Deep hypothermic circulatory arrest or tepid regional cerebral perfusion: impact on haemodynamics and myocardial integrity in a randomized experimental trial

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Abstract

\textbf{OBJECTIVES:} Organ protective management during aortic arch surgery comprises deep hypothermic (18°C) circulatory arrest (DHCA), or moderate hypothermia (28°C/‘tepid’) with regional cerebral perfusion (TRCP). The aim of this experimental study was to evaluate the effect of distinct organ protective management on hemodynamic performance and myocardial integrity.

\textbf{METHODS:} Ten male piglets were randomized to group DHCA (n = 5) or TRCP (n = 5) group and operated on cardiopulmonary bypass (CPB) with 60 min of aortic cross-clamping. Blood gas analysis was performed throughout the experiment. Haemodynamic assessment was performed using a thermodilution technique before and after CPB. Myocardial biopsies were taken 2 h after CPB and evaluated using terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate–biotin nick-end labelling assay and western blot analysis.

\textbf{RESULTS:} At reperfusion, levels of central venous saturation were significantly higher (P = 0.016) and levels of lactate significantly lower (P = 0.029) in the DHCA group. After CPB, thermodilution measurements revealed higher stroke volume and lower peripheral resistance in the TRCP group (P = 0.012 and 0.037). At the end of the experiment, no significant differences regarding laboratory and haemodynamic parameters were evident. All specimens showed enrichment of terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate–biotin nick-end labelling-positive cells exclusively at the left ventricular subendocardium with no difference between groups and equal concentrations of cyclo-oxygenase-2.

\textbf{CONCLUSIONS:} TRCP is associated with decreased peripheral resistance and higher stroke volume immediately after CPB. However, this beneficial effect is contrasted by signs of lower body hypoperfusion, which is expressed by lower central venous saturations and higher lactate levels. Distinct strategies of organ protection did not seem to affect apoptotic/necrotic and inflammatory changes in the left ventricular myocardium.

Keywords: Deep hypothermic circulatory arrest • Tepid regional cerebral perfusion • Aortic arch • Congenital cardiac surgery

INTRODUCTION

Deep hypothermic circulatory arrest (DHCA) and regional cerebral perfusion (RCP) are the standard organ-protective methods during surgical repair of congenital aortic arch anomalies. Randomized trials did not prove the superiority of any technique regarding their neuroprotective impact [1, 2].

As systemic cooling reduces metabolic rates and offers prolonged tolerance of intrinsic ischaemia, organ preservation is essential during complex cardiac surgery. However, several authors have emphasized the adverse systemic side effects of a lowered core temperature on enzymatic and biochemical systems [3] concerning a shift of the oxyhaemoglobin dissociation curve with metabolic acidosis [4], platelet dysfunction [5], disturbances of iron homeostasis and intracellular calcium overload [6]. Moreover, cerebral autoregulatory mechanism is severely affected by temperature caused by a state of ‘cold-induced vasoparesis’, in which cerebral vascular resistance increases with the reduction in temperature. It has been shown that autoregulation is intact during moderate hypothermia (25–32°C), and it is lost during deep hypothermia (18–22°C) [7].

Because of the adverse effects of deeper temperatures, moderate hypothermia in combination with RCP has been introduced in the context of adult aortic arch surgery and was shown to be a safe strategy regarding postoperative clinical outcomes [8]. ’Tepid’ RCP (TRCP) at approximately 28°C was recently adapted even on paediatric patients, and the results were encouraging [9, 10].
The clinical routine regarding congenital aortic arch repair in our department comprises either DHCA at 18°C or TRCP at 28°C. The aim of this experimental study was to evaluate the effect of distinct organ-protective techniques on haemodynamic performance and myocardial integrity.

MATERIALS AND METHODS

Experimental set-up

This project was conducted according to a protocol approved by the Institutional Animal Care and Use Committee of the University Hospital Erlangen and by the state authorities (Regierung Mittelfranken). Animals were housed at least for 3 days in our laboratory for adaptation and reduction in preoperative stress.

The study is part of a subgroup analysis regarding the validation of beating-heart aortic arch surgery [11]. Twenty male piglets aged between 4 and 5 weeks (German Landrace, mean weight 10.9 ± 1.0 kg) were referred to a prospective trial. Randomization of piglets undergoing cardiac arrest included DHCA (Group DHCA, n = 5) at 18°C or RCP at moderate hypothermia (28°C) core temperature (Group TRCP, n = 5).

Anaesthesia and surgical technique

The experimental set-up including anaesthesia and surgery has been previously described [11]. All piglets underwent mid-line sternotomy under general anaesthesia. Full blood was used for cardiopulmonary bypass (CPB) priming from a second piglet, and levels of haemoglobin in the piglets were kept at a normal range throughout the experiment. CPB commenced after cannulation of the aorta and right atrial appendage. Estimated flow was maintained with a rate of 100 ml/kg/min. Piglets were cooled to a particular target core temperature that was measured via a rectal probe. To ensure cerebral vasodilatation, we used the alpha-stat target core temperature that was measured via a rectal probe. To ensure cerebral vasodilatation, we used the alpha-stat target core temperature that was measured via a rectal probe. To ensure cerebral vasodilatation, we used the alpha-stat target core temperature that was measured via a rectal probe. To ensure cerebral vasodilatation, we used the alpha-stat target core temperature that was measured via a rectal probe. To ensure cerebral vasodilatation, we used the alpha-stat target core temperature that was measured via a rectal probe.

Measurements

Haemodynamic values including heart index, heart rate, pulse pressure variation, stroke volume, systemic vascular resistance, index of left ventricular contractility, stroke volume variation, global ejection fraction, global end-diastolic volume and extravascular lung water were measured using the PICCO system (PICCO, Pulsson Medical System, Munich, Germany) combining transcardiopulmonary thermodilution and pulse contour analysis [13].

Cardiac biopsies

Immediately after euthanasia, tissue probes were taken from the left side of the ventricular septum and prepared for western blot analysis by snap freezing in liquid nitrogen and for histological evaluation by immersing in paraformaldehyde solution for 24 h followed by paraffinization.

Protein lysates were assembled by adding urea lysis buffer. Protein concentration was measured with a spectrophotometer (Nanodrop 2000c, Thermo Fisher Scientific Inc., Waltham, MA, USA), and 10 µg of protein lysates were denaturated by 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted on a PVDF membrane (Immunoblot Polyvinylidene difluoride (PVDF) Membrane, Bio-Rad Laboratories, Inc., Munich, Germany) using the 'wet-blot' technique. For immunodeTECTION, primary rabbit polyclonal anti-cyclo-oxygenase-2 (COX-2) (1:1500, Cat. No. ABIN672471, antibodies-online GmbH, Aachen, Germany) was used.

A secondary goat-anti-rabbit peroxidase-conjugated antibody (1:1500, Cat. No. 7074, Cell Signaling Technology, Danvers, MA, USA) was used for visualization with an enhanced chemiluminescence system (ECL, Prime Western Blotting Detection Reagent, Bio-Rad). After stripping the membrane, re-staining with a monoclonal mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:50 000, Cat. No. ab9482, Abcam, Cambridge, UK) ensured equal loading.

Cell nuclei were visualized by 4', 6-diamidino-2-phenylindole (DAPI) staining. The detection of DNA fragmentation in apoptotic/necrotic cells was performed using the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate–biotin nick-end labelling (TUNEL) technique on triplicates of the paraffin sections using a commercially available assay (In Situ Cell Death Detection Kit, Fluorescein; Cat. No. 11684795910; Roche, Switzerland). By merging DAPI and TUNEL stainings, image overlays were created.

Furthermore, morphological changes in haematoyxlin and eosin staining were assessed by a blinded pathologist.

Figure 1: Experimental set-up with measurement points. CPB: cardiopulmonary bypass; DHCA: deep hypothermic circulatory arrest; TRCP: tepid regional cerebral perfusion.
Blood gas samples were taken at each measurement point, whereas troponin-I and NT-pro-BNP were measured at all points except T1. Thermodilution measurements were determined at T1, T4 and T5. After euthanasia, tissue samples were taken from the left side of the ventricular septum.

Statistics

Results are expressed as mean ± standard deviation. Continuous data were compared using a Student’s unpaired t-test or the non-parametric Mann–Whitney U-test according to distribution. Exclusion criteria included incidents regarding surgery or CPB-interfering investigations, preoperatively elevated laboratory levels of markers indicating cardiac injury and the need for defibrillation before cardiac arrest. Calculations were done with statistical software (IBM SPSS Statistics, version 22; SPSS Inc., Chicago, IL, USA). A P-value <0.05 was considered statistically significant.

RESULTS

Baseline characteristics

There were no significant differences concerning body weight, preoperative blood gas analysis and haemodynamic measurements between the groups. All animals survived until the end of the experiment.

Blood gas analysis

Levels of lactate in the DHCA group were significantly higher at T2 but lower during T3 (T2: DHCA, 4.0 ± 0.9 mmol/l vs TRCP, 2.6 ± 0.6 mmol/l, P = 0.042; T3: DHCA, 6.1 ± 1.8 mmol/l vs TRCP, 8.7 ± 0.6, P = 0.029). Levels of mixed venous oxygen saturation were higher in the DHCA group at T3 and remained at a higher level until T4 (T3: DHCA, 95 ± 3% vs TRCP, 85 ± 8%, P = 0.032 and T4: DHCA, 93 ± 2% vs TRCP, 87 ± 4%, P = 0.016) (Figs 2 and 3).

Haemodynamic measurements

At T4, systemic vascular resistance was significantly higher in the DHCA group (DHCA, 2707 ± 409 dyn/s/cm⁵ vs TRCP, 1700 ± 802 dyn/s/cm⁵, P = 0.037), whereas stroke volume was significantly higher in the DHCA group (DHCA, 17.4 ± 1.7 ml/m²/beat vs TRCP, 23.4 ± 3.8 ml/m²/beat, P = 0.012). At T4 and T5, no other significant differences in haemodynamic parameters were measured (Table 1).

Cardiac biopsies

Protein expression of COX-2 was detected in all piglets without significant differences between both groups, as evidenced by the western blot analysis. Equal expression of GAPDH provided proper loading control (Fig. 4).

TUNEL-positive nuclei were detected in the subendocardium of the left side of the ventricular septum in all specimens with similar distribution patterns in both groups, as shown in Fig. 5.

There were no morphological correlates as evaluated by haematoxylin and eosin staining.

DISCUSSION

This study mirrors our clinical routine regarding the surgical treatment of congenital aortic arch anomalies in an experimental set-up. When comparing TRCP with DHCA, no significant differences regarding haemodynamic performance or myocardial integrity were evident at the end of the examination. However, various temperature and flow-dependent peculiarities were observed during and early after CPB.

Statistical analysis

Before aortic cross-clamping (T2), significantly higher values of lactate were evident in the DHCA group, indicating that microcirculation seemed to be affected by the profound cooling process. Flow rates, blood gas management and systemic afterload reduction were uniform in both groups and conform to other experimental trials [14, 15]. Unfortunately, we could not measure systemic vascular resistance at those measurement points, as technically thermodilution measurements cannot be determined during CPB.

On the other hand, early on during reperfusion (T3), an exponential increase of lactate after TRCP implied an insufficient
Our findings counteract the results of Ganzel et al. [17], who demonstrated a lower expression of COX-2 after deep hypothermia (26–28 °C) with mild hypothermia (34 °C) [18, 19], with a hint for less inotropic support using milder temperature and autoregulation, which seems important particularly in neonates [20].

According to our clinical routine, we chose the alpha-stat regimen with elevated pCO2 levels to maintain both cerebral vasodilation and autoregulation, which seems important particularly during TRCP [12]. However, it is questionable whether a pH-stat management with deliberate addition of CO2 to maintain a temperature-corrected pCO2 of 40 mmHg would have influenced cardiac output, especially after profound cooling process in the DHCA group [21].

We have shown that levels of the cardiac ischaemia marker troponin-T were comparable between piglets undergoing DHCA and TRCP [11]. These results are in concordance with clinical studies observing no association between core temperature during CPB and perioperative levels of troponin [22, 23].

Similar distribution of TUNEL-positive cells among specimens showed that cell damage was not related to the chosen methods of organ protection. DNA fragmentation was exclusively found in the subendocardial layer of the left ventricle, which is notably the most vulnerable area to ischaemia [24]. Unfortunately, cardiac slices presented inhomogeneously as biopsies were taken manually and showed intrinsic morphological variety. Thus, a quantification based on percentage of TUNEL-positive cells in the subendocardial layer was not sensible. Fluorescent areas were correlated with haematoxylin and eosin sections, revealing no morphological changes. We believe that this is plausible with regard to the described duration of the apoptotic process as programmed cell death leading to microscopically detectable cell destruction [25].

Based on the literature, comparability is difficult due to different levels of temperature as well as the location and latency of tissue sampling. Two other swine models showed equal amount of apoptosis in moderate hypothermia compared with normothermia 6 h after CPB, confirming the thesis of this study that distinct temperature management of CPB has no impact on myocardial cell damage [26]. In contrast, a higher percentage of apoptotic cells detected via DNA laddering was found in the left ventricle after deep hypothermia in a piglet model when comparing DHCA with the normothermic CPB regimen, whereas tissue probes were collected at a not clearly defined period after CPB [27].

A significantly lower expression of COX-2 after deep hypothermia in cardiomyocytes was described in cell culture studies [28, 29]. However, the postulated role of hypothermia in downregulation of inflammation could not be demonstrated in our study in specimens taken 2 h after CPB. In another piglet model comparing moderate hypothermia with normothermia, tissue probes revealed a significant decrease of COX-2 only immediately after reaching the target temperature in animals treated with
moderate hypothermia but already equal levels before declamping [30]. Thus, hypothermia might provide additional benefit concerning myocardial inflammation during the cooling process itself, without evidence for a long-term impact.

Limitations

Limitations are inherent to the small group size and the experimental character of the study. A longer aortic cross-clamping period would have been perhaps more conclusive. Because of animal protection requirements, we were not permitted to use neonatal piglets and to extend the postoperative survival period, which might have shown more conclusive data. Serial temperature changes in various areas of the myocardium were not measured, and the input of distinct systemic on myocardial temperature remains unclear after infusion of a single-shot 4°C cold solution. This aspect might be even more important in paediatric patients with a higher surface-area-to-mass ratio. Therefore, no direct conclusions considering the impact on hypothermia itself can be drawn. This study lacks an assessment of lower body end-organ function. The results of this study should be transmitted with caution to paediatric patients with aortic arch obstruction, implicating that this population may provide more blood to the subdiaphragmatic part due to collateral vessels [16].

CONCLUSION

In conclusion, both methods on organ protection—DHCA and TRCP—show equal outcomes regarding haemodynamic performance or myocardial integrity. Despite an improved systemic microcirculation with TRCP, protection of the lower half of the body seems to be more effective on deeper temperatures during DHCA. Additional regional perfusion concepts of the lower body could enhance the outcome in patients if aortic cross-clamping periods are longer when using TRCP.

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