Resuscitation with 100% O₂ does not protect the myocardium in hypoxic newborn piglets

W B Børke, B H Munkeby, L Mørkrid, E Thaulow, O D Saugstad

Background: Perinatal asphyxia is associated with cardiac dysfunction secondary to myocardial ischaemia. Cardiac troponin I (cTnI) is a marker of myocardial necrosis. Raised concentrations in the blood are related to perinatal asphyxia and increased morbidity.

Objective: To assess porcine myocardial damage from enzyme release during hypoxaemia induced global ischaemia, and subsequent resuscitation with ambient air or 100% O₂. To investigate whether CO₂ level during resuscitation influences myocardial damage.

Design: Newborn piglets (12–36 hours) were exposed to hypoxaemia by ventilation with 8% O₂ in nitrogen. When mean arterial blood pressure had fallen to 15 mm Hg, or base excess to < −20 mmol/l, the animals were randomly resuscitated with ventilation with either 21% O₂ (group A, n = 29) or 100% O₂ (group B, n = 29) for 30 minutes. Afterwards they were observed in ambient air for another 150 minutes. During resuscitation, the two groups were further divided into three subgroups with different CO₂ levels.

Analysis: Blood samples were analysed for cTnI, myoglobin, and creatine kinase-myocardial band (CK-MB) at baseline and at the end of the study.

Results: cTnI increased more than 10-fold (p < 0.001) in all the groups. Myoglobin and CK-MB doubled in concentration.

Conclusion: The considerable increase in cTnI indicates seriously affected myocardium. Reoxygenation with 100% oxygen offered no biochemical benefit over ambient air. CK-MB and myoglobin were not reliable markers of myocardial damage. Normoventilation tended to produce better myocardial outcome than hyperventilation or hypoventilation.

METHODS

Approval

The experimental protocol was approved by the hospital’s ethics committee for animal studies under surveillance of the Norwegian Animal Experimental Board. The animals were cared for and handled in accordance with the European Guidelines for Use of Experimental Animals.

Surgical preparation

Seventy five newborn Landrace piglets (12 to 36 hours) were delivered from a local farmer (one hour transportation) on the day of the experiment. Seventeen were excluded, mainly because of low haemoglobin concentration at baseline, infection, metabolic acidosis, and diarrhoea. One was excluded because it died from hypoxaemia, one because of skull fracture, and three because of technical problems.

Abbreviations: cTnI, cardiac troponin I; PAP, pulmonary artery pressure; PIP, peak inspiratory pressure; PVR, pulmonary vascular resistance
General anaesthesia was induced with 4% halothane (Fluothane; AstraZeneca, Södertälje, Sweden), reduced to 1–1.5% mixed with room air/100% O₂ until the piglet was unconscious. An ear vein was cannulated, and pentobarbital 50 mg/ml (Haukeland University Hospital Pharmacy, Bergen, Norway) 15–20 mg/kg, fentanyl (Leptanal; Janssen Pharmaceutica, Beerse, Belgium) 10–30 μg/kg, and midazolam (Dormicum; Hoffmann La Roche, Basel, Switzerland) 0.4 mg/kg was given. Lidocaine (Xylocain; AstraZeneca) 1% was given as local anaesthetic before tracheotomy. General anaesthesia was continued throughout the experiment, given as a continuous infusion with fentanyl 50 μg/kg and midazolam 0.25 mg/kg (IVAC P2000 infusion pump). If necessary, a bolus of fentanyl (10 μg) or midazolam (1 mg) was added. Before cannulation of the femoral artery, a bolus of pancuronium (Pavulon; NV Organon Os, the Netherlands) 0.1 mg/kg was given to prevent shivering. A continuous intravenous infusion (saline 0.7% and glucose 1.25%) 20 ml/kg was given throughout the experiment. Glucose was regularly measured in the blood (Blood Gas Analyzer 860; Ciba Corning Diagnostics, Midfield, Massachusetts, USA), and intravenous infusion was occasionally adjusted to keep serum glucose in the selected range (2–10 mmol/l). Haemoglobin 50–110 g/l was confirmed after surgery (Co-oximeter 270; Instrumentation Laboratory, Lexington, Massachusetts, USA). Base excess and electrolytes were not adjusted.

After tracheotomy, a 3.5 mm Portex endotracheal tube (Portex Ltd, Hythe, Kent, UK) was inserted, with a ligature around the tube and trachea to prevent leakage and displacement of the tube. The piglets were ventilated mechanically with a pressure controlled ventilator, (Babyplog 8000+; Drägerwerk, Lubeck, Germany). Normoventilation (Paco2 4.5–6.0 kPa) and a tidal volume 10–15 ml/kg was achieved by adjusting the peak inspiratory pressure (PIP) or ventilatory rate. During surgery, stabilisation, and hypoxia, ventilatory rate was 30–40 breaths/minute. Inspiratory time (0.4 second) and positive end expiratory pressure (4 cm H₂O) were kept constant throughout the experiment. Inspired fraction of O₂ and end tidal CO₂ were continuously measured (Datex Normocap Oxy; Datex, Helsinki, Finland). The right femoral artery was cannulated with polyethylene catheters (Portex PE-50, inner diameter 0.58 mm). Rectal temperature was maintained at 38–40°C with a heating blanket and a radiant heating lamp.

Experimental protocol

One hour of stabilisation was allowed after surgery. Hypoxaemia was achieved by ventilation with a gas mixture of 8% O₂ in N₂ (AGA, Oslo, Norway), until either mean arterial blood pressure reached 15 or base excess \( < -20 \text{ mm Hg} \). CO₂ gas was added to produce a Paco₂ of 8.0–9.5 to imitate a situation of birth asphyxia. Before the start of resuscitation, the piglets were block randomised by the fact that, in neonates and newborn animals, pulmonary vasodilation follows hyperoxaemia or oxygen treatment. Whether resuscitation was with ambient air or 100% O₂ made no difference to the outcome. This is despite the fact that, in neonates and newborn animals, pulmonary vasodilation follows hyperoxaemia or oxygen treatment.
From this, one could expect that reduced pulmonary artery pressure (PAP) would follow a decrease in pulmonary vascular resistance (PVR), with a secondary decrease in right ventricular workload. We did not, however, find any evidence of reduced myocardial enzyme release in the piglets treated with 100% oxygen (fig 3). Our results showed a trend in the opposite direction. In all the piglets resuscitated with 100% O₂ (group B1, B3), except the normoventilated ones, the increase in cTnI release was greater than in the piglets resuscitated with ambient air (NS) (figs 2 and 3). Other mechanisms are thought to influence the outcome. Further investigations are necessary to resolve this.

In newborn piglets, as in neonates, asphyxia is one of the mechanisms that may induce pulmonary vasoconstriction and increased PVR. PVR is also augmented by acidosis and hypercapnia. Medbo et al studied early changes in pulmonary haemodynamics during hypoxia and reoxygenation in normoventilated newborn piglets. They found that PAP increased significantly in the first few minutes, and then decreased at the end of the hypoxaemic period. During early reoxygenation, PAP rapidly increased significantly above baseline, before reaching values comparable to baseline. Fugelseth et al examined pulmonary haemodynamic changes in unsedated piglets in a long term follow up study. They confirmed the early transient increase in PAP, assessed by echocardiography three hours after global hypoxic-ischaemic brain injury. The use of different models—that is, anaesthetised versus unsedated piglets—is probably one reason for the different timing of PAP normalisation. The different ages may also be important: the piglets in the experiment of Fugelseth et al were aged 12–36 hours, whereas those in the other group were 3–5 days old. Susceptibility to alterations in PVR may be greater closer to birth.

This model has been used previously to study cellular effects on other organs during hypoxia and reoxygenation. With respect to the myocardium, a haemodynamic approach is necessary as well as cellular mechanisms. With regard to blood pressure (fig 1, table 2), all the piglets followed the same pattern. The large decrease suggested cardiac involvement and ventricular dysfunction. Blood pressure, however, has a multifactorial regulation. The piglets

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**Table 1** Baseline values

<table>
<thead>
<tr>
<th>Group</th>
<th>CO₂ level</th>
<th>No of piglets</th>
<th>Weight (kg)</th>
<th>Hb (g/l)</th>
<th>Hypoxaemia duration (min)</th>
<th>BE (mmol/l)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 21% O₂</td>
<td>Low</td>
<td>9</td>
<td>1.9 (0.1)</td>
<td>7.6 (1.1)</td>
<td>63 (6)</td>
<td>1.71</td>
<td>7.45</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>10</td>
<td>1.6 (0.1)</td>
<td>7.8 (2.2)</td>
<td>75 (10)</td>
<td>0.52</td>
<td>7.45</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>10</td>
<td>1.6 (0.1)</td>
<td>8.4 (1.3)</td>
<td>56 (8)</td>
<td>1.68</td>
<td>7.42</td>
</tr>
<tr>
<td>B 100% O₂</td>
<td>Low</td>
<td>9</td>
<td>1.8 (0.1)</td>
<td>7.0 (1.3)</td>
<td>61 (7)</td>
<td>0.68</td>
<td>7.45</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>10</td>
<td>1.7 (0.1)</td>
<td>8.0 (1.3)</td>
<td>65 (5)</td>
<td>0.10</td>
<td>7.43</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>10</td>
<td>1.7 (0.1)</td>
<td>7.6 (1.6)</td>
<td>67 (11)</td>
<td>0.28</td>
<td>7.43</td>
</tr>
</tbody>
</table>

Values are mean (SEM).

**Table 2** Mean arterial pressure (MAP) and heart rate

<table>
<thead>
<tr>
<th>Group</th>
<th>CO₂ level</th>
<th>MAP (mm Hg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>Start resusc</td>
</tr>
<tr>
<td>A 21% O₂</td>
<td>Low</td>
<td>72 (4)</td>
<td>23 (3)</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>71 (6)</td>
<td>21 (3)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>74 (6)</td>
<td>23 (3)</td>
</tr>
<tr>
<td>B 100% O₂</td>
<td>Low</td>
<td>78 (3)</td>
<td>20 (2)</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>74 (4)</td>
<td>26 (5)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>71 (4)</td>
<td>20 (2)</td>
</tr>
<tr>
<td>All groups</td>
<td></td>
<td>74 (5)</td>
<td>22 (2)</td>
</tr>
</tbody>
</table>

Values are mean (SEM).

**Table 3** Cardiac troponin I (cTnI) concentration at baseline and end point

<table>
<thead>
<tr>
<th>Group</th>
<th>CO₂ level</th>
<th>cTnI at baseline (µg/l)</th>
<th>cTnI at end point (µg/l)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 21% O₂</td>
<td>Low</td>
<td>0.115 (0.02)</td>
<td>2.340 (0.70)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>0.077 (0.02)</td>
<td>1.074 (0.24)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.087 (0.03)</td>
<td>1.771 (0.61)</td>
<td>20</td>
</tr>
<tr>
<td>B 100% O₂</td>
<td>Low</td>
<td>0.092 (0.01)</td>
<td>3.109 (1.32)</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>0.114 (0.03)</td>
<td>1.506 (0.40)</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.091 (0.01)</td>
<td>3.416 (1.49)</td>
<td>38</td>
</tr>
</tbody>
</table>

Values are mean (SEM). Ratio between cTnI levels describes the difference between the groups (NS).
Myocardial damage and hypoxaemia in neonatal pigs

in each group were given equivalent fluid therapy, 20 ml/kg throughout the experiment. None of the piglets were supported with inotropic drugs. Glucose concentration was kept within the selected range.

With respect to base excess, the piglets showed similar patterns, with a large decrease during hypoxia, a slower increase during resuscitation, and near normalisation at the end of the experiment. None of the animals were acid/base corrected.

In this study, the piglets with low or high CO₂ showed a greater release of cTnI than the piglets in the normoventilated group (group 2, fig 2), suggesting more serious cardiac deterioration. The piglets in group 3, with high CO₂, were normoventilated and supplied with CO₂ gas. Raised CO₂ concentration leads to vasoconstriction in the lungs and increased PVR and PAP. To achieve the low PaCO₂ in the hyperventilated groups (group 1), PIP and ventilatory rate were both altered during the 30 minutes of resuscitation. The considerable increase in PIP leads to increased intrathoracic pressure, which again may cause increased pressure in the pulmonary artery and increase the right ventricle workload. Several studies have shown the effect of increased intrathoracic pressure on preload. Reduced venous return to the right ventricle is one of the mechanisms. Low inspiratory CO₂ concentration leads to pulmonary vasodilatation. The decrease in PaCO₂, however, seems to play a minor role related to the large increase in intrathoracic pressure. The decrease in CO₂ is not capable of compensating for the effects of the PIP induced increase in intrathoracic pressure. The piglets going through a period of hyperventilation also had a higher heart rate than the other piglets at the end of the experiment (p < 0.01). In this group, heart rate also normalised, but at a lower rate. Increased heart rate reduces the time for coronary perfusion, which affects the myocardium, and may further increase myocardial susceptibility to impairment.

CONCLUSION

All groups in this hypoxia/reoxygenation model showed a significant release of cTnI, confirming severe cardiac dysfunction. Both haemodynamic and cellular mechanisms play a major part in cardiovascular regulation and influence the myocardial dysfunction caused by ischaemia-reoxygenation injury. Reoxygenation with 100% oxygen offered no biochemical protection against troponin release compared with ambient air. Normoventilation tended to produce a better myocardial outcome than hyperventilation or hypoventilation, as assessed by enzyme release.

ACKNOWLEDGEMENTS

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