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Effects of post-resuscitation administration with sodium hydrosulfide on cardiac recovery in hypoxia-reoxygenated newborn piglets ☆

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ABSTRACT

Hydrogen sulfide may protect multiple organ systems against ischemic–reperfusion injuries. It is unknown if treatment with sodium hydrosulfide (NaHS, a hydrogen sulfide donor) will improve myocardial function and minimize oxidative stress in hypoxic-reoxygenated newborn piglets. Mixed breed piglets (1–5 day, 1.5–2.5 kg) were anesthetized and acutely instrumented for the measurement of systemic, pulmonary and regional (carotid, superior mesenteric and renal) hemodynamics and blood gas parameters. The piglets were induced with normocapnic alveolar hypoxia (10–15% oxygen, 2 h) followed by reoxygenation with 100% (1 h) then 21% oxygen (3 h). At 10 min of reoxygenation, either NaHS (10 mg/kg, 5 ml) or saline (5 ml) was administered intravenously for 30 min (5 min bolus followed by 25 min of continuous infusion) in a blinded, block-randomized fashion ($n=7$/group). Plasma lactate and troponin I levels and tissue markers of myocardial oxidative stress were also determined. Two hours hypoxia caused cardiogenic shock ($45 \pm 3\%$ of respective normoxic baseline), reduced regional perfusion with metabolic acidosis (pH 6.94 ± 0.02). NaHS infusion significantly improved recovery of cardiac index ($84 \pm 3\%$ vs. $72 \pm 5\%$ in controls), systemic oxygen delivery ($84 \pm 3\%$ vs. $72 \pm 5\%$ in controls) and systemic oxygen consumption ($102 \pm 5\%$ vs. $84 \pm 6\%$ in controls) at 4 h of reoxygenation. NaHS had no significant effect on systemic and pulmonary blood pressures, regional blood flows, plasma lactate and troponin I levels. The myocardial glutathionine ratio was reduced in piglets treated with NaHS (vs. controls, $P < 0.05$). Post-resuscitation administration of NaHS improves cardiac function and systemic perfusion and attenuates myocardial oxidative stress in newborn piglets following hypoxia-reoxygenation.

1. Introduction

Despite recent advances in obstetrical care and neonatal resuscitation, asphyxia remains a major cause of neonatal mortality and morbidity. Of the estimated 4 million annual deaths in the 4-week period following birth, approximately 23% are a result of asphyxia (Lawn et al., 2006; WHO, 2005). Besides mortality, cardiovascular dysfunction along with myocardial stunning, pulmonary hypertension, and derangement of vascular autoregulation are commonly observed in neonates after asphyxia, resulting in low cardiac output, hypotension, and end-organ hypoperfusion (Martin-Ancel et al., 1995; Shah et al., 2004). Etiologies of myocardial injury after asphyxia and reperfuson/reoxygenation are multifactorial; the production of reactive oxygen species and the resultant oxidative stress contribute to the cardiac dysfunction during recovery.

Hydrogen sulfide (H_2S), an endogenous second messenger, has been shown to cause vasodilatation, to enhance antioxidant activities, to attenuate inflammation and to minimize apoptosis (for review see Dongo et al., 2011; King and Lefer, 2011; Szabo, 2007). Because of its properties, numerous studies have been carried out in the last decade to explore the effectiveness of H_2S on cytoprotection in ischemia–reperfusion (I–R) injury. Either exogenous or endogenous increases of H_2S have been shown to exert cardio- and hepato-protective effects in cultured cells, isolated hearts as well various animal models of regional or global myocardial ischemia (King and Lefer, 2011; Szabo, 2007; Nicholson and Calvert, 2011). Recently, it has been shown that treating the animal with H_2S, in the form of sodium hydrosulfide (NaHS) or sodium sulfide, can also significantly attenuate tissue injury in other end organs, including intestine (Henderson et al., 2010), kidney (Hunter et al., 2012) and brain (Li et al., 2011) after I–R.

As cellular hypoxia and reoxygenation are two essential elements of I–R, there are similarities between I–R and hypoxia-reoxygenation (H–R) (Allison et al., 1990; Karmazyn et al., 1993;

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Tan et al., 1998). Despite substantial studies have examined the cytoprotective effect of H_2S using various I–R models, little information is available regarding the beneficial effect of H_2S in newborn subjects which have unique cardiovascular responses to H–R. As compared with adults, the neonatal heart has been shown to have limited reserves for contractility, cardiac output and energy (Ishiyama et al., 2006; Price, 2011). Further neonates have reduced anti-oxidative capacity (Buonocore et al., 2001; Thibeault, 2000) and thus are more susceptible to H–R injury. Indeed, the syndrome of hypoxia-related myocardial dysfunction occurs in about 30% of asphyxiated infants (Joynt and Cheung, 2009). Using an established porcine model of neonatal H–R, our primary objective was to evaluate whether H_2S had any potential cardioprotective effect in neonates after H–R. In addition to systemic and regional hemodynamics, changes in systemic oxygen transport as well as myocardial oxidative stress markers were also measured in piglets after post-resuscitation administration with NaHS. We hypothesized that NaHS would improve cardiac function and reduce myocardial oxidative stress injury in asphyxiated newborn piglets.

2. Methods

All experiments were conducted in accordance with the guidelines and approval of the Animal Care and Use Committee (Health Sciences), University of Alberta. Twenty-one newborn mixed breed piglets 1–5 days of age weighing 1.5–2.5 kg were obtained on the day of experimentation from University Swine Research Unit.

2.1. Animal preparation

Anesthesia was induced initially with isofluorane at 5% and maintained at 2–3%. Inhalational anesthesia with isofluorane was discontinued once mechanical ventilation was commenced. Subsequently anesthesia was maintained with intravenous fentanyl 5–50 μg/kg/h and midazolam 0.2–0.5 mg/kg/h and pancuronium 0.05–0.1 mg/kg/h. Additional doses of fentanyl (10 μg/kg) and acepromazine (0.25 mg/kg) were also given as needed for anesthetic purposes. Fractional inspired oxygen concentrations (FiO_2) were continuously measured and maintained between 0.21 and 0.25 to maintain PaO_2 between 60 and 80 mmHg. Percutaneous oxygen saturation was measured by a pulse oximeter (Nellcor, Hayward, California), and heart rate and blood pressure were measured with a Hewlett Packard 78833B monitor (Hewlett Packard Co, Palo Alto, California). Intravenous fluids consisting of 5% dextrose at 10 ml/kg/h and 0.9% NaCl at 2 ml/kg/h were used to maintain glucose levels and hydration. Piglet body temperature was maintained at 38.5–39.5 °C using overhead warmer and a heating pad.

A 5-French Argyle double-lumen catheter was inserted into the femoral vein, up to the level of the right atrium for administration of fluids and medications. A 5-French Argyle single-lumen catheter was inserted into the femoral artery to the distal aorta and attached to a pressure transducer for continuous systemic measurement of arterial pressure to determine mean arterial pressure (MAP). Endotracheal intubation via a tracheostomy was performed, and pressure-controlled assisted ventilation (Sechrist infant ventilator model IV-100; Sechrist Industries, Anaheim, CA) was commenced at a respiratory rate of 16–20 breaths/min and pressure of 19/4 cm H_2O. A left flank incision was used to open the retroperitoneum. The superior mesenteric artery and the left renal artery were encircled with a 3-mm and 2-mm transonic flow probe to measure intestinal and renal blood flow, respectively. In the third intercostal space a left anterior thoracotomy was performed and a 6-mm transonic flow probe (6SB; Transonic Systems Inc, Ithica, NY) was placed around the main pulmonary artery to measure blood flow which served as the surrogate of cardiac output. Following this a 20 G Arrow (Arrow International, Reading, PA) catheter was inserted and secured in the base of the main pulmonary artery to continuously measure pulmonary artery pressure (PAP). All incisions were covered or closed to minimize evaporative heat loss. Transonic flow probes were connected to a small animal flow meter. Transonic flow probes and pressure transducer outputs were digitized and recorded by a converter board in a computer equipped with custom Asyst programming software (Data Translation, Ontario, Canada).

The piglets were allowed to recover from surgical instrumentation (75–90 min) until baseline hemodynamic measures were stable. Ventilator rate was adjusted to keep the $PaCO_2$ 35–45 mmHg (Table 2) during the experimental period. Heart rate, MAP, PAP, cardiac output and regional blood flows were continuously monitored throughout the experiment.

2.2. Experimental protocol

The piglets were block-randomized into 2 experimental groups ($n=7$ per group) that underwent H–R in a blinded fashion. A third sham-operated group of piglets ($n=7$) underwent complete instrumentation without H–R and delivery of medications.

In the H–R groups, normocapnic alveolar hypoxia was induced. These piglets were ventilated with a FiO_2 of 0.10–0.15 by increasing the inhaled concentration of nitrogen gas relative to oxygen for 2 h, aiming for PaO_2 of 30–40 mmHg, to produce clinical asphyxia with severe metabolic acidosis and systemic hypotension (Johnson et al., 2007). This was followed by reoxygenation with 100% oxygen for 1 h and then 21% oxygen for 3 h. This protocol would result in cardiogenic shock at 2–4 h after reoxygenation. At 10 min of reoxygenation, piglets received a blinded treatment either with NaHS (10 mg/kg) or saline (placebo, control). NaHS was used for the present study because it has been shown to be effective against I–R injury and it is easy to prepare. The dosage and regimen of NaHS was based on our pilot study and consistent with similar dosages used in swine (Drabek et al., 2011; Osipov et al., 2009; Simon et al., 2008). Blinding was maintained by reconstituting all doses of NaHS and normal saline in a standard volume (5 ml) immediately before administration. The medication was given intravenously over 30 min (5 mg/kg was given as 5 min bolus followed by another 5 mg/kg over 25 min continuous infusion). A laboratory technician uninvolved in the experiment prepared the medications. At the end of the experiment, the piglet was euthanized with an overdose of pentobarbital (100 mg/kg, i.v.). Left ventricle was removed rapidly and flash-frozen in liquid nitrogen and stored at −80 °C for subsequent analysis.

2.3. Biochemical analysis

Myocardial tissues were homogenized with 10 μl/mg of 50 mM phosphate buffer containing 1 mM EDTA (pH 7.0). The tissue levels of oxidized and total glutathione (GSSG and GSH, respectively) and lipid hydroperoxides (LPO) were measured using commercially available assay kits (#703002 and #705002, respectively, Cayman Chemical, Ann Arbor, MI). Tissue lactate was assayed by enzymatic spectrometric methods. Plasma cardiac troponin-I concentration was measured using a commercially available ELISA kit (#2010-4-HS, Life Diagnostics, West Chester, PA). The protein content was determined by bicinchoninic acid assay kit (Sigma-Aldrich Canada Ltd., Oakville, ON).

2.4. Statistical analysis

All results are expressed as mean ± S.E.M. Two-way repeated measures and 1-way analysis of variance and Krushal–Wallis test