Selective cerebral perfusion using moderate flow in complex cardiac surgery provides sufficient neuroprotection. Are children young adults?

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Abstract

OBJECTIVE: Selective cerebral perfusion (SCP) is commonly applied during the correction of complex congenital cardiac defects. In this study, we assessed the impact of different flow levels of SCP on potential brain ischaemia.

METHODS: Fifteen piglets (7–10 kg, age 3–4 weeks) received SCP via the right common carotid artery during cardiopulmonary bypass at 25°C for 90 min. Regular brain perfusion (1 ml/g brain weight/min), moderate hypoperfusion (0.5 ml/g/min) and extensive hypoperfusion (0.25 ml/g/min) were evaluated. Clinical parameters and tissue oxygenation index (TOI) were registered online until 3 h of reperfusion. Hematoxylin and eosin (HE) staining and immunohistological analyses for apoptosis inducing factor (AIF) and nitrotyrosine (NO-Tyr) were performed on sections of the hippocampus.

RESULTS: Intracerebral pressure remained stable throughout the study. Haemodynamic parameters, blood gas and lactate measurements were stable until the end of the study. Extensive hypoperfusion led to a moderate reduction of TOI. NO-Tyr immuno-positive cells were 15.7% at regular cerebral perfusion, 23.9% at moderate hypoperfusion (P = n.s.) and 46.1% at extensive hypoperfusion (P < 0.05). AIF immuno-positive nuclei were present in 8.3% of the hippocampus cells after regular perfusion, in 10.8% after moderate hypoperfusion (P = n.s.) and in 17.9% after extensive hypoperfusion (P < 0.05).

CONCLUSIONS: SCP using a moderate SCP flow regime demonstrates comparable results to normal brain perfusion while after extensive hypoperfusion significant morphological brain injury could be found. Thus moderate, but not extensive, hypoperfusion might have the potential to prevent perfusion-related cerebral oedema and an increasing risk of brain injury.

Keywords: Selective cerebral perfusion • Cerebral protection • Nitrotyrosine • Apoptosis inducing factor • Congenital heart surgery

INTRODUCTION

Cerebral protection is one of the most important objectives when performing complex cardiac and aortic arch surgical procedures in congenital as well as in the adult cardiac surgery. During cardiopulmonary bypass (CPB) hypothermic perfusion has been applied for decades and corrections on the aortic arch had been performed under deep hypothermic circulatory arrest (DHCA) [1, 2]. However, prolonged duration of DHCA could be found to be a predictor for impaired long-term neurodevelopmental outcomes [3].

More recently, selective cerebral perfusion (SCP) has been applied to achieve adequate cerebral protection during surgical corrections of the aortic arch [4–7]. Using these strategies, standard corrections can be performed under moderate hypothermia or even normothermia [8, 9]. However, as there is no clear evidence in favour of normothermia, some degree of hypothermia provides a clear safety margin and SCP may be used in different temperature strategies to reduce the risk of cerebral ischaemic/reperfusion injury [4–7, 10]. Despite an almost routine application of SCP, little is known about the potential of different cerebral flow regimes of SCP to protect the brain. No data regarding the incidence of early cerebral injury and the associated impairment of neurocognitive functional status and the potential extent of cerebral damage in relation to different perfusion modes is available. Thus, the aim of this experimental animal study was to evaluate the potential for cerebral protection using specific markers for diffuse brain damage during different controlled SCP strategies at moderately hypothermic CPB of 25°C.
MATERIALS AND METHODS

The study was performed on 15 piglets aged between 3–4 weeks with body weights of 7–10 kg. Using sealed envelopes, the animals were randomized into three groups. Animals in each group were subjected to 90 mins of (1) regular SCP (1 ml/g brain weight/min), or (2) moderate hypoperfusion (0.5 ml/g/min) or (3) extensive hypoperfusion (0.25 ml/g/min) prior to each experiment. One procedure per day was performed in a standard experimental operative theatre. Approval was obtained from the governmental offices prior to this study and all animals received humane care in compliance with the 'Guide for the Care and Use of Laboratory Animals' prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, Washington, DC, USA, revised in 1996. General anaesthesia was applied for all surgical interventions.

For induction animals received Atropine at a dose of 0.02 mg/kg and Azaperone (Stresnil™) at a dose of 4 mg/kg intramuscularly. Intravenous access was then accomplished via a peripheral vein; the piglets received 4 mg/kg of Thiopental (Trapanal™), and 1 mg of Pancuronium intravenously. Tracheotomy was performed under additional local anaesthesia (5–7 ml lidocaine 2%). A 5 French endotracheal tube was used; Isoflurane at 0.5–2 Volume% and controlled ventilation were administered and the animals received continuous infusion of Fentanyl at a dose of 25 μg/kg/h and Midazolam (Dormicum™) at a dose of 0.2 mg/kg/h via a central venous line.

Also, lines for measurement of intracranial pressure and brain temperature (placed slightly parasagittally), femoral arterial pressure and internal jugular vein oxygen saturation were inserted. The Doppler probes for carotid artery blood flow measurement as well as the electrodes for an ECG and registration of the tissue oxygenation index (TOI) [11]. After median sternotomy, thymectomy and pericardiotomy, both subclavian arteries and the left carotid artery were prepared.

A schematic illustration on the operative set-up is given in Fig. 1. Arterial access for CPB was established via a Y-piece, and a 2.6-mm cannula and a 2.0-mm cannula (Stockert, Munich, Germany) were introduced into the proximal aortic arch and the common brachiocephalic trunk, respectively. Venous return was obtained using a standard 14 French cannula placed in the right atrium. During SCP the common brachiocephalic trunk was cross-clamped proximally to the cannulation site and the left carotid artery and both subclavian arteries were snared down.

Systemic body perfusion was continued to avoid significant body ischaemia affecting metabolic and haemodynamic stability during the experiments. Blood cardioplegia was administered via a needle vent. An additional vent was placed via the left atrial appendage if required.

All experiments were performed under moderate hypothermia of 25°C. During cooling and re-warming, a pH-stat strategy and an α-stat strategy were chosen, respectively. Hyperoxygenation aiming at an arterial pO2 between 200 and 600 mmHg was performed throughout the entire experiment.

Haemodynamic and laboratory parameters were measured online with registration at eight specific moments: (a) before starting CPB under normothermia; (b) after aortic cross-clamp; (c) after 45 min of SCP; (d) after 90 min of SCP; (e) after full re-warming to 37°C; (f) after one; (g) after two; and (h) after 3 h of reperfusion. The animals were then sacrificed and rapid brain removal was performed. Tissue samples of the left and right hippocampus as the most sensitive watershed area of the brain were analyzed.

Figure 1: Schematic drawing of the anatomy of piglets in the presence of a common arterial trunk when performing SCP.
were taken and preserved in alcohol and snap frozen in liquid nitrogen until further analysis.

The specific sections CA1, CA2, CA3 and CA4 of both sides of the hippocampus were analysed regarding signs of ischaemic/reperfusion injury and diffuse scattered neurological damage or cerebral oedema. Standard histological examinations were performed initially. Immunohistology was performed to evaluate the amount of the neurones per visual field showing positive nitrotyrosine (NO-Tyr) staining. An Axiolab (Zeiss, Jena, Germany) microscope at 1000× magnification, an F4 camera (Nikon, Japan) and the KS 300 software (Zeiss) were used for quantitative evaluation. Apoptosis inducing factor (AIF) was quantified on histological sections of the CA1 region of the hippocampus after indirect immune-staining using an anti-AIF primary antibody (monoclonal mouse anti-AIF IgG, Santa Cruz Biotechnology, Santa Cruz, USA; 1:100) and a horseradish peroxidase labelled secondary anti-mouse antibody (Sigma, Deisenhofen, Germany) followed by tyramide signal amplification and AEC staining for visualization according to standard protocols. Results are given in percent of neurones exhibiting nuclear translocations related to the total number of investigated neurones within a visual field (400× magnification).

Continuous variables are expressed as the mean ± standard error of the mean or as the median and categorical data as proportions. Normal distribution of all metric variables was tested by the one-sample Kolmogorov–Smirnov test. For post hoc multiple comparisons between the groups the Bonferroni correction was used. Categorical variables were compared using the χ2 test and dependent continuous variables were compared by two-tailed Student’s t-test or the Mann–Whitney U-test as appropriate. Statistical analysis was performed using the SPSS 17.0 statistical software package. A P < 0.05 was considered to indicate statistical significance. All authors had full access to the data and take full responsibility for its integrity.

### RESULTS

The experiments were successfully performed in all 15 animals with random assignment to the three groups. Access to the heart, cannulation and conduction of CPB was uneventful. Throughout the observational period the animals were stable regarding heart rate, arterial blood pressure and central venous pressure. Intracranial pressure remained stable without any signs of cerebral oedema. Routine blood gas analyses demonstrated good arterial and central venous oxygenation indicative of sufficient cardiac output and CPB support, respectively. Initial lactate levels were low in all groups, but they significantly increased during reperfusion. A detailed overview on the perioperative haemodynamic and laboratory findings is given in Table 1.

Cerebral perfusion as measured on the right carotid artery with all other head and neck vessels being snared is given in Table 2. These results indicate that the three different groups received the target level of cerebral blood flow during the 90 min interval of SCP. During reperfusion a significant increase in carotid artery flow was seen in the two low-flow groups, whereas there was no significant increase in the normal perfusion group.

The TOI is given in Table 3. The results indicate relatively stable levels throughout the study in the normal perfusion and the moderate hypoperfusion group. In comparison, during extensive hypoperfusion there was a clear decrease in the TOI during the period of SCP and a significant increase at reperfusion.

Results on specific histological markers for ischaemic/reperfusion tissue damage are given in Figs 2 and 3. In Fig. 2 results for NO-Tyr are shown. Relative cell numbers per visual view were 15.7% at normal perfusion, 23.9% at moderate hypoperfusion and 46.1% at extensive hypoperfusion (P < 0.05 against the two others). Figure 4 shows a representative view of the NO-Tyr-immunohistology in the normal perfusion vs the severe hypoperfusion group.

Results for AIF are demonstrated in Fig. 3. Nuclear translocation was present in 8.3% at normal perfusion, 10.8% at moderate hypoperfusion and 17.9% at severe hypoperfusion (P < 0.05 vs the two others). No difference between both sides of the hippocampus could be found. A typical view showing AIF translocation during severe hypoperfusion in a section CA1 of the left hippocampus is given in Fig. 5.

### DISCUSSION

Different techniques to perform complex surgical aortic arch correction are used clinically. For various reasons, DHCA remains the standard technique in aortic arch surgery, especially in congenital heart surgery [1]. In recent decades, moderate

Table 1: Haemodynamic, functional and laboratory parameters

<table>
<thead>
<tr>
<th>Total = 15</th>
<th>Severe hypoperfusion (0.25 ml/g)*</th>
<th>Moderate hypoperfusion (0.5 ml/g)*</th>
<th>Normal perfusion (1 ml/g)*</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td>Heart rate (min)</td>
<td>126 ± 5</td>
<td>123 ± 4</td>
<td>109 ± 3</td>
<td>n.s.</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>44 ± 1</td>
<td>44 ± 2</td>
<td>45 ± 1</td>
<td>n.s.</td>
</tr>
<tr>
<td>CVP (mmHg)</td>
<td>7 ± 1</td>
<td>8 ± 1</td>
<td>7 ± 1</td>
<td>n.s.</td>
</tr>
<tr>
<td>ICP (mmHg)</td>
<td>9 ± 1</td>
<td>9 ± 1</td>
<td>10 ± 1</td>
<td>n.s.</td>
</tr>
<tr>
<td>pO₂ (mmHg)</td>
<td>342 ± 13</td>
<td>322 ± 17</td>
<td>344 ± 13</td>
<td>n.s.</td>
</tr>
<tr>
<td>S₅0₂ (%)</td>
<td>68 ± 3</td>
<td>65 ± 3</td>
<td>72 ± 2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Lacₐ, pre-ECC</td>
<td>4.1 ± 0.5</td>
<td>3.4 ± 0.4</td>
<td>3.2 ± 0.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>Lacₐ, at reperfusion</td>
<td>6.5 ± 0.5</td>
<td>7.9 ± 1.2</td>
<td>4.4 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

MAP: mean arterial pressure; CVP: central venous pressure; ICP: intracranial pressure; pO₂: Oxygen pressure at the arterial blood gas analysis; S₅0₂: Venous oxygen saturation; Lacₐ: arterial lactate; ECC: extracorporeal circulation.

*Brain perfusion during SCP in ml/g brain weight per minute.
Table 2: Right carotid artery flow (in ml/min) at eight different moments of the experiment

<table>
<thead>
<tr>
<th>Total = 15</th>
<th>Severe hypoperfusion (0.25 ml/g)</th>
<th>Moderate hypoperfusion (0.5 ml/g)</th>
<th>Normal perfusion (1 ml/g)</th>
<th>P-value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Before ECC = baseline</td>
<td>73 ± 5</td>
<td>81 ± 5</td>
<td>70 ± 11</td>
<td>n.s.</td>
</tr>
<tr>
<td>(b) x-clamp</td>
<td>10.3 ± 1</td>
<td>24.3 ± 2</td>
<td>52.1 ± 2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(c) 45 min x-clamp</td>
<td>9.9 ± 1</td>
<td>25.1 ± 3</td>
<td>44.5 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(d) 90 min x-clamp</td>
<td>10.4 ± 1</td>
<td>22.8 ± 2</td>
<td>51.4 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(e) 37°C</td>
<td>53 ± 10</td>
<td>52 ± 11</td>
<td>52 ± 6</td>
<td>n.s.</td>
</tr>
<tr>
<td>(f) 1 h reperfusion</td>
<td>43 ± 6</td>
<td>49 ± 11</td>
<td>54 ± 7</td>
<td>n.s.</td>
</tr>
<tr>
<td>(g) 2 h reperfusion</td>
<td>45 ± 6</td>
<td>37 ± 11</td>
<td>58 ± 14</td>
<td>n.s.</td>
</tr>
<tr>
<td>(h) 3 h reperfusion</td>
<td>55 ± 7</td>
<td>44 ± 18</td>
<td>60 ± 12</td>
<td>n.s.</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

Phases b, c, d indicate measurements during SCP. ECC: extracorporeal circulation; x-clamp: aortic cross-clamp; 37°C: time point when full re-warming to 37°C was achieved; SCP: selective cerebral perfusion.

Table 3: TOIs as non-invasively measured using epicrani near infrared spectroscopy (NIRO) measurements during the eight different phases of the experiment

<table>
<thead>
<tr>
<th>Total = 15</th>
<th>Severe hypoperfusion (0.25 ml/g)</th>
<th>Moderate hypoperfusion (0.5 ml/g)</th>
<th>Normal perfusion (1 ml/g)</th>
<th>P-value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Before ECC = baseline</td>
<td>60 ± 1</td>
<td>57 ± 3</td>
<td>56 ± 2</td>
<td>n.s.</td>
</tr>
<tr>
<td>(b) x-clamp</td>
<td>55 ± 3</td>
<td>56 ± 4</td>
<td>55 ± 2</td>
<td>n.s.</td>
</tr>
<tr>
<td>(c) 45 min x-clamp</td>
<td>48 ± 5</td>
<td>47 ± 2</td>
<td>52 ± 2</td>
<td>n.s.</td>
</tr>
<tr>
<td>(d) 90 min x-clamp</td>
<td>49 ± 4</td>
<td>48 ± 3</td>
<td>52 ± 3</td>
<td>n.s.</td>
</tr>
<tr>
<td>(e) 37°C</td>
<td>60 ± 4</td>
<td>56 ± 3</td>
<td>54 ± 3</td>
<td>n.s.</td>
</tr>
<tr>
<td>(f) 1 h reperfusion</td>
<td>62 ± 4</td>
<td>50 ± 2</td>
<td>59 ± 4</td>
<td>n.s.</td>
</tr>
<tr>
<td>(g) 2 h reperfusion</td>
<td>57 ± 1</td>
<td>54 ± 6</td>
<td>59 ± 3</td>
<td>n.s.</td>
</tr>
<tr>
<td>(h) 3 h reperfusion</td>
<td>57 ± 2</td>
<td>57 ± 5</td>
<td>58 ± 3</td>
<td>n.s.</td>
</tr>
<tr>
<td>P-value</td>
<td>0.007</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
</tr>
</tbody>
</table>

Phases c and d indicate measurements during SCP. 37°C: time point when full re-warming to 37°C was achieved; SCP: selective cerebral perfusion.

Figure 2: No-Tyr measurements on sections of the hippocampus for the three different groups (five animals each) with a total of 15 animals. Groups are divided according to severe hypoperfusion (0.25 ml/g brain weight/min), moderate hypoperfusion (0.5 ml/g brain weight/min) and normal perfusion (1 ml/g brain weight/min).

Figure 3: Translocation of AIF into the nucleus in percent as measured on sections of the hippocampus for the three different groups (five animals each) with a total of 15 animals. Groups are divided according to severe hypoperfusion (0.25 ml/g brain weight/min), moderate hypoperfusion (0.5 ml/g brain weight/min) and normal perfusion (1 ml/g brain weight/min).
Aortic arch surgery appears to be safe when using SCP at normal hypothermia of 25–30°C has been provided and has demonstrated clinical feasibility when additional SCP was applied. Although SCP has been applied in several studies [4–7], no evaluation of the effectiveness of different SCP flow and temperature strategies has been performed so far. Thus, here we evaluated the influence of different SCP flow rates on ischaemic/reperfusion injury of the brain using markers for diffuse damage in such watershed areas of the hippocampus.

A standard piglet model mimicking the clinical situation in congenital heart surgery was used. Compared with the clinical situation, however, in our animal model additional aortic arch perfusion during the period of SCP with occlusion of all head vessels was justified in order to avoid severe end-organ damage that might have obscured the overall results on postoperative cerebral function due to haemodynamic and metabolic instability caused by global metabolic derangement. This allowed us to focus exclusively on the potential brain damage and to exclude the effects of global body ischaemia that, of course, also might influence the overall outcome of patients undergoing aortic arch surgery in daily routine.

The results of non-invasive NIRS indicate rather unspecific changes throughout the experiments. However, there was a significant increase in the TOI during reperfusion in the low-flow group. Even so, the NIRS as a non-invasive and easily applicable monitoring of the cerebral oxygenation during these operations remains useful for further clinical practice [12].

There were several important findings in the present study. Aortic arch surgery appears to be safe when using SCP at normal or moderately reduced SCP flow conditions under moderate (25°C) hypothermia. Severe hypoperfusion of one-fourth of the normal flow, however, leads to a significant increase in cerebral damage. The diffuse ‘scattered’ pattern of cerebral injury may be an indicator for diffuse cerebral injury and might be closely associated with neurodevelopmental retardation in the clinical setting.

There is a continuous risk of suffering neuropsychological deficits during complex cardiac surgical procedures. This may lead to neurological dysfunction, impaired motor activities, speech disturbances and an impaired intelligence level [13–15]. Recently, long-term results after correction for relatively simple congenital cardiac defects have been published. Corrective surgery with low-flow CPB combined with circulatory arrest was associated with reduced a neurodevelopmental outcome [16]. The use of DHCA remains controversial and further studies on the improvement of perioperative care is required [17]. Thus, the present study on SCP under moderate hypothermia without circulatory arrest is important for further improvement in patient care.

When evaluating the present results, we did not find any gross cerebral perfusion deficits indicative of major stroke. This is due to the fact that some degree of cerebral perfusion and additional global protection by moderate hypothermia were present. We specifically intended to evaluate for potential end-stream perfusion deficits in such watershed areas as the hippocampus.

The diffuse hippocampus damage may be indicative of clinically present neurodevelopmental dysfunctions. To test for such damage, besides using routine haemodynamic and histological monitoring, we selected NO-Tyr and AIF as markers of potential cerebral damage.

NO-Tyr is a highly sensitive marker for early ischaemic/reperfusion injury caused by the liberation of NO due to low-flow ischaemia reacting with oxygen-derived radicals and resulting in the formation of peroxynitrite, which can then lead to the formation of NO-Tyr. Proteins with modified tyrosine residues (NO-Tyr) may lose or change their function, and cell function may be altered as a consequence of NO-Tyr formation. Cells exhibiting enhanced NO-Tyr formation may be destroyed later on. The CA1 region of the hippocampus is a so-called watershed area in the brain and therefore is especially sensitive to hypoperfusion. In addition, ischaemic/reperfusion injury can lead to DNA strand breaks and the induction of apoptosis. While complete apoptosis takes >6 h to develop, one of the earliest events in the induction of apoptosis is the translocation of AIF to the nucleus, which can be observed within the first hours. Thus, it was possible to investigate AIF within the time frame of our

Figure 4: No-Tyr-immunohistology on section CA1 of the hippocampus in an animal having severe hypoperfusion (0.25 ml/g brain weight/min) of the brain (left) compared with an animal having normoperfusion (1 ml/g brain weight/min) of the brain (right).

Figure 5: Translocation of AIF (red arrows) into the nucleus in section CA1 of the hippocampus in an animal having severe hypoperfusion (0.25 ml/g brain weight/min) of the brain.
experiments. AIF has the potential to delineate early cerebral changes in the presence of potential alterations caused by the perioperative perfusion and cerebral protection strategies. Cells with positive nuclear AIF translocation can be assumed to undergo apoptosis as a subsequent process and might be destroyed later on. Results from AIF and NO-Tyr staining are markers for scattered neuronal cell damage in watershed areas such as the hippocampus. These results may account for the intellectual, developmental and psychological deficits seen after complex congenital cardiac surgery. According to our results, sufficient SCP management may potentially attenuate these adverse outcomes.

As mentioned above, we found no difference in ischaemia/reperfusion between the left and right hippocampi. This finding indicated a good bilateral collateralization through the pig’s Circle of Willis and extracranial arterial branches. However, this finding can only serve as an uncertain reference for the transfer in neonates and infants with aortic arch malformations.

The lack of neuropsychological testing, which is practically impossible in any animal model, is probably the most important shortcoming of the present study. However, based on the present findings, delineating the relatively safe from the rather risky perfusion strategies will be of significant benefit for further clinical applications [18]. Future studies will have to focus on additional protective strategies (i.e. specific medical therapies) to further decrease cerebral ischaemic/reperfusion injury.

The current findings demonstrate the potential for additional cerebral protection by means of SCP. Comparable to recent clinical studies, moderate flow rates seem to be safe and may have the potential to avoid perfusion-related brain injury and oedema [19]. Thus, moderate (25°C) instead of deep (18°C) hypothermia might be a considerable option in congenital heart surgery requiring circulatory arrest [20].

In summary, regarding the apoptotic markers, sufficient brain protection has been documented when applying normal perfusion of 1 ml/g brain weight/min and moderate hyperoxygenation of 0.5 ml/g/min at moderate hypothermia (25°C). However, further human studies are warranted to verify the protective potential of SCP in moderate hypothermia [21]. As shown here, extensive cerebral hyperoxygenation of 25% of normal levels is associated with worse cerebral oxygenation and significantly increased brain damage and thus should be avoided.

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**Conflict of interest:** none declared.

**REFERENCES**


**APPENDIX. CONFERENCE DISCUSSION**

Dr R. Bonser (Birmingham, UK): The perfusate conditions and the pressure and flow conditions for optimal selective antegrade perfusion both in children and adults are just not known, and your work has taken us a step further.

One thing that we've heard yesterday, and work that has been done in Randall Griep's laboratory, suggests that very high perfusion pressures during SACP may be detrimental. As SACP progresses, there is a gradual increase in
Intracranial pressure and an increase in cerebrovascular resistance but you didn't find this in your experiments.

Disturbingly, in your results you appeared to show some lactate generation in your lower perfusion groups. The brain is an obligatory consumer of only glucose, and it is anaerobic glucose metabolism that generates lactate. When the brain does generate lactate, which it usually does not do in adult SACP, that lactate generation is a real danger signal.

So the question I ask you is, did you see changes in cerebrovascular resistance across your groups over time? And did you see pressure changes? And thirdly, could you comment more on the lactate generation that you've observed.

**Dr Emrich**: To answer your first question, we didn't see any differences in intracranial pressures. We didn't do any assessments of the vascular resistance. We had five pigs per group so that was really just a hypothesis-creating study to maybe do a little bit more on that topic later on. The lactate I gave was serum lactate, a whole body lactate, so that probably has nothing to do with the metabolism in the brain. But I found it interesting that we had the same setup in all the animals, but the lactate still is higher in the high perfusion group, and I don't know where that came from.

**Dr F. Santini** (Verona, Italy): By reducing the perfusion flow, I think you increase the chance that your perfusion will be unevenly distributed in the brain. How can you ensure by just checking on the hippocampus instead of any other spot in the brain that your perfusion will be evenly distributed?

**Dr Emrich**: That will be done in further studies, of course. We took the hippocampus because, as a watershed area, that is the most sensitive for ischaemia due to malperfusion or hypoperfusion. So that's where we could see damage or no damage, which is why we chose the hippocampus. We just wanted to know whether we could reduce the flow rates. We know that perfusion can lead to blood-brain barrier damage. We know that it can lead to cerebral oedema, more in hyperperfusion than in normal perfusion or hypoperfusion. But in our clinical practice we see so many seizures after these aortic cases and similar things, so it was just a study to review our SCP strategies and, with further research, we may come to the conclusion that we might be able to do hypoperfusion.

**Dr W. Harringer** (Braunschweig, Germany): Did you do any assessment, any further assessment, of brain oedema? Probably not after what you've said.

**Dr Emrich**: Well, what we did is that we took sections of the brain and weighed them directly after explant and then we dried them for 48 h and then weighed them again. But that's a very not sensitive procedure. We didn't find any differences there. And microscopically we saw some pericellular oedema but not a severe cerebral oedema.