextend infusion to replace blood loss (40 mL/kg) over the next hour; then, the animals received 10 mL/kg lactated Ringer's solution (maintaining fluid) for an additional hour. From this point, we attempted to wean the animals from mechanical ventilation. Animals that were weaned successfully were extubated and transferred to the vivarium for observation. We killed animals that failed to be weaned off the ventilator 6 h after fluid resuscitation. This model design is based on Institutional Animal Care and Use Committee policy, and was used to examine resuscitation outcomes previously [12]. During the recovery phase, we treated animals for acute pain with 60 μg/kg intramuscular buprenorphine. We administered antibiotic (80 mg/kg intravenous cefazolin) before extubation. We kept the animals for 3 d to assess survival and neurologic outcomes.

Two members of the laboratory team who were blinded to treatment assessed neurologic evaluations each day. The Neurological Deficit Score for pigs assigns values for deficits in neurologic function; a score of 0–10 is normal and a score of 100 is brain death [13]. The Cerebral Performance Category is a more global assessment of neurologic function in which a category of 1 is normal and 5 is brain death. After the last functional neurologic evaluation (72 h), the animals were anesthetized, ventilated, and re-instrumented for monitoring of hemodynamics. After obtaining blood samples, hemodynamic, and blood gas data, we humanely killed the animals while they were still under anesthesia, with 10 mL sodium pentobarbital, a method that is consistent with the recommendation of the Panel on Euthanasia of the American Veterinary Medical Association.

2.3. Regional blood flow measurement using colored microspheres (Series 2)

We measured organ regional blood flow in a separate group of control ($n = 5$) and BIIB513-treated pigs ($n = 5$) with the same preparation as described above, with the following modifications: We placed a catheter into the carotid artery and further advanced it into the left ventricle for microsphere injection. Briefly, we injected a bolus of 6×10^6 colored microspheres (Dye-Trak; Triton Technologies, San Diego, CA) into the left ventricle at baseline, immediately after hemorrhage (H30), immediately before resuscitation (H90), and 2 h after resuscitation (R120). We collected the reference blood samples from the abdominal aorta by a withdrawal pump started 20 s before the injection of the microspheres at a withdrawal rate of 5 mL/min, and continued for 2 min after the injection of microspheres. We performed microsphere extraction, purification, and quantification according to the manufacturer's instructions, described in our previous studies [14]. We calculated blood flow as the ration of the tissue-derived microsphere numbers to reference arterial blood-derived