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Tissue-specific changes in glutathione content of hypoxic newborn pigs reoxygenated with 21% or 100% oxygen ☆

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Abstract

We compared the responses towards oxidative stress in the liver, lung, brain, heart, kidney and small intestine of hypoxic newborn animals resuscitated with 21% or 100% oxygen. After stabilization, piglets (1–3 days, 1.6–2.0 kg, $n=8$/group) were randomized to receive 2 h of alveolar hypoxia ($FiO_2=0.10–0.14$) followed by reoxygenation with 21% or 100% oxygen for 1 h and then another hour with 21% oxygen. Controls were sham-operated without hypoxia–reoxygenation. At the end of the experiment, tissues from liver, lung, brain, heart, kidney and small intestine were collected and tested for GSH, GSSG and lipid peroxidation levels and histological examination. In normoxic controls, liver had the highest GSH level, followed by brain, heart, lung, small intestine and kidney which had the highest level of oxidative stress markers (GSSG level and GSSG:GSH ratio). Hypoxic–reoxygenated piglets had the highest GSSG levels and GSSG:GSH ratio in the kidney. Hypoxic piglets resuscitated with 100% oxygen had higher GSSG:GSH ratios in the lung and liver, but not in the kidney, brain, heart and small intestine, than controls, which were not different from the 21% group. No significant differences in peroxidation and histological tissue damage were found between groups in the liver and lung. We concluded that although hypoxic piglets resuscitated with 100% oxygen have higher oxidative stress in the liver and lung than with 21% oxygen, there are no significant differences in peroxidation and histological tissue damage acutely.
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Keywords: Glutathione; Organs; Hypoxia–reoxygenation; Newborn; (Pig)

1. Introduction

GSH is a tripeptide, synthesized from the amino acids glutamate, cysteine and glycine by two ATP-dependent enzymes-glutamate cysteine ligase and GSH synthetase (Dickson and Forman, 2002). GSH is the most abundant non-enzymatic antioxidant in the human body with the highest content found in the liver (Lopez-Torres et al., 1993). As a non-enzymatic antioxidant, GSH reacts with oxygen free radicals with the oxidation of the sulfhydryl (–SH) group to disulfide and the formation of GSSG (Bast et al., 1991). The GSH and GSSG levels, and the ratio of GSSG to GSH content have been used to measure tissue oxidative stress and as the markers of cellular GSH redox state.

In different organs of the human body the cellular GSH redox state can be affected in varying degrees to hypoxia and reoxygenation. Jenkinson et al. (1988) demonstrated GSSG formed in the lung during reoxygenation after hypoxia. Reuter and Klinger (1992) showed that the increased tissue GSSG level

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was followed by an increase in plasma GSSG concentration, which was directly correlated with oxidative stress in the liver (Denno et al., 1995). Denno et al. (1995) demonstrated a hypoxia-related decline in the GSH level followed by a decrease in GSSG level in cultured hepatocytes, while Kretzschmar et al. (1990) noted relatively stable hepatic cellular GSSG:GSH ratio in vivo. Park et al. (1991) have shown that in hypoxic isolated rat hearts, there was a decline in the GSH level, an increase in the GSSG level and an increase in the GSSH:GSH ratio, with no significant decrease in GSH level during reoxygenation.

The newborn may be less able to counter the oxidative stress because of low levels of protective antioxidants including vitamin E, GSH peroxidase, superoxide dismutase and catalase (Jenkinson et al., 1988; Reuter and Klinger, 1992). When hypoxic newborns recover following the resuscitation, there is hypoxia–reoxygenation injury at multiple organs which can be caused by oxygen free radicals produced during hypoxia and reoxygenation (Bast et al., 1991). Common complications include myocardial dysfunction, renal failure, necrotizing enterocolitis, hepatic dysfunction and pulmonary edema (Martín-Ancel et al., 1995). Although the standard approach to resuscitation is to use 100% oxygen, it is reasonable to begin resuscitation with an oxygen concentration of less than 100% or to start with no supplementary oxygen (i.e., room air) (American Heart Association, American Academy of Pediatrics, 2006). Several studies have shown the equivalence of 21% oxygen with 100% oxygen used in the resuscitation of hypoxic animals and infants (Tan et al., 2005). The oxidative stress of hypoxia and reoxygenation was increased with the use of 100% oxygen compared to 21% oxygen in the reoxygenation. Indeed the complications following asphyxia may be related to the hypoxia and reoxygenation injury. However, due to the potential differences in innate antioxidant capacity and in the regional oxygen delivery to different organs, the differential effects of reoxygenation on various hypoxic organs deserve further investigation including the effect of increased oxidative stress on tissue damage.

Therefore, we compared the GSH content in the liver, lung, brain, heart, kidney and small intestine in hypoxic newborn piglets reoxygenated with 21% or 100% oxygen. We hypothesized that there would be tissue-specific changes in GSH content with the most dramatic change in the liver and the higher oxidative stress in piglets resuscitated with 100% oxygen than that with 21% oxygen. To follow-up the effect of oxidative stress during hypoxia and reoxygenation, we also compared the biochemical marker of peroxidation and histological damage in the organ that had increased oxidative stress between groups.

2. Materials and methods

The study conformed to the regulations of the Canadian Council on Animal Care and was approved by the Health Sciences Animal Welfare Committee, University of Alberta.

2.1. Neonatal hypoxia–reoxygenation protocol

The protocol has been previously described (Haase et al., 2005). Briefly, 24 newborn piglets (1–3 day-old, 1.6–2.0 kg) were initially anesthetized with isofluorane, which was discontinued when a tracheostomy tube was inserted and the animals were mechanically ventilated with a neonatal ventilator (Model IV-100, Sechrist Inc., Anaheim, CA). Baseline ventilation was pressure controlled at 16/4 cm H_2O at 12–18 breaths/min with an inspired oxygen concentration (FiO_2) of 0.21–0.25. Muscle paralysis was achieved by pancuronium (0.1 mg/kg bolus followed by an infusion of 0.05–0.1 mg/kg/h), sedation and analgesia was provided by continuous infusions of midazolam (0.1–0.2 mg/kg/h) and fentanyl (5–10 μg/kg/h). A dextrose-saline infusion at 15 ml/kg/h maintained glucose and hydration.

Via a groin incision, 5 F single lumen catheters (Sherwood Medical Co., St Louis, MO) were placed in the femoral vein and artery. Medications, as earlier described, were infused through the femoral vein. Samples for blood gases were taken from the femoral artery. Stabilization was defined as a heart rate and blood pressure within 10% of pre-surgical levels; arterial pO_2 60–80 mm Hg; pCO_2 35–45 mm Hg; pH 7.35–7.45; rectal temperature 38.5–39.5 °C.

Animals were block-randomized into three groups ($n=8$ each). After an hour of stabilization, systemic normocapnic hypoxia was induced by decreasing the FiO_2 to 0.10–0.14 for 2 h to achieve an arterial pO_2 of 40–50 mm Hg and mean blood pressure of 25–35 mm Hg. Resuscitation was with 21% oxygen for 2 h in the 21% reoxygenated group or 100% oxygen for 1 h followed by another hour with 21% oxygen in the 100% reoxygenated group. Control piglets underwent no hypoxia or reoxygenation. At the end of the experiment, animals were euthanized with pentobarbital (100 mg/kg), tissue samples from liver, lung, left ventricle of the heart, kidney and small intestine were collected, freeze-clamped in liquid nitrogen and kept at −80 °C until subsequently tested for GSH content and lipid peroxidation. The tissue was also preserved in formalin for histological examination. The fronto-parietal cortex of the brain was preserved in isopentane at −80 °C and saved for analysis later.

2.2. Determination of GSH and GSSG levels

Tissue response to oxidative stress was measured by determining GSSG and GSH levels by a commercially available GSH assay kit (#703002, Cayman Chemical, Ann Arbor, MI). Fifty milligrams of tissue was homogenized in 500 μl of MES buffer (0.2 M 2-(*N*-morpholino) ethanesulphonic acid, 0.1 M phosphate and 2 mM EDTA, pH 6.0). After centrifugation at 10,000 ×*g* for 15 min at 4 °C, the supernatant was removed and deproteinated. The GSSG was reduced to GSH by GSH reductase in the assay cocktail of the kit containing 5,5′-dithio-bis-2-nitrobenzoic acid, glucose-6-phosphate dehydrogenase, GSH reductase, $NADP^+$ and glucose-6-phosphate. GSH combines with 5,5′-dithio-bis-2-nitrobenzoic acid. The absorbance of the yellow product (5-thio-2-nitrobenzoic acid) was read at 405 nm to give the GSH when compared to standards. GSSG was quantified by first derivatizing the GSH in the tissue with 2-vinylpyridine and then assaying as for GSH. GSH and GSSG levels were expressed as μmol/g wet weight of tissue. The GSSG:GSH ratio of the sample was calculated.

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