Antegrade cerebral perfusion reduces apoptotic neuronal injury in a neonatal piglet model of cardiopulmonary bypass

Valerie Y. Chock, MD,^a Gabriel Amir, MD,^b Corrine R. Davis, PhD,^c Chandra Ramamoorthy, MD,^d R. Kirk Riemer, PhD,^b Dustin Ray,^d Rona G. Giffard, MD, PhD,^d and V. Mohan Reddy, MD^b

Objective: Neonates with congenital heart disease might require surgical repair with deep hypothermic circulatory arrest, a technique associated with adverse neurodevelopmental outcomes. Antegrade cerebral perfusion is thought to minimize ischemic brain injury, although there are no supporting experimental data. We sought to evaluate and compare the extent of neurologic injury in a neonatal piglet model of deep hypothermic circulatory arrest and antegrade cerebral perfusion.

Methods: Neonatal piglets undergoing cardiopulmonary bypass were randomized to deep hypothermic circulatory arrest or antegrade cerebral perfusion for 45 minutes. Animals were killed after 6 hours of recovery, and brain tissue was stained for evidence of cellular injury and for the apoptotic markers activated caspase 3 and cytochrome c translocation from mitochondria to cytosol.

Results: Piglets from the antegrade cerebral perfusion group exhibited less apoptotic or necrotic injury (4 ± 3 vs 29 ± 12 cells per field, $P = .03$). The piglets undergoing antegrade cerebral perfusion also had less evidence of apoptosis, with fewer cells staining for activated caspase 3 (57 ± 8 vs 93 ± 9 cells per field, $P = .001$) or showing cytochrome c translocation (6 ± 2 vs 15 ± 4 cells per field, $P = .02$).

Conclusions: The use of antegrade cerebral perfusion in place of deep hypothermic circulatory arrest reduces evidence of apoptosis and histologic injury in neonatal piglets. Neonates with congenital heart disease might benefit from antegrade cerebral perfusion during complex cardiac surgery to improve their overall neurologic outcome.

From the Departments of Neonatology,^a Cardiothoracic Surgery,^b Comparative Medicine,^c and Anesthesia,^d Stanford University School of Medicine, Stanford, Calif.

Received for publication May 23, 2005; revisions received Sept 2, 2005; accepted for publication Sept 13, 2005.

Address for reprints: Valerie Chock, MD, Division of Neonatology, Stanford University Medical Center, 750 Welch Rd, Suite 315, Stanford, CA 94305 (E-mail: vchock@stanford.edu).

J Thorac Cardiovasc Surg 2006;131:659-65
0022-5223/\$32.00

Copyright © 2006 by The American Association for Thoracic Surgery

doi:10.1016/j.jtcvs.2005.09.005

Neonates with congenital heart disease are at high risk for neurologic injury.^{1,2} Although the cause of injury is likely multifactorial, one strategy to reduce the risk of neurologic injury has been to modify intraoperative techniques. Specifically, although cardiopulmonary bypass (CPB) with deep hypothermic circulatory arrest (DHCA) allowed surgeons to attain a bloodless operative field for optimal operating conditions, an increased risk of seizures occurred postoperatively,³ and a higher incidence of poor motor scores, visual spatial skills, and speech scores were found at the 4- and 8-year developmental follow-up examinations.⁴ A newer technique involves the use of antegrade cerebral perfusion (ACP) during hypothermic CPB to selectively perfuse the brain while allowing for repair of complex congenital heart lesions. However, there are limited experimental data to support its use.⁵⁻⁷

We used a piglet model of CPB to compare the degree of neurologic injury after ACP or DHCA. Injury was assessed histologically, and specific markers of apoptotic injury were compared. A piglet model was chosen because of its similarity in weight and brain development at birth to the human neonate.⁸ In addition, this piglet model of CPB with DHCA has already been shown to induce apoptotic brain

Abbreviations and Acronyms

ACP	= antegrade cerebral perfusion
CPB	= cardiopulmonary bypass
CREB	= cAMP response element binding protein
DHCA	= deep hypothermic circulatory arrest
H&E	= hematoxylin and eosin
NMDA	= N-methyl-D-aspartate
TUNEL	= terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling

injury^{9,10} by morphology, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining, and caspase 3 and caspase 8 activation in neurons of the neocortex and hippocampus.

We studied specific markers of apoptosis because apoptosis is known to play a prominent role in the evolution of hypoxic-ischemic injury in the neonatal brain.¹¹ Caspase 3 was assessed because it is a major effector protease in the execution of cellular apoptosis. We also studied cytochrome c translocation because it is released from mitochondria and participates in forming the apoptosome, which activates caspase 3 through the intrinsic pathway. We hypothesized that the piglets undergoing ACP would have reduced neurologic injury on brain histology, specifically with less caspase 3 activation and cytochrome c translocation. These initial studies assessed the induction of apoptosis at an early survival time point.

Methods**Experimental and Surgical Protocol**

All procedures were carried out according to a protocol approved by the Stanford University Animal Care and Use Committee. Sixteen neonatal piglets (2 weeks of age, with an average weight 5 kg) were anesthetized with intramuscular ketamine and xylazine and intubated, and a midline sternotomy was performed. The ascending aorta (10F), right atrium (18F), and right carotid artery (6F for ACP) were cannulated after heparinization (400 IU/kg). Anesthesia was maintained with isoflurane and intravenous fentanyl, and paralysis was achieved with pancuronium before initiation of bypass. Rectal and nasopharyngeal temperatures were continuously monitored. Use of these peripheral temperature probes approximate deep brain temperatures within 1°C.¹² Intraoperative hemodynamic monitoring was achieved through a femoral artery catheter and jugular venous central line. Two control piglets were anesthetized, cannulated, and heparinized but not started on CPB. Additional naive control piglets (n = 3) did not undergo operations and were killed.

Cardiopulmonary Bypass and Perfusion

The CPB circuit consisted of a roller pump, membrane oxygenator (Minimax; Medtronic Bio-Medicus, Minneapolis, Minn), and sterile 1/4-inch tubing. The circuit was primed with blood previously harvested from a donor pig mixed with crystalloid prime solution

(Normasol; Abbot Labs, Chicago, Ill) to maintain the hematocrit value at greater than 30%. In addition, dexamethasone (30 mg/kg), heparin (2500 U), mannitol (0.5 g/kg), and sodium bicarbonate (20 mEq) were added to the priming solution. CPB was then initiated, and the animal was cooled to 18°C over 40 minutes at a pump flow of 200 mL · kg⁻¹ · min⁻¹ by using pH-stat arterial blood gas management. The piglets were randomized to DHCA (n = 7) or ACP (n = 7) for 45 minutes. For the arrest period, the aorta was clamped, and cold cardioplegic solution was administered to the aortic root (Plegisol, Abbot labs). For those animals in the ACP group, the proximal innominate artery was clamped, and ACP flow rate was maintained at 40 mL · kg⁻¹ · min⁻¹ with continued pH-stat management.

This ACP flow rate correlates with cerebral blood flow of 24 ± 5 mL · kg⁻¹ · min⁻¹, as measured with a carotid flow probe.¹³ In this study we found that an ACP flow rate of 40 mL · kg⁻¹ · min⁻¹ corresponded with cerebral flow rates of 19 to 24 mL · kg⁻¹ · min⁻¹ and cerebral capillary oxygen saturations of 50% to 70%. Lower flow rates resulted in cerebral capillary oxygen saturations of less than 40%, reflecting unacceptable tissue ischemia. Near-infrared spectroscopy measurements by others demonstrate the need for regional perfusion of 20 mL · kg⁻¹ · min⁻¹ to maintain baseline cerebral saturations in neonates undergoing regional low-flow perfusion.⁶

The piglets were then rewarmed to 37°C by using a combined pH-stat and alpha-stat strategy for arterial blood gas management. They were weaned from bypass, and hemoconcentration was performed to achieve a hematocrit value of 45%. The piglets were extubated and allowed to recover for 6 hours before death.

Histology

Piglets were killed with sodium pentobarbital and phenytoin (Euthanol; MTC Pharmaceuticals, Cambridge, Ontario, Canada) and perfused with 1 L of chilled saline and 1 L of 4% paraformaldehyde. Brains were removed and immersed overnight in 4% paraformaldehyde for fixation. Five-millimeter coronal blocks were cut and routinely processed into paraffin. Sections were cut from each block at a thickness of 5 μm and either stained with hematoxylin and eosin (H&E) or reserved for immunohistochemistry.

Immunohistochemistry

Paraffin sections were rehydrated, treated with 0.5% H₂O₂ for 30 minutes to inactivate endogenous peroxidases, and blocked with 2% horse serum for 30 minutes. Primary antibody to caspase 3 (rabbit monoclonal, 1:2000; PharMingen, San Diego, Calif) or cytochrome c (mouse monoclonal, 1:200; Santa Cruz Biotechnology, Santa Cruz, Calif) was added and incubated for 2 hours. After incubation with the species-appropriate secondary IgG antibody (Vector Laboratories, 1:200) for 45 minutes, the sections were washed and incubated with an avidin-biotin complex (ABC 1:100, Vector Laboratories), and immunoreactivity was visualized with 3,3'-diaminobenzidine tetrahydrochloride (Sigma, St Louis, Mo). For negative controls, the primary antibody was omitted.

Counting Protocol

Ten randomly generated 400× fields of H&E-stained caudate nucleus, putamen, hippocampus, cortex, and cerebellum were examined for each animal by using bright-field microscopy. All

Figure 1. A, Schematic illustration of a typical coronal brain section scanned at 100 $\times$ showing localization of injured neurons after hematoxylin and eosin staining, as marked by *red dots*. Significant injury was found in the striatum, with scattered injury in the cortex. B, Piglets undergoing deep hypothermic circulatory arrest (DHCA) had significantly more histologic injury compared with that seen in piglets undergoing antegrade cerebral perfusion (ACP); 29 vs 4 apoptotic or necrotic cells per high-power field [HPF] in caudate nucleus and putamen. *Comparison between piglets undergoing DHCA and piglets undergoing ACP, $P = .03$. Error bars indicate standard error of the mean. Control piglets had less than 1 apoptotic cell per HPF. There was a total of 18 piglets ($n = 6$ piglets undergoing DHCA, $n = 7$ piglets undergoing ACP, and $n = 5$ control piglets). Squares in panel A represent typical striatal regions in which we quantified cell death. Photomicrographs from these regions in 2 different piglets show necrotic neurons in putamen with inset at 400 $\times$ (C) and apoptotic neurons in putamen with inset at 400 $\times$ (D). Scale bar = 50 μ m.

slides were blinded before examination by a pathologist. Cell injury was defined as necrotic or apoptotic by morphology, and counts were averaged for the ten 400 $\times$ fields. Necrotic cells were identified on the basis of pyknotic or absent nuclei and shrunken hyper eosinophilic cytoplasm and angular cell margins. Apoptotic cells had fragmented or condensed nuclei with shrunken cytoplasm and rounding of cytoplasmic margins. Positively stained cells for activated caspase 3 and cytochrome c translocation were counted in a similar manner in caudate nucleus, putamen, neocortex, hippocampus, and cerebellum by a blinded observer. Cytochrome c release from mitochondria was counted when staining was distributed diffusely through the cytoplasm.

Statistical Analysis

Data are presented as means $\pm$ the standard error of the mean. One-way analysis of variance with the post-hoc Newman-Keuls test was used to compare results between groups. Statistical anal-

yses demonstrated no significant differences in the histology of brains of the control piglets that underwent cannulation with anesthesia and those of control piglets that did not. Therefore these animals ($n = 5$) were pooled into a single control group for histology but kept as 2 distinct groups for immunohistochemistry.

Results

This neonatal piglet model of CPB enabled documentation of cellular injury caused by DHCA. Figure 1, A, is a schematic illustration of a coronal brain section depicting regions of injury after H&E staining. Damage was predominantly in the striatum, with isolated multifocal areas of cortical injury. Representative photomicrographs of brain injury comprised of both necrosis and apoptosis are shown in Figure 1, C and D. Injured cells included mainly neurons but also some astrocytes and microglia. Although not quantified separately,

Figure 2. Piglets undergoing deep hypothermic circulatory arrest (DHCA) exhibit widespread positive immunostaining for activated caspase 3 (A), whereas piglets undergoing antegrade cerebral perfusion (ACP) have less caspase 3 staining (B), and naive control piglets have very minimal staining (C). All photomicrographs are taken at 400 $\times$ in striatal tissue (scale bar = 50 μ m). Piglets undergoing DHCA had significantly more cells positive for caspase 3 ($*P = .001$) compared with piglets undergoing ACP (D). There was a total of 19 piglets with an average of 10 high-power fields (HPF) counted per piglet in caudate and putamen ($n = 7$ piglets undergoing DHCA, $n = 7$ piglets undergoing ACP, $n = 2$ anesthesia control piglets [Ctrl anesth], and $n = 3$ naive control piglets [Ctrl naive]). Error bars indicate standard error of the mean.

both necrotic and apoptotic injury was seen. In more severely injured piglets, extensive cellular changes were seen not only in the striatum but also minimally in the cortex, cerebellum, and hippocampus. However, because of the limited amount of injury in these regions, analysis was restricted to the striatum.

Cellular damage in the striatum, as determined by means of bright-field examination of H&E-stained sections, was 7-fold higher in piglets undergoing DHCA compared with that seen in piglets undergoing ACP ($P = .03$; Figure 1, B). Tissues from one piglet in the DHCA group had severe freeze artifact and was excluded from the histologic study. Control piglets that were anesthetized and cannulated but not started on CPB ($n = 2$) and normal control piglets ($n = 3$) had an average of less than 1 apoptotic cell per ten 400 $\times$ fields. Injury in piglets undergoing DHCA was a combination of necrosis and apoptosis, whereas in piglets undergoing ACP, our pathologist's impression was that injury appeared mostly apoptotic by morphology.

Figures 2, A through C, and 3, A through C, show differences in the intensity of immunostaining in the striatum for activated caspase 3 and cytochrome c in the DHCA, ACP, and control groups. Both neurons and glial cells showed positive staining for activated caspase 3, whereas cytochrome c-positive cells were neurons. Control piglets showed minimal positive staining cells for either antibody. Immunostaining was also done in the hippocampus, cortex, and cerebellum, but because of the milder injury in these brain regions, differences did not reach statistical significance (data not shown).

Quantification of immunohistochemistry is shown in Figures 2, D, and 3, D. Piglets undergoing DHCA had more than 1.5 times more activated caspase 3-positive cells compared with those seen in piglets undergoing ACP ($P = .001$). Similarly, piglets undergoing DHCA had more than 2.5 times more cells with cytochrome c translocation compared with those seen in piglets undergoing ACP ($P = .02$).

A difference was suggested between the control groups for activated caspase 3 and cytochrome c immunostaining. There was a trend toward more staining for activated caspase 3 and greater cytochrome c translocation in control piglets that underwent cannulation after achievement of anesthesia compared with that seen in the completely naive piglets. Both control groups had significantly less staining for both antibodies when compared with that seen in piglets undergoing DHCA ($P < .05$) but not when compared with that seen in piglets undergoing ACP.

Discussion

We demonstrated that the technique of ACP reduced apoptotic brain injury compared with DHCA in a neonatal piglet model of CPB. Furthermore, apoptotic injury occurred primarily in the striatum of the brain. In addition, we found decreased activated caspase 3 and cytochrome c immunostaining in the piglets undergoing ACP.

Other porcine models of CPB have also found less severe injury in a regional low-flow perfusion group.^{5,14} In an adult pig model of hypothermic ACP, Hagl and colleagues⁵ found earlier electroencephalographic recovery and lower intracranial pressure increase compared with that seen in their pigs undergoing 90 minutes of DHCA. During a neonatal piglet study with 90 minutes of DHCA, a cerebral flow rate of $10 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, and a 7-day recovery period, Myung and associates¹⁴ found a trend toward less severe injury in their ACP group by means of H&E staining and decreased apoptosis by means of TUNEL staining. We chose a more clinically relevant time for DHCA of 45 minutes, an ACP flow rate of $40 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, and an earlier 6-hour time point to analyze histologic injury. We also used more specific measures of apoptosis: activated caspase 3 analysis and cytochrome c analysis.

Length of circulatory arrest, duration of recovery, and degree of cerebral perfusion are key variables differentiating our model from others. In an adult pig model of DHCA, it takes at least 75 to 90 minutes of hypothermic circulatory arrest to consistently produce cerebral damage by histopathology.⁵ Perhaps longer arrest times would have resulted in increased injury in additional brain regions and even more pronounced protection in our ACP compared with DHCA groups. Similarly, after flow is restored after bypass, brain oxygen levels still remain decreased, even at 2 hours of recovery.^{15,16} As injury continues to evolve beyond a 6-hour recovery, delayed effects, such as inflammation, will require analysis with longer survival intervals. Inflammatory mediators contributing to brain injury might not peak until 72 hours to 1 week after injury.¹⁷

In this global model of brain ischemia, we demonstrated a selective vulnerability by brain region, with striatum being the most sensitive to injury. Other investigators using this piglet model saw more cortical and hippocampal injury and

less striatal injury^{9,10}; however, they used a 90-minute DHCA time. Different mechanisms of neuronal injury might occur in different selectively vulnerable brain regions at various times. Striatum is involved in control of motor behavior and is especially vulnerable to hypoxic-ischemic injury in the neonate.¹⁸ Indeed, after severe perinatal asphyxia in term infants, characteristic injury occurs in the basal ganglia region, as determined by means of magnetic resonance imaging.¹⁹ Consistent with our findings, significant striatal damage occurs after cardiac arrest in newborn piglets.²⁰

Several mechanisms might account for striatal vulnerability in our model. Increased levels of striatal dopamine, a mediator of neuronal injury, and increased striatal orthotyrosine, a measure of free radical generation, were reported in a piglet model of DHCA in comparison with a low-flow bypass condition.²¹ Others have further shown the dopaminergic system of piglet striatum to be very sensitive to local oxygen pressure, with extracellular dopamine increasing as tissue oxygenation decreases.^{22,23} Increased striatal dopamine after hypoxia and ischemia in piglets was also associated with decreased cAMP response element binding protein (CREB) phosphorylation²⁴ and suppression of Na^+ , K^+ -adenosine triphosphatase activity,²⁵ mechanisms potentially contributing to neuronal death in this region. After hypoxia-ischemia, altered N-methyl-D-aspartate (NMDA) receptors and decreased intracellular calcium clearance are also found in piglet striatum, implicating excitotoxic injury as an additional factor in striatal vulnerability.^{26,27} In addition to excessive dopaminergic excitatory activity, after a hypoxic insult, neonatal piglet striatal neurons express almost no heat shock protein 72, suggesting that protective mechanisms in this brain region at this developmental stage are limited.^{26,28}

Although apoptosis can be identified on the basis of typical changes in cellular morphology and DNA fragmentation, these characteristics are not entirely specific for apoptosis. In the setting of ischemia, mixed and intermediate morphologies are often observed. Therefore we investigated several key apoptotic proteins. Cytochrome c is translocated from dysfunctional mitochondria into cell cytoplasm in response to ischemia activating the intrinsic pathway of apoptosis.²⁹ It forms a complex with Apaf-1, which activates caspase 9 to activate caspase 3. Caspase 3 can then initiate cleavage of DNA. We found significantly increased cells with activated caspase 3 and translocated cytochrome c in the DHCA group of piglets compared with that in the ACP group at 6 hours of reperfusion, suggesting that the protective effects of ACP might be due to a decrease in apoptotic activity. In addition, there was no difference in apoptotic markers between the ACP and control groups, despite increased histologic injury in the ACP group. This discrepancy might reflect differences in the timing of cel-

Figure 3. Piglets undergoing deep hypothermic circulatory arrest (DHCA) exhibit increased immunostaining for translocated cytochrome c (A), whereas piglets undergoing antegrade cerebral perfusion (ACP) showed fewer cells with cytochrome c staining (B), and naive control piglets had only minimal staining (C). Photomicrographs are taken at 400 $\times$ in striatal tissue (scale bar = 50 μ m). Piglets undergoing DHCA had significantly more cells positive for cytochrome c (* P = .02) compared with those seen in piglets undergoing ACP. There was a total of 19 piglets with an average of 10 high-power fields (HPF) counted per piglet in caudate and putamen (n = 7 piglets undergoing DHCA, n = 7 piglets undergoing ACP, n = 2 anesthesia control piglets [Ctrl anesth], and n = 3 naive control piglets [Ctrl naive]). Error bars indicate standard error of the mean.

lular morphologic changes. Ditsworth and colleagues⁹ found a similar increase in caspase 3 activity from 4 to 72 hours after DHCA in piglets, whereas cytosolic cytochrome c levels, as determined by means of Western blotting, peaked at 1 hour after DHCA and remained mildly increased for up to 72 hours. Their study further found an increase in caspase 8 levels at 24 hours of reperfusion after DHCA.⁹ This finding might reflect an additional contribution to apoptosis through the extrinsic pathway and might be related to later inflammatory effects of DHCA, which we were unable to assess with our early 6-hour reperfusion time point.

Both apoptosis and necrosis occur in striatum after transient global ischemia, although it is impossible to distinguish between these 2 histologic injuries on the basis of clinical outcome alone.¹¹ Neurologic injury is typically a spectrum between apoptosis and necrosis. However, apoptosis tends to occur in regions with less severe ischemia,³⁰ whereas necrotic injury is the form of neuronal death in

piglet striatum after severe asphyxic cardiac arrest.³¹ Although not quantified, the observation that piglets undergoing DHCA had more necrotic cells and piglets undergoing ACP had more apoptotic cells further supports the attenuation of brain injury caused by ACP.

As an animal model, our study is subject to several experimental constraints. As previously discussed, the benefit of using ACP in our model is restricted by a shorter circulatory arrest time and early survival time point. Although clinically relevant, our relatively short circulatory arrest time of 45 minutes might preclude assessment of the protective effects of ACP when typical time limits have been exceeded during surgical repair. Histologic evaluation at 6 hours of survival prevents us from analyzing the evolution of apoptosis in multiple brain regions. Furthermore, it is unclear to what extent increased apoptotic brain injury correlates with clinical deficits of motor integration and neurologic function in our model. In a similar piglet model

of DHCA, despite ongoing histologic cell death, neurologic function improved over a 7-day survival period.¹⁰ In addition, species differences might limit application of our findings to human neonates with congenital heart disease, although piglet size and brain maturation is similar to that of the term human newborn.⁸

Our study supports the use of ACP during hypothermic CPB in this piglet model by a reduction in histologic injury and specific apoptotic markers. Moreover, striatum was the most vulnerable brain region to injury, a finding consistent with known patterns of hypoxic-ischemic injury in the neonate. Application of our model with longer survival periods and investigation of other markers of injury at later time points might yield additional insight into the protective role of ACP and the mechanism of cerebral injury during CPB.

We thank Sandy Perez for assistance with manuscript preparation, Beth Hoyte for help with the figures, and Pauline Chu for making paraffin sections.

References

- Mahle WT, Tavani F, Zimmerman RA, Nicolson SC, Galli KK, Gaynor JW, et al. An MRI study of neurological injury before and after congenital heart surgery. *Circulation*. 2002;106(suppl 1):I109-14.
- Limperopoulos C, Majnemer A, Shevell MI, Rohlicek C, Rosenblatt B, Tchervakov C, et al. Predictors of developmental disabilities after open heart surgery in young children with congenital heart defects. *J Pediatr*. 2002;141:51-8.
- Bellinger DC, Jonas RA, Rappaport LA, Wypij D, Wernovsky G, Kuban KC, et al. Developmental and neurologic status of children after heart surgery with hypothermic circulatory arrest or low-flow cardiopulmonary bypass. *N Engl J Med*. 1995;332:549-55.
- Newburger JW, Jonas RA, Wernovsky G, Wypij D, Hickey PR, Kuban KC, et al. A comparison of the perioperative neurologic effects of hypothermic circulatory arrest versus low-flow cardiopulmonary bypass in infant heart surgery. *N Engl J Med*. 1993;329:1057-64.
- Hagl C, Khaladj N, Peterss S, Hoeffler K, Winterhalter M, Karck M, et al. Hypothermic circulatory arrest with and without cold selective antegrade cerebral perfusion: impact on neurological recovery and tissue metabolism in an acute porcine model. *Eur J Cardiothorac Surg*. 2004;26:73-80.
- Pigula FA, Nemoto EM, Griffith BP, Siewers RD. Regional low-flow perfusion provides cerebral circulatory support during neonatal aortic arch reconstruction. *J Thorac Cardiovasc Surg*. 2000;119:331-9.
- Kilpack VD, Stayer SA, McKenzie ED, Fraser CD Jr, Andropoulos DB. Limiting circulatory arrest using regional low flow perfusion. *J Extra Corpor Technol*. 2004;36:133-8.
- Dobbing J, Sands J. Comparative aspects of the brain growth spurt. *Early Hum Dev*. 1979;3:79-83.
- Ditsworth D, Priestley MA, Loepeke AW, Ramamoorthy C, McCann J, Staple L, et al. Apoptotic neuronal death following deep hypothermic circulatory arrest in piglets. *Anesthesiology*. 2003;98:1119-27.
- Kurth CD, Priestley M, Golden J, McCann J, Raghupathi R. Regional patterns of neuronal death after deep hypothermic circulatory arrest in newborn pigs. *J Thorac Cardiovasc Surg*. 1999;118:1068-77.
- Martin LJ, Al-Abdulla NA, Brambrink AM, Kirsch JR, Sieber FE, Portera-Cailliau C. Neurodegeneration in excitotoxicity, global cerebral ischemia, and target deprivation: a perspective on the contributions of apoptosis and necrosis. *Brain Res Bull*. 1998;46:281-309.
- Amir G. Deep brain hyperthermia routinely occurs during rewarming from cardiopulmonary bypass. Presented at: Society of Thoracic Surgeons 41st Annual Meeting; 2005 Jan 23-26; Tampa, Florida.
- Amir G, Ramamoorthy C, Riemer R, Davis C, Hanley F, Reddy V. Visual light spectroscopy reflects flow related changes in brain oxygenation during regional low flow perfusion and deep hypothermic circulatory arrest. Presented at: Western Thoracic Surgical Association 30th Annual Meeting; 2004 June 26-29; Maui, Hawaii.
- Myung RJ, Petko M, Judkins AR, Schears G, Ittenbach RF, Waibel RJ, et al. Regional low-flow perfusion improves neurologic outcome compared with deep hypothermic circulatory arrest in neonatal piglets. *J Thorac Cardiovasc Surg*. 2004;127:1051-7.
- Schears G, Shen J, Creed J, Zaitseva T, Wilson DF, Greeley WJ, et al. Brain oxygenation during cardiopulmonary bypass and circulatory arrest. *Adv Exp Med Biol*. 2003;510:325-30.
- DeCampi WM, Schears G, Myung R, Schultz S, Creed J, Pastuszko A, et al. Tissue oxygen tension during regional low-flow perfusion in neonates. *J Thorac Cardiovasc Surg*. 2003;125:472-80.
- Wiessner C, Gehrman J, Lindholm D, Topper R, Kreutzberg GW, Hossman KA. Expression of transforming growth factor-beta 1 and interleukin-1 beta mRNA in rat brain following transient forebrain ischemia. *Acta Neuropathol (Berl)*. 1993;86:439-46.
- Johnston MV. Selective vulnerability in the neonatal brain. *Ann Neurol*. 1998;44:155-6.
- Miller SP, Ramaswamy V, Michelson D, Barkovich AJ, Holshouser B, Wycliffe N, et al. Patterns of brain injury in term neonatal encephalopathy. *J Pediatr*. 2005;146:453-60.
- Martin LJ, Brambrink A, Koehler RC, Traystman RJ. Primary sensory and forebrain motor systems in the newborn brain are preferentially damaged by hypoxia-ischemia. *J Comp Neurol*. 1997;377:262-85.
- Schultz S, Creed J, Schears G, Zaitseva T, Greeley W, Wilson DF, et al. Comparison of low-flow cardiopulmonary bypass and circulatory arrest on brain oxygen and metabolism. *Ann Thorac Surg*. 2004;77:2138-43.
- Yonetani M, Huang CC, McGowan J, Lajevardi NS, Pastuszko A, Delivoria-Papadopoulos M, et al. Effect of hemorrhagic hypotension on extracellular level of dopamine, cortical oxygen pressure and blood flow in brain of newborn piglets. *Neurosci Lett*. 1994;180:247-52.
- Pastuszko A, Saadat-Lajevardi N, Chen J, Tammela O, Wilson DF, Delivoria-Papadopoulos M. Effects of graded levels of tissue oxygen pressure on dopamine metabolism in the striatum of newborn piglets. *J Neurochem*. 1993;60:161-6.
- Zaitseva T, Creed J, Antoni D, Wilson DF, Pastuszko A. CREB phosphorylation following hypoxia and ischemia in striatum of newborn piglets: possible role of dopamine. *Brain Res*. 2005;1040:169-77.
- Zaitseva T, Shen J, Schears G, Creed J, Wilson DF, Pastuszko A. Effect of catecholamines on activity of Na(+), K(+)-ATPase in neonatal piglet brain during posthypoxic reoxygenation. *Comp Biochem Physiol A Mol Integr Physiol*. 2002;132:139-45.
- Guerguerian AM, Brambrink AM, Traystman RJ, Hagan RL, Martin LJ. Altered expression and phosphorylation of n-methyl-D-aspartate receptors in piglet striatum after hypoxia-ischemia. *Brain Res Mol Brain Res*. 2002;104:66-80.
- Ooboshi H, Sadoshima S, Yao H, Nakahara T, Uchimura H, Fujishima M. Inhibition of ischemia-induced dopamine release by omega-conotoxin, a calcium channel blocker, in the striatum of spontaneously hypertensive rats: in vivo brain dialysis study. *J Neurochem*. 1992;58:298-303.
- Murphy SJ, Song D, Welsh FA, Wilson DF, Pastuszko A. The effect of hypoxia and catecholamines on regional expression of heat-shock protein-72 mRNA in neonatal piglet brain. *Brain Res*. 1996;727:145-52.
- Ouyang YB, Tan Y, Comb M, Liu CL, Martone ME, Siesjo BK, et al. Survival- and death-promoting events after transient cerebral ischemia: phosphorylation of akt, release of cytochrome c and activation of caspase-like proteases. *J Cereb Blood Flow Metab*. 1999;19:1126-35.
- Ruan YW, Ling GY, Zhang JL, Xu ZC. Apoptosis in the adult striatum after transient forebrain ischemia and the effects of ischemic severity. *Brain Res*. 2003;982:228-40.
- Martin LJ, Brambrink AM, Price AC, Kaiser A, Agnew DM, Ichord RN, et al. Neuronal death in newborn striatum after hypoxia-ischemia is necrosis and evolves with oxidative stress. *Neurobiol Dis*. 2000;7:169-91.