Selective antegrade cerebral perfusion at two different temperatures compared to hypothermic circulatory arrest – an experimental study in the pig with microdialysis

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Received 2 December 2008; received in revised form 5 March 2009; accepted 6 March 2009

Abstract

Hypothermic arrest and selective antegrade cerebral perfusion (SACP) is widely used during aortic arch surgery. The microdialysis technique monitors biomarkers of cellular metabolism and cellular integrity over time. In this study, the cerebral changes during hypothermic circulatory arrest (HCA) at 20 °C and HCA with SACP at two different temperatures, 20 and 28 °C, were monitored. Twenty-three pigs were divided into three groups. A microdialysis probe was fixated into the forebrain. Circulatory arrest started at a brain and body temperature of 20 °C or 28 °C. Arrest with/without cerebral perfusion (flow 10 ml/kg, max carotid artery pressure 70 mmHg) lasted for 80 min followed by reperfusion and rewarming during 40 min and an observation period of 120 min. The microdialysis markers were registered at six time-points. The lactate/pyruvate ratio (L/P ratio) and the lactate/glucose ratio (L/G ratio) increased significantly (P<0.05), during arrest, in the HCA group. The largest increase of glycerol was found in the group with tepid cerebral perfusion (28 °C) and the HCA group (P<0.05). This study supports the use of SACP over arrest. It also suggests that cerebral metabolism and cellular membrane integrity may be better preserved with SACP at 20 °C compared to 28 °C.

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Keywords: Aortic surgery; Hypothermic circulatory arrest; Selective antegrade cerebral perfusion; Cerebral protection; Pig model; Microdialysis

1. Introduction

Cerebral injury is a threat during complex aortic arch surgery [1] and optimal cerebral protection is of crucial importance. Reducing the metabolism by cooling is the mainstay of cerebral protection and is used alone with hypothermic circulatory arrest (HCA) or in conjunction with cerebral perfusion [2].

Selective antegrade cerebral perfusion (SACP) is widely used and there are clinical and experimental [3] evidence of its superiority to HCA alone.

The microdialysis technique is an established clinical experimental method to monitor cellular metabolism [4] and is often used in neurointensive care after neurosurgery, trauma and stroke.

In this experimental study we used a porcine model including cardiopulmonary bypass (CPB), HCA and SACP.

The aim of the study was to explore the changes in cerebral energy metabolism and cellular integrity biomarkers by microdialysis during HCA (20 °C) alone and HCA with SACP at two different temperatures, 20 and 28 °C.

2. Material and methods

2.1. Study design

The study involved experiments on 23 domestic pigs (31.2 ± 2.5 kg) divided into three separate groups. In the first group (HCA, n=7) cerebral protection was achieved with HCA alone, at 20 °C. In the second group (SACP20, n=7) body temperature and cerebral perfusion temperature was 20 °C, and in the third group (SACP28, n=9) body temperature and cerebral perfusion temperature was 28 °C.

This project was approved by the Animal Research Ethical Committee of Uppsala University (C 284/05) and the animals were treated in compliance with the European Convention on Animal Care.

2.2. Anesthesia

Induction by intramuscular injection of 6 mg/kg tiletamine/zolazepam (Zoletil®; Reading Laboratories, Carros, France) and xylazine/atropine (Rompum®; Bayer AG, Leverkusen, Germany) 2.2 mg/kg and 0.04 mg/kg, respectively, followed by intravenous 5–10 μg/kg Fentanyl (Fentanyl®).
50 μg/ml, Pharmalink, Pharmaceuticals Ltd, Roscrea, Ireland). After intubation artificial ventilation was instituted using a Servo I ventilator (Siemens Electromedic Group, Danvers, MA). Tidal volume was 10 ml/kg, FiO2 50% and respiratory frequency adjusted to an end-tidal carbon dioxide tension between 5.2 and 5.6 kPa. Ventilation was reduced to 5 ml/min during CPB cooling and reperfusion and disconnected during HCA/SACP.

An infusion of 7.5 ml/kg/h glucose (Rehydrex, Fresenius Kabi, Sweden, glucose 25 g, Na+ 70 mmol, Cl− 45 mmol, Ac− 25 mmol) with 4 g ketamin (Ketalar® 50 mg/ml, Pfiezer), 40 mg pancuronium bromide (Pavulon® 2 mg/ml, Organon Teknika) and 0.5 mg fentanyl in every 1000 ml was used. Body temperature was measured rectally, in the brain and in the main pulmonary artery.

2.3. Perioperative management

Arterial and central venous catheters were inserted in the femoral and carotid arteries and the jugular vein for pressure monitoring, blood sampling and cardiac output measurement. The skull was prepared with two parallel burr holes. The microdialysis probe was placed in the first burr hole (see section 2.5.). The second was used for the temperature probe.

Ringer’s solution (Ringer-acetate®; Fresenius Kabi, Sweden; Na+ 131 mmol, K+ 4 mmol, Ca2+ 2 mmol, Mg2+ 1 mmol, Ac− 30 mmol, Cl− 110 mmol) (6 ml/kg/h) and colloid (Voluven® 60 mg/ml, Fresenius Kabi, Sweden) was infused.

2.4. Surgical preparation and CPB

After sternotomy heparin (Heparin® 5000 IU/ml; Løvens Kemiske Fabrik, Denmark) 400 U/kg was given to ACT over 400 s. The ascending aorta was cannulated with a 16 F arterial cannula and separate venous cannulae were used (28 F).

Priming of the CPB circuit was done with Ringer-acetate solution (600 ml), mannitol (200 ml) and 2500 U of heparin.

Non-pulsatile CPB (Jostra Heart-Lung Machine HL-15Ts, Sweden), using alpha-stat pH management, was initiated at a flow rate of 70–100 ml/kg/min and the flow was adjusted to a perfusion pressure not exceeding 70 mmHg. After cooling to a brain temperature of 20 °C or 28 °C, SACP was instituted by redirection of flow to the brachiocephalic artery from where both carotid arteries originate [3]. The right subclavian artery was snared.

Cardiac arrest was induced by infusion of cardioplegia (Cardioplegic St Thomas type I solution, Ringer-acetate 1000 ml, K+ 16 mmol, Mg2+ 16 mmol, procainum 1 mmol, Cl− 49 mmol, tribonate 10 ml) at a minimum of 15 ml/kg.

After declamping the aorta, rewarming was started. The temperature difference between blood and water was always below 10 °C.

After weaning epinephrine (Adrenalin®, NM Pharma; Stockholm, Sweden) and fenylefrin hydrochloride (Fenylefrin-hydroklorid® 10 mg/ml, Apoteksbolaget, Umeå, Sweden), were used as required.

2.5. Microdialysis

The microdialysis probe was inserted obliquely into the superficial area of the forebrain. The microdialysis probe (CMA-70 brain MD probe with 10 mm polyamide membrane; CMA/Microdialysis, Stockholm, Sweden) was perfused with artificial cerebrospinal fluid (containing Na+ 148 mM, Ca2+ 1.2 mM, Mg2+ 0.9 mM, K+ 2.7 mM and Cl− 155 mM) by a micro injection pump (CMA 107 MD pump, CMA/Microdialysis) at a rate of 2 μl/min. A 30-min period of calibration was followed by three 20-min periods of basal level measurement before start of CPB. The microdialysate samples were collected in 20-min fractions and stored in a freezer.

The interstitial concentrations of brain tissue glucose, lactate, pyruvate, glycerol and glutamate were analyzed enzymatically (CMA 600 Microdialysis Analyzer, Microdialysis, CMA/Microdialysis).

The analyzer was automatically calibrated and quality controlled according to the manufacturers instructions. The lactate/pyruvate ratio (L/P ratio) and lactate/glucose ratio (L/G ratio) were calculated.

2.6. Experimental protocol

The animals were cooled during 40 min to a brain and body temperature of 20 °C or 28 °C. Arrest or SACP lasted for 80 min. Reperfusion continued during 40 min (37 °C). After termination of CPB the animals were observed for 2 h. The microdialysis samples were recorded at six time-points; at baseline, end of cooling, end of HCA/SACP, end of rewarming and in the middle and end of the observation time.

2.7. Statistics

The data were entered in an Excel spreadsheet and analyzed using SPSS 15.0. Microdialysis values, hemodynamic values, hematocrit, pH, pCO2, and temperature are expressed as mean ± S.D.

The statistical analysis for the microdialysis results were performed on their relative changes. Significance between groups at selected time-points were analyzed with the Kruskal–Wallis test. If significance between groups existed the Mann–Whitney test was used to compare significance between two groups at each time-point.

A difference of P<0.05 was considered to be statistically significant.

3. Results

3.1. Comparability of experimental groups

Baseline values for temperature, hemodynamics, respiratory parameters and acid-base data were without significant differences between the groups (see Table 1).

One animal in the SACP group died of hemorrhage and was replaced.
3.2. Microdialysis

The analyzed microdialysis samples showed the following significant differences.

Glucose was higher in group SACP_28 compared to SACP_20 after CPB cooling (1030±620 vs. 517±330 μmol/l) and at the end of cerebral perfusion/arrest (1610±1660 vs. 352±314 μmol/l) (Fig. 1a).

A difference was also registered at the end of CPB warming between SACP_28 and HCA (1950±1900 vs. 410±462 μmol/l) (Fig. 1a).

Lactate showed an increase in all groups during the experiment. This was most pronounced in SACP_28 with a difference compared to SACP_20 at the end of cerebral perfusion/arrest (1880±1214 vs. 720±180 μmol/l) (Fig. 1b).

Pyruvate had the most pronounced increase in SACP_28 (Fig. 1c).

Compared to SACP_20 there was a difference at end of cooling (46±32 vs. 17±17 μmol/l) and compared to HCA there were differences both at end of cooling (46±32 vs. 32±14 μmol/l) and at end of cerebral perfusion (73±51 vs. 8.4±8 μmol/l).

L/P ratio showed a peak at the end of arrest in HCA (430±480) which was different from both SACP_20 (25±5) and SACP_28 (25±11).

However, there was also a difference between SACP_20 and SACP_28 at this timepoint (Fig. 1d).

L/G ratio showed a peak at the end of arrest in group HCA (409±470) with a difference from both SACP_20 (4.7±6) and SACP_28 (1.6±1). At the end of CPB warming a difference was seen between HCA and SACP_20 (7.8±7 vs. 1.7±2). At the end of the experiment these changes were normalized (Fig. 1e).

Glycerol showed an increase in both group HCA and SACP_28 (Fig. 2a). There were differences between HCA and SACP_28 at end of perfusion/arrest (22.5±7 vs. 6.5±4 μmol/l) but also at end of CPB warming (37±21 vs. 11±6 μmol/l). There was also a difference between SACP_28 and SACP_20 at end of perfusion (62±65 vs. 6.5±4 μmol/l) and at end of CPB warming (74±74 vs. 11±6 μmol/l).

Glutamate was without differences between the groups. However, a tendency to a peak was noted at the end of CPB warming in group SACP_28 (Fig. 2b).

3.2.1. Individual microdialysis results from group SACP_28 animals

The microdialysis results in SACP_28 showed great heterogeneity dividing this group into two subgroups. Glucose, lactate, pyruvate, glycerol and glutamate showed almost no increase in half of the animals but a considerable peak in the other subgroup (Fig. 3).

4. Discussion

The main findings of this study support the hypothesis that cerebral energy metabolism in pigs is best preserved with SACP compared to HCA alone. It also may support the notion that SACP with colder perfusate (20 °C) could be superior to tepid perfusate (28 °C) regarding cellular integrity.

Cerebral metabolism is dependent on substrate delivery from the blood but also on cellular mitochondrial function and membrane integrity. The microdialysis technique measures local metabolic changes and typical patterns of biomarkers are identified in the interstitial fluid if the energy metabolism or cellular integrity is altered.

The ratios of the energy substrates (L/P ratio, L/G ratio) are less susceptible to changes in probe recovery than the actual values of the substrates themselves. This makes them more accurate markers and quantitative measures of changes in energy metabolism over time [5]. These ratios are especially used to identify cellular anaerobic metabolism (mitochondrial dysfunction) and to follow the substrate
Fig. 1. Energy metabolites and their ratios shown as mean values ± S.D. Number of asterisks show group. White asterisks indicate significance ($P<0.05$) compared to black asterisks. *, HCA; **, SACP$_{20}$; ***, SACP$_{60}$. 

Fig. 2. Time course of cerebral, cell membrane and cell integrity, metabolites shown as mean values ± S.D. Number of asterisks show group. White asterisks indicate significance compared to black asterisks. *, HCA; **, SACP; ***, SACP.

4.1. Energy metabolism and cell damage during HCA and SACP

Microdialysis findings in the HCA group indicated anaerobic metabolism compared to SACP. In group SACP, lactate was significantly higher compared to the SACP group but the L/P ratio was unaffected. This can be explained with a preserved oxidative phosphorylation in which lactate and pyruvate both will increase as a consequence of increased glycolysis.

In group SACP, the L/P ratio was increased compared to the group SACP. The increase was significant but very low compared to the L/P ratio in the HCA group which had overt ischemia. This may be explained by a reduction of pyruvate due to reduced metabolism with colder perfusate. Our results are supported by other microdialysis markers of ischemia [9, 10].

Experimental studies on piglets from Schultz et al. [11, 12], concluded worse cerebral metabolism with HCA (18 °C) alone compared to intermittent low-flow CPB (20 ml/kg/min). A recent microdialysis study [13] compared HCA followed by different CPB flows and confirmed the same metabolic results as above.

The increase of glycerol in HCA is most probably of ischemic origin. In contrast, the peak of glycerol in group SACP could be by another mechanism as the ischemic trauma was less severe (see below).

4.2. SACP at moderate temperatures

The results in the group SACP split the group into two subgroups with two separate biochemical patterns (Fig. 3). One possible explanation of this is that SACP with 28 °C, in this experiment, is close to the border of unsafe protection and that the cellular response is of the type on/off reaction. The combination of hyperglycolysis and disturbed cellular integrity could reflect a situation of augmented oxidative stress that may lead to permanent cell damage. These results could give further support to the observation that tepid SACP could be insufficient.

There are, to our knowledge, no microdialysis studies comparing metabolic effects of different perfusate temperatures. However, there are experimental studies supporting cold perfusate as cellular damage decreases, and psychometrical tests are better performed at follow-up [14, 15].

5. Limitations

The microdialysis method reflects metabolism in a focal brain area surrounding the catheter membrane. However, we assume that the interventions used in this study have a global impact on cerebral metabolism suggesting that our results are representative for the whole brain.
Fig. 3. Time course of individual microdialysis results from group SACP. The group is divided into two subgroups. Half of the animals show almost no increase of the microdialysis biomarkers but the other half show a considerable increase.
Serum values of glucose, lactate and glycerol were not taken together with the microdialysis samples and it is unknown how the plasma levels relate to interstitial values in the present study. There were no true randomizations but the experiments were mixed during the study period.

6. Conclusion

This study supports the use of SACP over HCA, it also may suggest that cerebral metabolism and cellular membrane integrity is better preserved with SACP at 20 °C than with SACP at 28 °C under the given conditions.

References


EComment: Selective antegrade cerebral perfusion and metabolic suppression

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doi:10.1510/icvts.2008.200048A

We read with great interest the article by Jonsson and co-workers dealing with the topic of hypothermic circulatory arrest (HCA) and different temperatures for selective antegrade cerebral perfusion (SACP) [1]. In their study, they were able to show an advantage of SACP at 20 °C compared to HCA alone or SACP at 28 °C by using microdialysis. Interestingly, their findings were not consistent in the study groups. From these data they speculated that 28 °C does not provide sufficient brain protection in their setup. These findings basically confirm the results from two studies from our group [2, 3]. In the first study we were able to demonstrate the advantages of additional SACP compared to HCA alone [2]. Despite an improved neurophysiological recovery and lower intracranial pressures (ICP), SACP significantly reduced tissue acidosis in the brain. In another study, the impact of different temperatures for SACp was elucidated [3]. SACp at 20 °C provided adequate brain protection in comparison to the potential detrimental effects of moderate (30 °C) or profound (10 °C) temperatures. This was proven by histopathology, sagittal sinus saturation as well as molecular genetics. Again, in both studies as well as previous ones, the ICP was a reliable indicator for neurological injury [4]. In this context we are still not able to answer the questions whether the raise in ICP is a reflection of cerebral injury or just causes aggravation. As the authors mentioned in their limitations, no serum values of the different microdialysis parameters were analyzed. Therefore, it is not possible to distinguish the source of metabolites. In this context, the integrity of the blood brain barrier is not well known. This could be of utmost importance for further clinical investigations for non- or less-invasive detection of cerebral injury [5].

References