Published in final edited form as:

Pediatr Crit Care Med. 2011 March ; 12(2): e79–e86. doi:10.1097/PCC.0b013e3181e89e91.

EFFECT OF DEEP HYPOTHERMIC CIRCULATORY ARREST FOLLOWED BY LOW FLOW CARDIOPULMONARY BYPASS ON BRAIN METABOLISM IN NEWBORN PIGLETS: COMPARISON OF PH-STAT AND ALPHA-STAT MANAGEMENT

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Abstract

Objective—To compare the effects of pH-stat and alpha-stat management prior to deep hypothermic circulatory arrest (DHCA) followed by a period of low flow (two rates) cardiopulmonary bypass (CPB) on cortical oxygenation and selected regulatory proteins: Bax, Bcl-2, Caspase-3 and phospho-Akt.

Design—Piglets were placed on CPB, cooled with pH-stat or alpha-stat management to 18°C over 30 min, subjected to 30 min DHCA and 1h low flow at 20 (LF-20) or 50 ml/kg/min (LF-50), rewarmed to 37°C, separated from CPB, and recovered for 6 h.

Subjects—Newborn piglets, 2–5 days old, randomly assigned to experimental groups.

Measurements and main results—Cortical oxygen was measured by oxygen-dependent quenching of phosphorescence; proteins were measured by western blots. The means from 6 experiments ± standard error (SEM) are presented as % of alpha-stat. Significance was by t-test. For LF-20, cortical oxygenation was similar for alpha-stat and pH-stat, whereas for LF-50 it was significantly better using pH-stat. For LF-20, the measured proteins were not different except for Bax in the cortex (214±24%, p=0.006) and hippocampus (118% ± 6%, p=0.024) and Caspase 3 in striatum (126% ± 7%, p=0.019). For LF-50, in pH-stat group: **In cortex**, Bax and Caspase-3 were lower (72±8%, p=0.001 and 72±10%, p=0.004, respectively) and pAkt was higher (138±12%, p=0.049). **In hippocampus**, Bcl-2 and Bax were not different but pAkt was higher 212±37% (p=0.005) and Caspase 3 lower (84 ± 4%, p=0.018). **In striatum**, Bax and pAkt did not differ but Bcl-2 increased (146±11%, p=0.001) and Caspase-3 decreased (81±11%, p=0.042).

Conclusions—In this DHCA-LF model, when flow was 20ml/kg/min there was little difference between alpha-stat and pH-stat management. However for LF-50, pH-stat management resulted in better cortical oxygenation during recovery and Bax, Bcl-2, pAkt and Caspase-3 changes consistent with lesser activation of pro-apoptotic signaling with pH-stat than with alpha-stat.

Keywords

Cardiopulmonary bypass; Circulatory arrest; alpha-stat; pH-stat; Oxygen; Apoptosis

INTRODUCTION

Congenital heart disease, the most common significant birth defect, affects 8 per 1000 live births and requires cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA) surgery. The early, enthusiastic use of DHCA, particularly in neonates, has been tempered by the finding of significant neurological morbidity associated with prolonged exposure, as observed in long-term follow up studies (1–4). There is, therefore, a pressing need to find better strategies, ones which would diminish brain injury and developmental abnormalities caused by DHCA.

Over the last several years, pH-stat versus alpha-stat blood gas management during CPB has been investigated in an attempt to establish which of these techniques results in lesser DHCA-dependent brain injury (5,6). Some clinical studies have suggested that, in infants, pH-stat provide some neuronal (7,8) and myocardial protection (9) compared to alpha-stat strategy. Other studies (10) have indicated that use of alpha-stat versus pH-stat acid-base management strategy during reparative infant cardiac operations with DHCA-CPB was not consistently related to either improved or impaired early neurodevelopmental outcomes. At the present time many other clinical and animal studies suggest that one or the other strategy is more beneficial. Alpha-stat has been reported to better preserve autoregulation of cerebral vasculature (5,11,12) and to be more neuroprotective, presumably due to fewer cerebral microemboli (14–16) but to cause more intracellular acidosis (16) and disturbance of cerebral oxygenation (17). On the other hand, pH-stat has been reported to provide increased cerebral blood flow and oxygenation, increased rate of homogenous cooling, and lowered cerebral vascular resistance during the rewarming stage (18–23). Priestley et al. (23) reported that, with DHCA in piglets, pH-stat CPB management resulted in better neurologic outcome than alpha-stat. The mechanism of protection was not related to hemodynamics, hematocrit, glucose, or brain temperature. Similarly, our earlier study using the piglet model of DHCA indicated that pH-stat can prolong “safe” time of DHCA and provides some protection of the brain against ischemic injury (24). However this strategy had also been reported to increase microembolism (25,26).

The present study compares the regional responses of brain oxygenation and metabolism to pH-stat and alpha-stat management in a newborn piglet model of DHCA followed by two levels of low flow cardiopulmonary bypass of 20 or 50 ml/kg/min. Our hypothesis was that pH-stat management would result in improved brain oxygenation during post-bypass recovery and decreased apoptotic activity. These would be also dependent on the rate of the flow of cardiopulmonary bypass following DHCA in experimental model of newborn piglets.

MATERIALS AND METHODS

Animal model

Newborn piglets, 3–5 days of age (1.4–2.5 kg), were anesthetized with halothane, and tracheotomy was performed. The piglets were then connected to a ventilator, paralyzed with pancuronium bromide (0.2mg/kg) and anesthesia was maintained with fentanyl (30µg/kg) and isoflurane (1%). Femoral venous and arterial cannulas were placed for collection of blood samples and blood pressure monitoring, respectively. With the head of the animal in a stereotaxic holder, the scalp was removed and a cranial window, approximately 8 mm in

diameter, was created over the right parietal hemisphere for measurement of cortical oxygenation. The CPB protocol was then started. At the end of experiments, the animals were euthanized with 4M KCl while anesthetized, brain immediately removed, dissected and stored frozen at -80°C for later analysis.

All animal procedures strictly followed the NIH Guide for the Care and Use of Laboratory Animals and have been approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

CPB Technique

The circuit was primed with Plasmalyte-A and 25% albumin. Donor whole blood was then added to maintain a hematocrit of 25–30%. Heparin (1000 units), fentanyl (50 μg), pancuronium (1 mg), CaCl_2 (500 mg), methylprednisolone (60 mg), cefazolin (250 mg), furosemide (2 mg), and NaHCO_3 (25 meq) were added to the pump prime. A membrane oxygenator (Lilliput) was used as well as a roller pump system (Cobe) and arterial filter (Terumo). For CPB, a median sternotomy was performed. Prior to cannulation, 500 units of heparin was administered IV. The ascending aorta was cannulated as well as the right atrial appendage.

DHCA technique and experimental protocol

The protocols and techniques used during these studies duplicated those practiced in a clinical setting. The initial full CPB flow was set at 150 ml/kg/min. During CPB anesthesia was maintained using isoflurane (1%) and fentanyl (30 $\mu\text{g/kg}$). Pancuronium 0.2 mg/kg was also used in the CPB prime. Once CPB was begun, the piglets were cooled to a nasopharyngeal temperature of 18°C over 25–30 minutes. In the pH-stat group, the temperature-corrected arterial blood pH was maintained between 7.35 and 7.45 by the addition of CO_2 (typical fresh gas flow 1.5 l/min with 50 ml/min of CO_2 in CPB sweep). In the alpha-stat group, the non-temperature corrected pH was maintained at 7.4 (typical fresh gas flow 300 ml/min with no added CO_2).

The animals were then subjected to 30 min of DHCA followed by 1h of low flow CPB at 20 ml/kg/min (LF-20) or 50 ml/kg/min (LF-50). All animals were then rewarmed to 37°C during a 30 min period using alpha-stat management with a flow of 150 ml/kg/min, after which they were separated from CPB, maintained for recovery for 6 h under anesthesia (isoflurane (1%) and fentanyl (10 $\mu\text{g/kg/h}$) and then euthanized with 4M KCl. After euthanasia, frontal cortex, striatum and hippocampus were rapidly isolated and kept frozen at -80°C for later analysis.

Measurements of oxygen pressure and oxygen distribution by the oxygen dependent quenching of phosphorescence

Cortical oxygen pressure was measured using oxygen dependent quenching of phosphorescence. The technical basis for determining the distribution of oxygen in the microcirculation of tissue from the distribution of phosphorescence lifetimes in the serum of blood has been described in detail (27,28).

Briefly, a near infrared oxygen sensitive phosphor, Oxyphor G2 (29), was injected IV at approximately 1.5 mg/kg. The measurements were made using a multi-frequency phosphorescence lifetime instrument (PMOD 5000). The excitation light (635 nm), modulated by the sum of 37 sinusoidal waves with frequencies spaced between 100 Hz and 40 kHz, was carried to the tissue through a 3 mm light guide. The phosphorescence ($\lambda_{\text{max}}=790\text{ nm}$) emitted from the tissue was collected through a second light guide, placed against the tissue at approximately 6 mm (center to center) from the excitation light-guide.

This positioning of the light-guides allowed effective sampling of brain tissue oxygenation down to approximately 6 mm under the neocortical surface. The phosphorescence was optically filtered (3 mm thick 695 nm long pass Schott glass) and the signal from the detector amplified, digitized and analyzed to give distribution of phosphorescence lifetimes (oxygen histogram) in the volume of tissue sampled by the light.

Western blotting

One hundred mg of frozen tissue samples were homogenized in a buffer containing 2% SDS, 10 mM Tris-HCl (pH 7.4) freshly supplemented with NaF (10 mM), Na pyrophosphate (10 mM), Na_3VO_4 (1 mM), Na_2MoO_4 (1 mM), phenylarsine oxide (1 μM), and aprotinin, leupeptin, pepstatin (10 $\mu\text{g}/\text{ml}$) each. The homogenate was boiled for 5 min after addition of SDS-PAGE sample buffer. Protein concentration was determined in a homogenate aliquot with a BCA Protein Assay kit (Pierce). An equal amount of protein from each sample was separated by 12.5% SDS-PAGE and transferred onto a nitrocellulose membrane (Hybond C, Amersham Pharmacia Biotech.). The membranes were then incubated in a blocking solution (phosphate buffered saline (PBS), pH 7.4, containing 5% non-fat milk powder) for 1 h at room temperature, followed by overnight incubation with antibodies generated against Bax (N-20) and Bcl-2 (Santa Cruz Biotechnology); phospho-Akt, Caspase-3 (Cell Signaling Technology), and β -actin (ABCAM) which served as a loading control. After being washed in PBS containing 0.05% Tween 20 (PBS-T; Sigma–Aldrich), the membranes were incubated for 1 h with peroxidase-conjugated goat anti-rabbit or anti-mouse secondary antibodies (Amersham Pharmacia Biotechnology). The final reaction of secondary antibodies binding was visualized using enhanced chemiluminescence (ECL–Western Blotting Detection Reagents, Amersham Pharmacia Biotechnology); membranes were exposed to x-ray film.

Data analysis

Autoradiographic films were analyzed using Scion Image software (NIH). Each blot contained two sets of samples, one for the alpha-stat and another for the pH-stat group. The data are presented as the mean $\pm$ SEM for six independent experiments. The data were normalized by assigning a value of 100 to the mean from the alpha-stat group and presenting the pH-stat values as a percent of the corresponding mean for the alpha-stat. Because this is an exploratory study we did not adjust for type 1 errors and statistical significance of differences were assessed using a two-tailed t-test with $p < 0.05$ considered significant. The 4 individual proteins are independent parameters and when testing for differences in the effects of alpha and pH stat management on each brain region this may be regarded as 4 comparisons. Applying the Bonferroni correction for multiple comparisons, significance at the 95% for a single difference would be equivalent to $p < 0.0125$ calculated by the two tailed t-test. Our hypothesis, that pH stat would result in better protection of the brain, predicts a direction for the changes in protein levels and a one tailed t-test would be appropriate. If readers wish to apply both corrections, significance at the 95% level would be at $p < 0.025$ calculated for the two tailed t-test.

RESULTS

Dependence of cortical oxygen pressure on pH-stat and alpha-stat management during cooling, circulatory arrest and low flow bypass and post bypass recovery in newborn piglets

Histograms of the distribution of oxygen in the microcirculation of the brain cortex are shown in Figures 1 (LF-20) and 2 (LF-50). Each histogram is the mean $\pm$ SEM for 6 experiments. The panels are the oxygen histograms for just before cooling, at the end of the 60 min low flow period, and at 60 and 120 min of recovery. Since there were substantial

differences among animals with respect to collected phosphorescence, the oxygen histograms were normalized to have a total signal (sum of y values for all oxygen pressure values less than 140 mm Hg) equal to 1.0. Both the intensities (amplitudes) and lifetimes of phosphorescent signals decrease with increasing oxygen pressures. Thus, for two equal volumes of tissue, containing equal amounts of the Oxyphor and excited by equal numbers of photons, the accuracy in signal measurement is higher for lower oxygen pressures. The decrease in signal with increasing oxygen pressure (decrease in signal to noise) results in asymmetric broadening of oxygen histograms as seen in the “tail” effect on the high oxygen end of the histogram. In the histograms, the oxygen pressures above the median should be used for only qualitative comparisons.

The histograms for alpha-stat and pH-stat management before beginning cooling were not significantly different as expected for similarly treated animals. For LF-20 (Fig. 1), the oxygen pressures in the low flow period were still well below control values and there is little difference between the pH-stat and alpha-stat groups. During recovery the oxygen pressures remained well below control values and the differences between pH-stat and alpha-stat groups were minimal, with some evidence for greater broadening of the oxygen distribution in alpha-stat as compared to pH-stat group. At the 120min of recovery the peak oxygen pressures were about 20mm Hg compared to control values of 35–40 mm Hg, suggesting the oxygen delivery had been seriously damaged in both groups.

For LF-50 (Fig. 2), at the end of the low flow period the histogram for alpha-stat was broader than for pH-stat, with both having significant amounts of tissue with near zero oxygen pressures. During recovery, the heterogeneity of the oxygen distribution becomes progressively greater in the alpha-stat group but not in the pH-stat group. At 120min recovery the histogram for alpha-stat management was distinctly bimodal and substantially different from that for pH-stat. The histograms for pH-stat throughout the recovery period were similar in shape to those observed for control animals, but values were shifted to lower oxygen pressures.

Effect of pH-stat management as compared to alpha-stat management on the levels of selected proteins in different regions of the newborn piglet brain

(1) Frontal cortex—When LF-20 was used, a significant difference between alpha-stat and pH-stat was observed only in the level of Bax. As can be seen in Figure 3, Bax in the pH-stat group was $214 \pm 24\%$ of that in the alpha stat group ($p=0.006$). There were no significant differences in levels of Bcl-2, pAkt or Caspase-3 between the two groups. The absolute values for alpha-stat group with flow 20ml/kg/min were given in our early publication (30).

For LF-50, the levels of Bax were lower in the pH-stat group ($72 \pm 8\%$ of alpha-stat, $p=0.001$) as were those of Caspase-3 ($72 \pm 10\%$ of alpha-stat, $p=0.004$). The level of pAkt was significantly higher than in alpha-stat ($138 \pm 12\%$ of alpha-stat, $p=0.049$).

The calculated ratio of Bcl-2/Bax was increased in pH-stat group by 43% (from 1 to 1.43) as compared to alpha-stat group.

(2) Hippocampus—There were no significant differences in the levels of measured proteins except for a small increase ($118\% \pm 6\%$, $p=0.024$) in Bax between alpha-stat and pH-stat in LF-20 experimental group of animals (Figure 4).

In experimental groups with LF-50, significant difference between alpha-stat and pH-stat groups was observed in the level of pAkt, which increased to $212 \pm 37\%$ of alpha-stat ($p=0.005$) (Figure 4), and in Caspase 3, which decreased to $84 \pm 4\%$ of alpha stat ($p=0.018$).

(3) Striatum—As was the case for hippocampus, for LF-20 the differences in the levels of the measured proteins were small and not significant except for Caspase 3 ($126\% \pm 7\%$, $p=0.019$) between alpha-stat and pH-stat groups (Figure 5).

For LF-50, the level of Bcl-2 was significantly higher in pH-stat than in alpha-stat group ($146 \pm 11\%$ of alpha-stat, $p=0.001$), whereas the level of Caspase-3 was significantly lower ($81 \pm 11\%$ of alpha-stat, $p=0.042$) (Figure 5). There were no significant differences between alpha-stat and pH-stat in levels of Bax and pAkt. The calculated ratio of Bcl-2/Bax was increased in pH-stat group by 76% (from 1 to 1.76) as compared to the alpha-stat group.

DISCUSSION

The present study had two goals. First, to examine whether using pH-stat as contrary to alpha-stat management during cooling, prior to DHCA and CPB, resulted in significant differences in the cortical oxygenation and changes in the levels of several proteins that can play significant role in apoptotic activity in the brain of newborn piglet. Second, to determine if the responses of above parameters, with alpha-stat and pH-stat management, are dependent on the flow for CPB following DHCA in our model of newborn piglets.

The use of the DHCA-low flow experimental model in our study reflects changes in attitude and practice regarding the acceptable duration of DHCA. It is recognized by many centers that DHCA beyond 30 minutes can be detrimental neurologically. Therefore it is becoming increasingly common for cardiovascular surgeons who treat congenital heart defects such as hypoplastic left heart syndrome to perform the difficult arch repair under DHCA and then complete the remainder of the surgery with low flow.

The data show that during cooling the cortical oxygen pressure is significantly higher with pH-stat than with alpha-stat management, in agreement with our early study (24). This is also consistent with clinical and animal studies by other investigators reporting that the pH-stat management provided better oxygenation to the brain tissue during cooling (5,12,31–34). The increased oxygen level is consistent with the known vasodilatory effect of increased CO_2 in the brain and with reports of faster cooling of the newborn brain. Duebener et al. (12) reported that in piglets during cooling microvascular diameter decreased in alpha-stat group and significantly increased in pH-stat group by the end of cooling. In addition, during the first minutes of rewarming, the cerebral microvascular diameter was significantly larger when the pH-stat management was used. Kurth et al. (22) showed that in newborn piglets, pH-stat improved brain cooling efficiency during CPB and that all regions cooled more rapidly with using this management.

In our study, levels of oxygen in the cortical microcirculation were measured continuously throughout the experimental protocol. The oxygen measurements show that in model of DHCA with low flow 50 ml/kg/min, in pH-stat group, the oxygen distributions in the cortex during the 6hrs of recovery period are similar to those observed before surgery. This is in contrast to what is observed when alpha-stat management was used, where the oxygen distributions show a significant and progressive broadening during the post surgery period. The broadening following surgery using alpha-stat management is consistent with a loss of regulatory control in the microcirculation, resulting in more heterogeneity in the flow through the microcirculation. Basically it appears there are regions in the microcirculation with higher than normal flow and above normal oxygen levels but these are mixed with regions having below normal flow and oxygen levels. When functioning correctly, the resistance of the vessels (primarily the arterioles) is dynamically regulated to match oxygen delivery to oxygen consumption, resulting in a nearly gaussian distribution of oxygen (see precooling histograms). An abnormal, bimodal oxygen distribution is evidence that the

regulatory mechanisms which modulate arteriolar resistance to match the downstream oxygen consumption are no longer working properly. This has serious consequences because local regions of hypoxia can develop even when the total flow is sufficient. The disturbance in oxygen delivery can arise from the arterioles failing to modulate their caliber correctly in response to downstream signaling or to altered resistance in a subset of capillaries, possibly due to increased white cell adhesion to the capillary endothelium, or to local edema. Alone or in combination these could result in the observed maldistribution of blood flow in the microcirculation.

When a model of DHCA with low flow 20 ml/kg/min was used, there was little difference in cortical oxygen distributions between the alpha-stat and pH-stat groups. The oxygen histograms show that during recovery following LF-20 the tissue was substantially more hypoxic than following LF-50. This is consistent with there being substantially greater vascular and tissue injury when the flow of 20 ml/kg/min was used. It also suggests that the beneficial effect of pH-stat relative to alpha-stat management is dependent on the flow during the low flow period being high enough to perfuse all or most of the vasculature and to provide significant levels of oxygen to all of the tissue.

To determine if pH-stat and alpha-stat management resulted in differences in selected apoptosis-related proteins in newborn brain, we measured expression of selected proteins (Bcl-2, Bax, Caspase-3 and pAkt) that had been shown to regulate the apoptotic processes in the brain. The rationale for focus on apoptosis is that in neonates, apoptosis might be favored over necrosis as a cell death process after hypoxic-ischemic insult (35) and that activation of apoptotic activity can be detected after relatively short times of recovery. Yue et al. (36) suggested that immature neurons might be more prone to apoptotic death while terminally differentiated neurons would die by necrosis. In models of global ischemia, apoptotic neuronal death began within hours of reperfusion and continued for several days (35).

The results show that in DHCA followed by LF-20 the differences in measured apoptosis regulating proteins in striatum and hippocampus between the alpha-stat and pH-stat groups were small. There was a trend for greater indication of apoptotic activation in the pH-stat than in the alpha-stat group, as evidenced by significant increases in Bax in cortex and hippocampus and of Caspase 3 in striatum. Except for Bax in the cortex these changes are small and the statistical significance marginal, but taken together they indicate that, for DHCA-LF-20, pH-stat management does not significantly influence apoptotic protein expression when compared to alpha-stat management.

For DHCA followed by low flow at 50ml/kg/min, the use of pH-stat management during cooling, when compared to alpha-stat management, resulted in a higher Bcl-2/Bax ratio and lower level of Caspase-3 in the cortex and striatum. In hippocampus significant difference between groups was observed in the level of pAkt which was significantly higher in pH-stat group, and in the level of Caspase 3, which was significantly lower in the pH stat group. The data suggested that in this newborn piglet model, pH-stat management during cooling results in significantly diminished apoptotic activity compared to alpha stat in all three of the regions tested, frontal cortex, striatum, and hippocampus. The increase in the Bcl-2/Bax ratio in the cortex and striatum can be of major importance. Data from literature show that proteins of Bcl-2 family are key proteins in apoptotic pathways. They can either promote cell survival (Bcl-2) or promote cell death (Bax) (37). There is accumulating evidence that over expression of Bcl-2 protects against apoptosis (38) and ischemic neuronal death (39,40). On the other hand, Bax is a pro-apoptotic protein, and has been shown to promote cell death by activating the caspase cascade (41). The balance between Bcl-2 and Bax strongly influences the probability that the cells will die by apoptosis. An increased ratio of

Bcl-2/Bax protein has been shown, in hypoxic and hypocapnic piglets, to correlate with decreased susceptibility to apoptotic cell death in the brain (42,43). In our model of DHCA-low flow of 50ml/kg/min ratio of Bcl-2/Bax increased in striatum by 76% and in cortex by 43%, in pH-stat group as compared with alpha-stat group. In DHCA-low flow of 20 ml/kg/min ratio of Bcl-2/Bax is lower in all regions of piglets in pH-stat group as compared to alpha-stat group.

These results are consistent with observed changes in the level of Caspase-3. Activation of caspases, which are cysteine proteases, is an essential component of the process of apoptosis (44). In the brain, Caspase-3 plays a key role in the initiation of the apoptotic pathway (45) and is thought to be responsible for many cytological changes that characterize neuronal apoptosis (46). Thus, Caspase-3 is considered an early marker of activation of the apoptotic pathway.

Another protein, the pAkt protein kinase, which increased significantly in hippocampus and striatum in pH-stat group as compared to alpha-stat group, is implicated as a critical transducer of PI3-kinase-dependent survival signals generated by a variety of stimuli and growth factors (47–50). Phospho-Akt targets several key proteins that help to keep cells alive, including apoptosis regulators and transcription factors. For example, Bad is a pro-apoptotic member of the Bcl-2 family, that in its unphosphorylated form can bind to Bcl-x L and thus block cell survival (51). But the activation of pAkt induces the phosphorylation of Bad and promotes its interaction with the chaperone protein 14-3-3, which sequesters Bad in the cytoplasm and inhibits pro-apoptotic activity of Bad (52). Akt has been shown to affect, directly or indirectly, three transcription factor families: Forkhead, cAMP-response-element-binding protein (CREB) and NF-kappaB, all of which are involved in regulating cell survival. It is clear that pAkt is a potent kinase whose activation can protect neurons from death in various ways.

The limitations of this study was that the measurement all proteins was done only in one time point (after 6 h of post-bypass recovery), oxygen pressure was measured only in frontal cortex and is not clear how/if these observed changes will affect brain injury. Studies are in progress with longer time of recovery to establish possible correlation between presented in this paper changes and brain injury determined by Caspase-3 immunostaining and TUNEL technique.

In conclusion

pH-stat and alpha-stat gas management during cooling were compared in a newborn piglet model with 30min DHCA followed by 60 min with low flow 20 or 50 ml/kg/min. When a flow of 20 ml/kg/min was used, effect of pH-stat was not significantly protective relative to alpha-stat management. When the flow was increased to 50 ml/kg/min, pH-stat management resulted in significantly better cortical oxygenation during the recovery period and a reduction in protein changes associated with apoptotic cell death compared with alpha-stat management. These results are consistent with pH-stat management providing better protection for the brain than alpha-stat management when flow during the low flow period was high enough to provide substantial oxygen levels to the brain.

Acknowledgments

We thank Dr. Abbas F. Jawad, Associate Professor of Biostatistics in Pediatrics at the Children's Hospital of Philadelphia, for reviewing the statistical analysis used in this paper and for his thoughtful and lucid discussions of the relevant statistics. This research was supported in part by grants HL-58669 and NS031465 from the U.S. National Institutes of Health.

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Figure 1. Comparison of cortical oxygenation when using pH-stat or alpha-stat management during cooling, DHCA, low flow of 20 ml/kg/min and post-bypass recovery

Histograms of oxygen distribution in the microcirculation of the cortex were determined by the distribution of phosphorescence lifetimes, as described in Methods. The presented histograms are for control conditions, at the end of the 60 min of low flow, and after 60 and 120 min of recovery. The histograms are for 6 independent experiments presented as the mean $\pm$ SEM for each oxygen pressure.

Figure 2. Comparison of cortical oxygenation when using pH-stat or alpha-stat management during cooling, DHCA, low flow of 50 ml/kg/min and post-bypass recovery
 Histograms of the oxygen distributions in the microcirculation of the cortex were determined by the distribution of phosphorescence lifetimes as described in Methods. The presented histograms are for control conditions, at the end of the 60 min of low flow, and after 60 and 120 min of recovery. The histograms are for 6 independent experiments presented as the mean $\pm$ SEM for each oxygen pressure.

Figure 3. Expression of Bax, Bcl-2, phospho-Akt, and Caspase-3 in frontal cortex of newborn piglets: comparison pH-stat and alpha-stat management

The means for DHCA with pH-stat (n=6) $\pm$ SEM expressed in % to the corresponding values obtained for the alpha-stat group of piglets (n=6). p values for significant difference from alpha-stat values as determined by two tailed t-test. $p < 0.05$ was considered statistically significant.

Figure 4. Expression of Bax, Bcl-2, phospho-Akt, and Caspase-3 in hippocampus of newborn piglets: comparison pH-stat and alpha-stat management

The means for DHCA with pH-stat (n=6) $\pm$ SEM expressed in % to the corresponding values obtained for the alpha-stat group of piglets (n=6). p values for significant difference from alpha-stat values as determined by two tailed t-test. $p < 0.05$ was considered statistically significant.

Figure 5. Expression of Bax, Bcl-2, phospho-Akt, and Caspase-3 in striatum of newborn piglets: comparison pH-stat and alpha-stat management

The means for DHCA with pH-stat (n=6) $\pm$ SEM expressed in % to the corresponding values obtained for the alpha-stat group of piglets (n=6). p values for significant difference from alpha-stat values as determined by two tailed t-test. p < 0.05 was considered statistically significant.