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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.

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.

Table 1
Physiologic parameters in controls ($n=8$) and study piglets at normoxic baseline and 2 h of hypoxia (upper panel), and at 1 h and 2 h of reoxygenation with 21% and 100% oxygen (lower panel) ($n=8$ each)

Phase	Normoxic baseline		2 h of hypoxia	
Group	Controls	Study	Controls	Study
Arterial pO_2 (mm Hg)	78±6	76±3	77±7	43±4 [a, b]
Arterial pH	7.38±0.03	7.40±0.01	7.37±0.03	7.01±0.03 [a, b]
Mean arterial pressure (mm Hg)	70±8	66±4	56±5	31±2 [a, b]
Heart rate (bpm)	212±13	201±9	204±13	198±12

Phase	1 h of reoxygenation			2 h of reoxygenation		
Group	Controls	21%	100%	Controls	21%	100%
Arterial pO_2 (mm Hg)	80±6	76±8	298±34 [a, b]	74±8	75±9	71±9
Arterial pH	7.36±0.03	7.20±0.05 [a, b]	7.23±0.05 [a, b]	7.39±0.02	7.28±0.03 [b]	7.30±0.05
Mean arterial pressure (mm Hg)	62±10	42±7 [b]	40±4 [b]	49±8	36±6 [b]	33±4 [b]
Heart rate (bpm)	232±18	252±10	223±16	233±16	261±10 [b]	207±34

[a] $P<0.05$ vs. controls (one-way ANOVA).
[b] $P<0.05$ vs. baseline (one-way repeated measures ANOVA).

2.3. Examination of tissue damage in the organs with increased oxidative stress

Tissue damage in specific organs with increased oxidative stress was assessed by biochemical measurement for peroxidation (using a commercially available kit for the assay of lipid hydroperoxides, #705002, Cayman Chemical) and histological examination. Briefly, lipid hydroperoxides were extracted from the tissue sample homogenate into chloroform along with a deproteination procedure according to manufacturer's recommendation. The hydroperoxides were quantified by the rapid reaction with ferrous ions to produce ferric ions. The resulting ferric ions were detected using thiocyanate ion as the chromogen, of which the absorbance at 500 nm was measured and compared to standards. The detection limit of this method was 0.25 μM. For histological examination all tissues were preserved in 10% formalin for subsequent processing. In the event of increased oxidative stress compared to controls, the organ was then fixed in paraffin, and stained with hematoxylin and eosin. The samples were examined by a pathologist (IH), who was unaware of the experimental group of the samples. The pathologic injury was evaluated for vascular congestion and neutrophil infiltration, using a semi-quantitative scale, based on previously established criteria (Park et al., 1990).

2.4. Statistics

Data are expressed in mean±S.E.M. Differences between groups were analyzed by one-way analysis of variance or Kruskal–Wallis test for non-parametric data if equality or normality test failed. For post hoc testing, Tukey or Dunn's tests were used as appropriate. Significance was defined as $P<0.05$.

3. Results

Following 2 h of systemic normocapnic hypoxia, the animals had hypotension and acidosis compared with the controls (Table 1). The base deficit was also significantly greater than controls (21.6±1.1 and 3.0±0.4, respectively). The physiologic parameters recovered immediately upon reoxygenation with an increased arterial pO_2 in the 100% hypoxic–reoxygenated group compared to controls and 21% hypoxic–reoxygenated group (339±30 vs. 83±3 and 80±5 mm Hg, respectively, $P<0.05$). However, after 2 h of reoxygenation (1 h of 21% or 100% oxygen followed by 1 h of 21% oxygen), there were no significant differences between groups regarding the physiologic parameters. The hypoxic–reoxygenated piglets had lowered mean arterial blood pressure compared to the respective baseline (Table 1). The hypoxic piglets resuscitated with 21% oxygen also had lowered arterial pH but higher heart rate than the baseline values (Table 1). Arterial plasma lactate concentration was significantly higher in the 21% hypoxic–reoxygenated but not 100% group than that of controls (7.3±0.9 and 5.6±1.8 vs. 2.4±0.2 mM, respectively).

3.1. Tissue GSH and GSSG levels in control piglets

Liver had the highest GSH levels, followed by the brain, left ventricle of the heart, lung, small intestine and kidney (liver > brain, heart > lung, small intestine, kidney) (Table 2). There was

Table 2
Tissue contents of GSH and GSSG in controls and hypoxic piglets resuscitated with 21% and 100% oxygen ($n=8$ in each group)

	Controls	21% Reoxygenation	100% Reoxygenation
GSH content (μmol/g)			
Liver	2.67±0.18[a]	1.86±0.38[b]	2.40±0.21[a]
Brain	1.68±0.05	1.41±0.16	1.46±0.11
Left ventricle	1.52±0.07	1.67±0.09	1.50±0.06
Lung	1.20±0.09	1.39±0.12	1.43±0.06
Small intestine	1.18±0.16	1.25±0.11	1.24±0.12
Kidney	0.91±0.06	0.73±0.13	0.91±0.08
GSSG content (μmol/g)			
Liver	0.055±0.006	0.050±0.017	0.081±0.014
Brain	0.077±0.002	0.069±0.007	0.071±0.003
Left ventricle	0.084±0.010	0.078±0.007	0.073±0.004
Lung	0.012±0.002	0.014±0.001	0.015±0.001
Small intestine	0.038±0.008	0.064±0.018	0.064±0.014
Kidney	0.108±0.007[a]	0.118±0.010[a]	0.113±0.015[a]

[a]$P<0.05$ and [b]$P<0.1$ vs. other organs (one-way ANOVA).

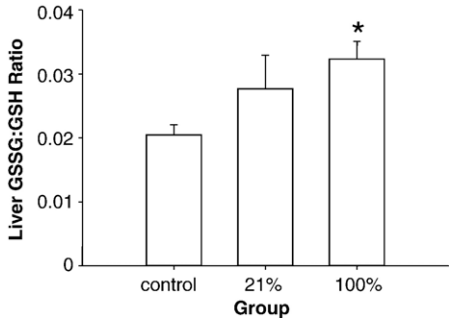

Fig. 1. GSSG:GSH ratio in the liver in controls, piglets resuscitated with 21% and 100% oxygen. * $P<0.05$ vs. controls (one-way ANOVA).

almost a 3-fold difference between the GSH levels of the liver and kidney. For the GSSG levels, kidney had the highest level, followed by the left ventricle of the heart, brain, liver, small intestine and lung with a 9-fold difference between that of kidney and lung (kidney > heart, brain > liver, small intestine > lung). Thus, the ratio of GSSG:GSH was found to be highest in the kidney and lowest in the lung with the heart, brain, small intestine and liver ranking between the two organs (kidney [0.119±0.003] > heart [0.055±0.005], brain [0.046±0.002] > small intestine [0.032±0.008] > liver [0.021±0.001] > lung [0.009±0.000]). The difference between the ratio of kidney and that of lung was 13-fold.

3.2. Tissue GSH, GSSG levels and GSSG:GSH ratio after hypoxia and reoxygenation

The tissue GSH content of different organs were compared in the two groups of hypoxic–reoxygenated piglets (Table 2). For 21% and 100% hypoxic–reoxygenated groups, the tissue GSH levels were highest in the liver and the tissue GSSG level was highest in the kidney compared to other organs, with a 2.5-fold difference between that of liver and the kidney ($P<0.05$–0.1), and an 8-fold difference between that of kidney and the lung ($P<0.05$), respectively. The tissue GSH and GSSG levels were not different between the other organs among the hypoxic–reoxygenated piglets. For the tissue GSSG:GSH ratios, it was highest in the kidney and lowest in the lung with no significant differences between the other organs (21% hypoxic–reoxygenated group: kidney > small intestine, brain, heart > liver > lung; 100% hypoxic–reoxygenated group: kidney > heart, small intestine, brain > liver > lung; both $P<0.001$). The differences between the ratio of kidney and that of lung were 13-fold and 12-fold in the 21% and 100% hypoxic–reoxygenated groups, respectively.

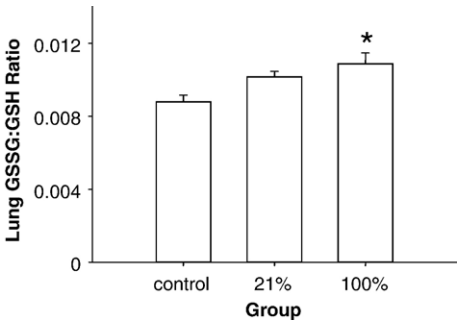

Fig. 2. GSSG:GSH ratio in the lung in controls, piglets resuscitated with 21% and 100% oxygen. * $P<0.05$ vs. controls (one-way ANOVA).

Table 3
Vascular congestion and neutrophil infiltration in the liver and lung of controls and hypoxic piglets resuscitated with 21% and 100% oxygen ($n=8$ in each group)

	Controls	21% Reoxygenation	100% Reoxygenation
Vascular congestion score			
Liver	1.1±0.8	1.4±0.6	1.2±0.5
Lung	1.4±0.4	2.1±0.4	2.1±0.3
Neutrophil infiltration score			
Liver	2.1±0.7	2.6±0.6	3.7±0.9
Lung	1.5±0.3	1.3±0.2	1.9±0.2

No significant differences were found in the tissue levels of GSH between controls and 21% and 100% hypoxic–reoxygenated groups in all tissue samples (Table 2). Neither was any significant difference found in the tissue GSSG levels between controls and the hypoxic–reoxygenated groups in all tissue samples (Table 2). Piglets in the 100% hypoxic–reoxygenated group had higher GSSG:GSH ratios in the lung and liver than the controls ($P=0.015$ and $P=0.026$, respectively), which were not significantly different from the 21% hypoxic–reoxygenated group (Figs. 1 and 2). There were no significant differences found in the ratio of GSSG:GSH between the control and hypoxic–reoxygenated groups in the brain, kidney, small intestine and heart (data not shown).

3.3. Tissue damage in the organs with increased oxidative stress

For the level of peroxidation, tissue concentrations of lipid hydroperoxides were measured in the liver and lung. The tissue concentrations of lipid peroxidation of 21% and 100% hypoxic–reoxygenated piglets were not different from that of controls (liver: 1.03±0.27 and 1.34±0.31 vs. 1.62±0.45 μmol LOOH/g protein of 21% and 100% hypoxic–reoxygenated and control groups; lung: 1.23±0.33 and 1.17±0.46 and vs. 1.06±0.24 μmol LOOH/g protein of 21% and 100% hypoxic–reoxygenated and control groups, respectively).

Tissue samples from the liver and lung were further examined for possible damage due to increased oxidative stress. There were no significant differences in the scores of vascular congestion and neutrophil infiltration between groups (Table 3).

4. Discussion

In this study of tissue GSH levels, which indicates the major non-enzymatic antioxidant capacity against oxidative stress, we observed the highest level in the liver and the lowest level in the kidney. After hypoxia and reoxygenation, GSSG levels and the ratios of GSSG:GSH were highest (suggesting an increased risk of oxidative stress) in the kidney. We also found significant differences in GSSG:GSH ratios in the liver and lung between controls and 100% hypoxic–reoxygenated piglets, indicating a higher oxidative stress occurred in these organs when 100%

oxygen is used in the resuscitation. Despite the increased oxidative stress, we did not observe an increase in significant tissue damage regarding the lipid peroxidation and histology. The response to oxidative stress may depend on the severity of hypoxemia. We believe that there was a severe hypoxic insult as evidenced by significant hypotension and arterial pH changes in this experimental model.

4.1. GSH content and oxidative stress in the liver of newborn piglets

In this study we confirmed previous reports that the highest GSH level is found in the liver of newborn piglets (Dickson and Forman, 2002). This supports the belief that the liver is the organ more resistant to oxidative stress in the body because of its abundant store of GSH that is used to scavenge oxygen free radicals generated during hypoxia and reoxygenation and thus protects the cell from the damaging effects of hypoxia and reoxygenation. As previously described, studies using cultured hepatocytes have shown a relative resistance to oxidative stress (Denno et al., 1995). Interestingly, the liver may also handle a high load of oxidants as a result of the release of oxygen free radicals by xanthine oxidase, myeloperoxidase and cytokines from the intestinal system (Koike et al., 1993). Our findings on the GSH content of the liver after hypoxia and reoxygenation support the antioxidant role of the liver because the content was lowered and was not different from other organs. Nonetheless, the liver remains to have a low GSSG:GSH ratio in the tissue.

4.2. GSH content and oxidative stress in the kidney of newborn piglets

We found that the tissue GSH level lowest, and the GSSG level and GSSG:GSH ratio highest in the kidney compared to other organs. These findings may suggest that the kidney is at a higher risk to oxidative stress and the related injury than other organs. In the hypoxic–reoxygenated piglets, the kidney also had the lowest GSH level and highest GSSG level and GSSG:GSH ratio (12–13-fold higher than from that of the lung). If the tissue GSH content reflects the balance of oxidative stress and antioxidant mechanisms, in the event of hypoxia and reoxygenation, the kidney may be more susceptible to the oxygen free radicals induced injury. Indeed, acute renal dysfunction is one of the commonest complications following asphyxia. Karlowicz and Adelman (1995) reported the prevalence of acute renal failure in 61% of the severely asphyxiated neonates. Vento et al. (2005) have recently shown the increased reno-tubular injury in neonates resuscitated with 100% oxygen compared to 21% oxygen. Interestingly, among the organs subjected to the pathological effect of oxygen free radicals generation, the protective effect of antioxidant is evident in the kidney with reduced oxidant-related injury and better functional recovery. The use of *N*-acetylcysteine has been found to be useful to combat contrast-induced nephropathy (Guru and Fremes, 2004), a condition believed to be related to the oxygen free radicals generation, and renal impairment after cardio-pulmonary bypass (Fischer et al., 2005) and neonatal asphyxia (Johnson et al., in press).

4.3. GSH content and oxidative stress in the lung of newborn piglets

Jenkinson et al. (1988) previously described that the GSSG levels increase dramatically during the reoxygenation period in the lung, indicating that there is oxygen free radicals generation and thus oxidative stress during reoxygenation. Although this may suggest that the lung is more susceptible to or has to deal with more oxidative stress during hypoxia and reoxygenation, this does not necessarily imply a greater degree of oxidant-related injury in the lung. We found very low GSH levels in the lung samples taken from control piglets but the organ also had the lowest GSSG:GSH ratio. Indeed, the lung also had the lowest GSSG:GSH ratio after hypoxia and reoxygenation. These findings are interesting because one would expect significant oxidative stress in the oxygen-rich environment. Our findings may suggest that other antioxidant systems are functionally important to protect the lung from the oxidative stress, thus resulting in lower GSSG:GSH ratios. Nozik-Grayck et al. (2000) showed that there is a developmental regulation of the superoxide dismutase in the neonatal lungs and the expression of this enzyme decreases during the first week of life. Further research to study the role and activity of other antioxidant systems in the tissue of neonatal subjects will help to understand the matter.

4.4. Resuscitation with 21% versus 100% oxygen

There is evidence that the resuscitation of perinatally asphyxiated neonates with 21% is as effective as with 100% oxygen (American Heart Association, American Academy of Pediatrics, 2006; Tan et al., 2005). Reuter and Klinger (1992) examined the changes in the tissue GSH content in organs including liver, lung and brain in neonatal rat pups after hypoxia and reoxygenation. They showed transient increases in tissue GSSG levels that subsequently returned to baseline values by 4 h after reoxygenation. In this study, we compared the differential oxidative stress at various tissues between 21% and 100% oxygen used in the resuscitation of hypoxic newborn piglets at 2 h after reoxygenation. Higher levels of oxidative stress markers in the plasma and tissues in the 100% reoxygenated group have been reported. In this study, it seems that the significant differences in the oxidative stress lie in the liver and the lung with the GSSG:GSH ratios highest in piglets resuscitated with 100% oxygen although the small sample size might have precluded us to detect significant changes in other organs. Nonetheless, the study confirms more oxidative stress in piglets resuscitated with 100% oxygen than 21% oxygen. It is known that in vitro the hypoxanthine–xanthine oxidase system generates more oxygen free radicals with increasing oxygen concentration. As the liver has the largest store of GSH in the body, it is predictable that the significant oxidative and thus oxygen free radicals detoxifying effect is observed in the liver. Giles et al. (2002) showed that prenatal hypoxia disrupted the normal developmental secretion of active superoxide dismutase in the lung of rabbits. Whereas the alveolar oxygen partial pressure is very high in the lung and even higher when a high oxygen concentration is used in the

resuscitation (approximately 700 mm Hg when 100% oxygen is used), this may induce excess stress on the antioxidant capacity of the lung during reoxygenation after hypoxia. Indeed, the lung also needs to handle the oxygen free radicals generated from xanthine oxidase system by neutrophils accumulated in the lung (Terada et al., 1992). Therefore these factors may be associated with the increased oxidative stress observed. Recently Munkeby et al. (2005) demonstrated that resuscitation with 100% oxygen compared with 21% oxygen is detrimental to the lung with greater oxidative stress and proinflammatory response in the pulmonary tissue. However, using the two crude but direct parameters of oxidative stress-induced injury (peroxidation and histological features of reoxygenation injury), we did not find more damaging effects in the liver and lung with higher oxidative stress. The increased oxidative stress may simply be a dose-response to higher oxygen content in the blood following hypoxia. Since our study was not originally powered to study the oxidant-induced injury, the negative findings require cautious interpretation. Indeed, although there were histological changes found, the short duration post-resuscitation (2 h) might not be sufficient for significant injury to be apparent. Nonetheless, caution is needed in the resuscitation with 100% oxygen in those neonates with compromised antioxidant capacity. Indeed, it has been demonstrated the increased oxygen radical absorbance capacity, which measures free radical scavenging activity against reactive oxygen species and the total antioxidant capacity, in the lung (Munkeby et al., 2005), heart (Borke et al., 2004) and brain (Munkeby et al., 2004) of hypoxic piglets resuscitated with 100% oxygen compared to 21% oxygen. By providing a specific assay of the GSH content of the tissue, our report suggests tissue specificity of the increased oxidative stress with 100% reoxygenation. This may indicate the effect of hyperoxemia is different in various organs (Huang et al., 2005).

5. Conclusions

Compared to 21% reoxygenation, normocapnic hypoxic piglets resuscitated with 100% induces higher oxidative stress mainly in the liver and lung. However, 21% and 100% reoxygenation do not differ in peroxidation and histological tissue damage acutely.

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