The Heart Surgery Forum #2009-1018
12 (4), 2009 [Epub August 2009]
doi: 10.1532/HSF98.20091018

Online address: http://cardenjennings.metapress.com

Antegrade Cerebral Perfusion during Deep Hypothermia Circulatory Arrest Attenuates the Apoptosis of Neurons in Porcine Hippocampus

Dr. Gu

Rong Zhao, MD, Qin Cui, MD, Shi-Qiang Yu, MD, Guo-Cheng Sun, MD, Hong-Bing Wang, MD, Zheng-Xiao Jin, MD, Chun-Hu Gu, MD, Ding-Hua Yi, MD

Department of Cardiovascular Surgery, Xijing Hospital, Fourth Military Medical University, Xi'an, China

ABSTRACT

Background: Cerebral damage is a major problem after reconstructive surgery of the aortic arch and the descending aorta. Current protective strategies, including deep hypothermia and antegrade cerebral perfusion (ACP), are used to prolong the tolerated duration of circulatory arrest. The aim of the study was to observe the influence of deep hypothermic circulatory arrest (DHCA) and ACP on neuronal apoptosis in the hippocampus. To further elucidate the mechanisms of neurologic injury and protection, we assessed the expression of the antiapoptotic protein Bcl-2 and the proapoptotic protein Bax.

Methods: We randomly divided 18 pigs into 3 groups: The control group (n = 6) received normal-temperature cardiopulmonary bypass (CPB), the DHCA group (core temperature, 18°C; n = 6) received DHCA for 90 minutes, and the third group (DHCA + ACP) (core temperature, 18°C; ACP, flow rate of 30 mL/kg per minute at a pressure of 15–25 mm Hg; n = 6) received DHCA for 90 minutes. Hippocampal tissue was sampled 2 hours after CPB was finished. Bcl-2 and Bax expression was examined by immunohistochemistry. Morphologic changes in hippocampal tissue were measured with transmission electron microscopy.

Results: Bax protein levels were significantly higher in the DHCA group than in the other 2 groups ($P < .05$), whereas Bcl-2 protein levels were significantly higher in the DHCA + ACP group than in the other 2 groups ($P < .05$). Obvious neuronal apoptosis was observed in the DHCA group but not in the controls, and few apoptotic neurons were seen in the DHCA + ACP group.

Conclusions: DHCA can induce neuronal apoptosis in the hippocampus. ACP during the DHCA period protects cerebral tissue by suppressing apoptosis through decreasing Bax expression and increasing Bcl-2 expression.

Received February 11, 2009; accepted March 3, 2009.

Correspondence: Dr. Chun-hu Gu, Cardiovascular Surgery, Xijing Hospital, Fourth Military Medical University, Xi'an, Shanxi, 710032, China; 86-29-84775309; fax: 86-29-83210092 (e-mail: guchunhu@fmmu.edu.cn). Dr. Ding-Hua Yi, Department of Cardiovascular Surgery, Xijing Hospital, Fourth Military Medical University, Xi'an, Shanxi, 710032, China; 86-29-84773898; fax: 86-29-83210092 (e-mail: zhaorong@fmmu.edu.cn).

INTRODUCTION

Since its first clinical use, deep hypothermic circulatory arrest (DHCA) has improved surgical outcomes, both in the correction of congenital cardiac defects in infants and in the repair of aortic arch aneurysms in adults [Swain 1991; Jonas 1996]. Although DHCA can protect the brain to some degree, the risk of injury to the central nervous system limits the duration of DHCA to 45 to 60 minutes [Bellinger 1995; Langley 1999]. Experiments have shown, however, that cerebral impairments can still occur during this time window [Mills 1980]. Selective antegrade cerebral perfusion (ACP) is now probably the most widely used adjunctive cerebral protective technique to supplement DHCA. Several groups have reported excellent clinical outcomes with very low rates of both mortality and neurologic morbidity in large series of patients [Kazui 2001; Di Eusanio 2003].

After 75 minutes of HCA at 18°C, experiments have revealed increases in cells staining positive for terminal deoxynucleotidyl transferase–mediated dUTP nick end-labeling (TUNEL), indicating DNA fragmentation, especially in the neocortex and hippocampus, with an absence of morphologic evidence for apoptosis [Ananiadou 2005]. The present study was undertaken to assess whether ACP during DHCA can decrease apoptosis in hippocampal tissues, and thus perhaps have clinical utility. To further elucidate the mechanisms of neurologic injury and protection, we assessed the expression of the antiapoptotic protein Bcl-2 and the proapoptotic protein Bax.

MATERIALS AND METHODS

Animal Preparation

Eighteen male juvenile pigs, 2.5 to 3.5 months of age and weighing 35 to 40 kg, were used for this study (provided by the Animal Center of the Fourth Military Medical University, Xi'an, China). All animals received human care in compliance with the *Guide for the Care and Use of Laboratory Animals* [Institute of Laboratory Animal Research 1996]. They were randomly allocated to 3 groups: The control group (6 pigs) received normothermic cardiopulmonary bypass (CPB); the DHCA group (6 pigs) received 90 minutes of DHCA; and the DHCA + ACP group (6 pigs) received 90 minutes of DHCA with ACP.

Table 1. Blood Gas Analysis of the DHCA and DHCA + ACP Groups (n = 6)*

Variable	Group	Before DHCA	After DHCA	After CPB
pH	DHCA	7.420 ± 0.078	6.963 ± 0.055	7.358 ± 0.056
	DHCA + ACP	7.417 ± 0.056	7.349 ± 0.081†	7.372 ± 0.085
Po_2, mm Hg	DHCA	409.2 ± 38.1	370.6 ± 24.9	402.6 ± 18.5
	DHCA + ACP	391.3 ± 43.9	351.3 ± 26.7	431.5 ± 24.9
Pco_2, mm Hg	DHCA	31.7 ± 1.4	30.5 ± 1.9	32.2 ± 2.3
	DHCA + ACP	72.3 ± 1.8	28.7 ± 1.7	31.5 ± 2.6
Sao_2, %	DHCA	97.5 ± 0.6	98.1 ± 0.3	98.5 ± 0.2
	DHCA + ACP	97.1 ± 0.7	97.7 ± 0.4	98.2 ± 0.3
$Sjvo_2$, %	DHCA	83.7 ± 0.3	60.5 ± 4.7	73.2 ± 0.7
	DHCA + ACP	82.6 ± 0.3	89.1 ± 6.6‡	74.1 ± 0.4
Hct, %	DHCA	19.5 ± 1.2	16.1 ± 0.4	27.6 ± 1.7
	DHCA + ACP	19.7 ± 0.9	15.8 ± 0.5	28.2 ± 1.3

*DHCA indicates deep hypothermic circulatory arrest; ACP, antegrade cerebral perfusion; CPB, cardiopulmonary bypass; Po^2, oxygen tension; Pco_2, carbon dioxide tension; Sao_2, arterial oxygen saturation; $Sjvo_2$, jugular vein oxygen saturation; Hct, hematocrit.

†$P < .05$, compared with the DHCA group.

‡$P < .01$, compared with the DHCA group.

Anesthesia and Care

Ketamine hydrochloride (10 mg/kg) and pentobarbital sodium (2.5 mg/kg, intravenously) were used for anesthesia, with pancuronium bromide (0.2 mg/kg, intravenously) used for muscular relaxation. Tracheal intubation was performed by mechanical ventilation with a Newport 100 ventilator (Newport Medical Instruments, Costa Mesa, CA, USA) and a mixture of 40% oxygen, 60% nitrous oxide, and 0.5% enflurane. A standard lead was used for sustainable electrocardiography, a 22-gauge catheter was placed in the femoral artery for continuous monitoring of arterial blood pressure, and another catheter was inserted into the jugular vein and positioned in the sagittal sinus vein for continuous monitoring of vein blood pressure and for obtaining samples of venous blood. Arterial pressure, end-expired carbon dioxide, electrocardiograms, blood gases, and nasopharyngeal and rectal temperatures were monitored continuously.

Surgical Procedures

The chest was opened via a right thoracotomy in the fourth intercostal space as previously described [Gu 2008]. A Stokert-Shiley roller pump (Shiley, Irvine, CA, USA), a Kewei membrane oxygenator (Kewei, Dongguan, China), and a 40-μm arterial filter were used for the CPB circuit. The circuit was primed with a bloodless solution consisting of 1000 mL of a compound sodium acetate solution, 80 mL mannitol, and 5000 IU heparin. Sodium bicarbonate was added as necessary to adjust the pH to 7.4. CPB was established by cannulation of the ascending aorta and the right atrium.

In the control group, a normothermic CPB was performed for 120 minutes without heart arrest or discontinuing mechanical ventilation. CPB was established at a flow rate of 100 mL/kg per minute, with the flow adjusted to maintain a mixed venous oxygen saturation of approximately 75%. In the DHCA group, the animals were cooled until the nasopharyngeal and rectal temperatures indicated core hypothermia of 28°C. Then, the ascending aorta was clamped, and heart arrest was induced with the infusion of cold blood cardioplegia through the aortic root cannula. When the rectal temperature reached 18°C, CPB was discontinued, the blood was drained into the oxygenator reservoir, and circulatory arrest was maintained for 90 minutes. CPB was then restarted, the aortic clamping was removed to restore the heart rhythm, and mechanical ventilation was restarted when the body temperature reached 30°C. CPB was discontinued after the body temperature reached 37.0°C. The pH was maintained at 7.40 by means of the pH-stat principle, and the arterial $Paco_2$ was maintained at 35 to 40 mm Hg, corrected for body temperature. In the DHCA + ACP group, the cooling and warming methods were the same as for the DHCA group. During circulatory arrest, cerebral perfusion was performed via cannulation of the innominate artery at a flow rate of 30 mL/kg per minute at a pressure of 15 to 25 mm Hg. After 90 minutes of circulatory arrest, the descending aortic cross-clamp was removed, and CPB was restarted. Systemic pressure was maintained above 60 mm Hg during reperfusion. After weaning off CPB and stabilization for 2 hours, we took blood samples and killed the animals by blood drainage through a right atrial cannula.

Collection of Blood Samples

Arterial, right atrium, and sagittal sinus vein blood samples were collected 5 times during the experiment: (1) at baseline (37°C) and before CPB; (2) during CPB, while cooling to a rectal temperature of 18°C just before DHCA; (3) immediately after DHCA; and (4) at the end of CPB for blood gas

analysis (GEM Premier 3000 Blood gas analyzer; Instrumentation Laboratory, Bedford, MA, USA).

Collection and Preparation of Hippocampus Tissue

At the end of the experiment (approximately 160 minutes after the onset of circulatory arrest), we perfused the brain with 1 L chilled 0.9% saline solution and then with 4% paraformaldehyde in 0.1 M phosphate-buffered saline solution (1 L, pH 7.4) through an aortic cannula to fix the brain in situ. The descending aorta was cross-clamped to avoid significant loss of the perfusate to the lower body. The brains were retrieved and immersed in 4% paraformaldehyde (4°C-8°C). Tissue was prepared for immunohistochemistry and TUNEL measurements, or fixed with electron microscope fixation solution for transmission electron microscopy (TEM).

Immunohistochemistry, TUNEL, and TEM Examination

Neuronal apoptosis was evaluated by in situ DNA fragmentation with TUNEL histochemistry and a Chemicon Apo-Direct Assay Kit (DingYi Technology, Shenzhen, China). The TUNEL assay was performed as described previously [Tseng 1997]. The expression of Bcl-2 and Bax protein in the hippocampal CA1 zone was detected via immunofluorescence staining (Wuhan Boxter Biological Reagent, Wuhan, China) according to the manufacturer's instructions. Positive- and negative-control slides were included in each assay. The results were observed with an Olympus microscope and imaging system (BX51; Olympus, Tokyo, Japan). Image analysis was done with the Scion image analyzer (Scion Corporation, Frederick, MD, USA). Four slices from the same plane were chosen from each animal. Five high-power fields were randomly chosen from each slice. Gray-scale images of cells with Bax and Bcl-2 staining showed darker staining with increased protein expression, and gray-scale levels were quantified. Tissues for TEM examination were prepared according to methods previously described [Gu 2008] and were observed with an Hitachi H-7500 TEM (Hitachi, Tokyo, Japan).

Image Analysis and Statistical Processing

All experimental data are reported as the mean ± SE. Variance was analyzed with the SPSS statistical software package (version 10.0; SPSS, Chicago, IL, USA), with multiple comparisons verified with the least significant difference *t* test. Statistical significance was set at a P level of <.05.

RESULTS

Blood Gas Analysis

All animals were hemodynamically stable before, during, and after CPB. Table 1 summarizes the blood gas analysis of the animals in the DHCA and the DHCA + ACP groups. The arterial oxygen saturation (Sao_2) and the jugular vein oxygen saturation ($Sjvo_2$) were not different in the DHCA and DHCA + ACP groups before circulatory arrest, but the pH and $Sjvo_2$ were lower after DHCA in the DHCA group than in the DHCA + ACP group ($P < .05$).

TEM Examination

Neurons in the control group showed no significant changes after CPB compared with neurons before CPB (Figure 1A). After DHCA, some neurons in the hippocampus had an apoptotic appearance: Mitochondria were clearly visible

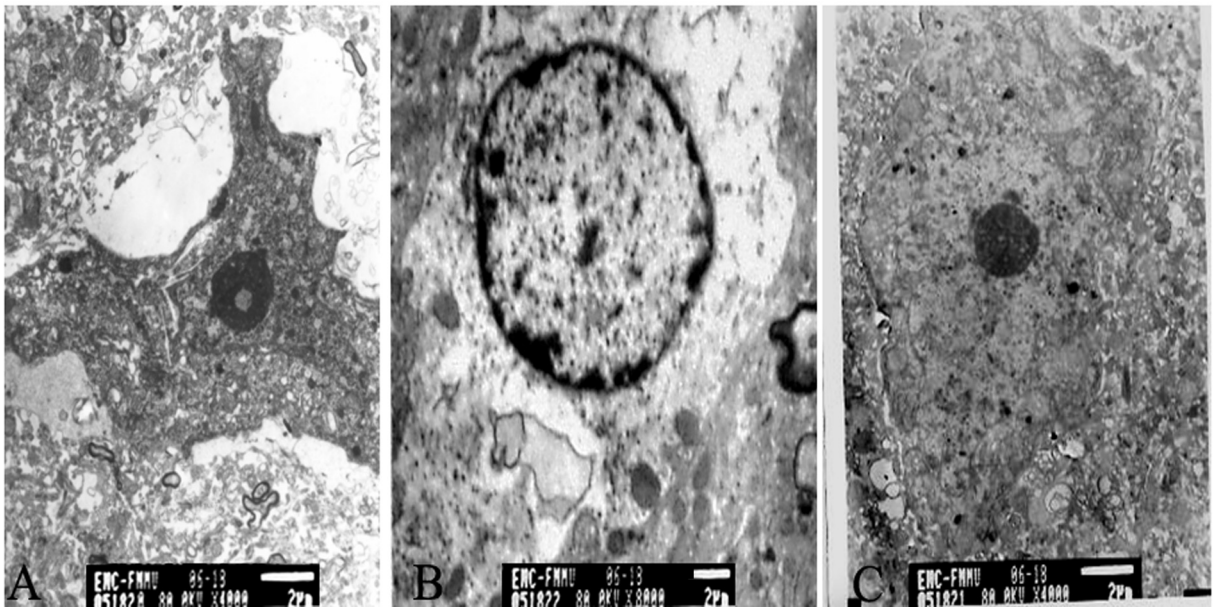

Figure 1. Hippocampal neuron imaged with transmission electron microscopy. A, Normal neuron (original magnification ×4000). B, A representative apoptotic hippocampal neuron after deep hypothermic circulatory arrest (DHCA). Mitochondria were clearly visible and swollen, chromatin was aggregated at the nuclear membrane, organelles were smaller, the endoplasmic reticulum was impaired, and dissolved nuclear chromatin was dispersed around the nucleus (original magnification ×8000). C, A representative hippocampal neuron after DHCA plus antegrade cerebral perfusion. There is no obvious swelling of the mitochondria, except for slight damage in the cytoplasm and slight nuclear swelling (original magnification ×4000).

Table 2. Bcl-2 and Bax Staining Intensity and Gray-Scale Values in the Hippocampal CA1 Area (n = 6)*

	DHCA	DHCA + ACP	NCPB
Bcl-2	231.3 ± 6.5	136.3 ± 2.9‡	175.2 ± 4.1†
Bax	127.8 ± 3.2	212.7 ± 2.7‡	178.3 ± 2.8†
Bcl-2/Bax	1.810	0.641	0.983

*DHCA indicates deep hypothermic circulatory arrest; ACP, antegrade cerebral perfusion; NCPB, normal control pig brain.

†$P < .05$, compared with the DHCA group.

‡$P < .01$, compared with the DHCA group.

and swollen, chromatin was aggregated at the nuclear membrane, organelles were smaller, the endoplasmic reticulum was impaired, and dissolved nuclear chromatin was dispersed around the nucleus (Figure 1B). On the other hand, only a few neurons in the hippocampus samples had an apoptotic appearance in the DHCA + ACP group. No obvious swelling of the mitochondria could be seen in most neurons, with the exception of slight damage in the cytoplasm and occasional slight nuclear swelling after treatment with DHCA + ACP (Figure 1C).

TUNEL and Immunohistochemistry Examination

In the DHCA group, TUNEL staining showed a positive nuclear signal, and the cytoplasm showed positive immunostaining for Bcl-2 and Bax (Figure 2). The DHCA group produced significantly higher TUNEL scores than the normal control animals in all brain regions examined (Table 2). The mean gray-scale value for Bcl-2 immunostaining in the hippocampal CA1 area was 175.2 ± 4.1 in the control group (Table 2). The DHCA group showed increased Bcl-2 immunostaining, with a gray-scale value of 231.3 ± 6.5. Bcl-2 immunostaining decreased in the DHCA + ACP group to 136.3 ± 2.9. The 3 groups were significantly different ($P < .01$, Table 2). The gray-scale immunostaining intensity for Bax in the hippocampal CA1 area was 178.3 ± 2.8 in the control group (Table 2), was decreased to 127.8 ± 3.2 in the DHCA group, and was increased to 212.7 ± 2.7 in the DHCA + ACP group ($P < .01$, versus the DHCA and control groups).

DISCUSSION

DHCA has been a standard technique in arch surgery and appears safe for short durations of cardiac arrest. The main rationale of DHCA is that cooling the patient on CPB to profound hypothermia causes sufficient cerebral metabolic suppression to permit a period of total circulatory arrest during which aortic arch anastomoses can be performed. However, DHCA is subject to a number of important physiological and clinical consequences that differ from those of CPB with continuous perfusion. Selective ACP is now probably the most widely used adjunctive cerebral protective technique to supplement DHCA. Recently, acute neuronal injury in a porcine animal model was reported to occur in various regions of the brain after short-term DHCA at 18°C [Ananiadou 2005]. In our study, we documented that DHCA alone can induce neuronal apoptosis in the hippocampus. ACP during the DHCA period protects cerebral tissue by suppressing apoptosis through decreasing Bax expression and increasing Bcl-2 expression.

Oxygen extracted in the brain is used both to support electrophysiological function and to maintain cellular integrity. Clinical studies of selective ACP have confirmed continued cerebral blood flow and reduced transcranial oxygen extraction immediately after arrest, ameliorating the oxygen debt otherwise accrued during DHCA [Strauch 2003]. In our study, Sao_2 and $Sjvo_2$ were not different in the DHCA and DHCA + ACP groups before circulatory arrest, but the $Sjvo_2$ was lower after DHCA in the DHCA group than in the DHCA + ACP group. The brain has a large metabolic requirement for oxygen and a low tolerance for hypoxia. Hypothermia can reduce oxygen consumption and the cerebral metabolic rate

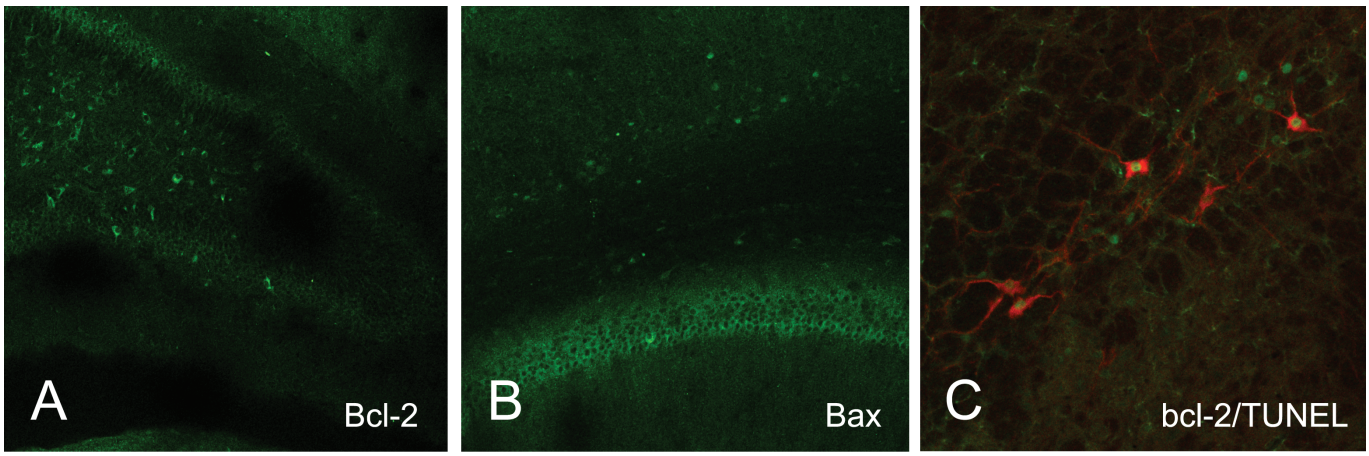

Figure 2. Immunofluorescence staining of Bcl-2 (A, green) and Bax (B, green), and double staining of Bcl-2 and TUNEL (terminal deoxynucleotidyl transferase–mediated dUTP nick end-labeling) (C, red and green, respectively) in the pig hippocampus following treatment with deep hypothermic circulatory arrest.

but cannot eliminate them completely. During DHCA, no oxygen or blood was provided to the brain, although there was sufficient blood perfusion in the DHCA + ACP group. Consequently, the pH was significantly lower in the DHCA group. The lower pH caused more oxygen to diffuse from the same amount of blood in the DHCA group, compared with the DHCA + ACP group. The results are similar to those of a previous report [Greeley 1991]. DHCA can induce neuronal apoptosis and necrosis [Kurth 1999], starting early in perfusion and lasting for days after surgery. This process may contribute to postoperative neurologic dysfunction. We found that normothermic CPB alone did not induce neuronal apoptosis but DHCA did. We also found that the addition of ACP reduced this induction.

Neuronal apoptosis requires regulated pathways involving multiple gene interactions [Lossi 2003]. Bcl-2 and Bax influence apoptosis by binding to each other. More Bcl-2 suppresses cell apoptosis, whereas more Bax drives apoptosis [Harris 2000]. Bax requires other cofactors to regulate apoptosis, usually Bcl-2 and p53. Bax can form a homodimer (Bax–Bax) to induce apoptosis or form a heterodimer with Bcl-2 (Bcl-2–Bax) to suppress apoptosis [Kaneda 1999].

Bcl-2 is encoded by an oncogene originally isolated from a follicular lymph cell tumor. The gene product is a transmembrane protein with 239 amino acid residues and is primarily localized in the mitochondrial membrane, the endoplasmic reticulum membrane, and the nuclear membrane [Tsujimoto 1984]. Bcl-2 lowers the permeability of the mitochondrial membrane to inhibit the release of cytochrome c, thereby down-regulating caspase activation and suppressing apoptosis [Swanton 1999].

Bcl-2 knock-out mice show an increased infarction volume and greater neurologic deficits after brain ischemia compared with wild-type mice [Hata 1999]. Adding Bcl-2 back into this model prevented this potentiation [Antonawich 1999]. The neuronal and astrocyte apoptosis induced by ischemia also involves the Bcl-2 family [Plesnila 2002]. Thus, increasing Bcl-2 expression should reduce the infarction area.

Bax contains 192 amino acid residues and shares a 21% homology with Bcl-2. Bax itself, the Bax–Bax homodimer, and the Bcl-2–Bax heterodimer primarily regulate apoptosis [Oltvai 1993]. Bax induces apoptosis [Jurgensmeier 1998], and Bax deficiency reduces hippocampal damage following ischemia in mice [Gibson 2001].

In the present study, Bcl-2 had the strongest expression in the DHCA + ACP group and the weakest expression in the DHCA group, where Bax expression was the strongest. Because cerebral tissue is still perfused during normothermic CPB, the balance between Bcl-2 and Bax is still present. DHCA disrupts the balance between Bcl-2 and Bax by up-regulating Bax, leading to apoptosis. The addition of ACP increased Bcl-2 expression and decreased Bax expression, suppressing apoptosis. Nevertheless, neuronal apoptosis during DHCA is complicated, with Bcl-2 and Bax constituting only one component of a complex system. Other regulatory factors can also contribute to cerebral protection during ACP, and these factors will need to be studied further.

REFERENCES

Ananiadou OG, Drossos GE, Bibou KN, Palatianos GM, Johnson EO. 2005. Acute regional neuronal injury following hypothermic circulatory arrest in a porcine model. Interact Cardiovasc Thorac Surg 4:597-601.

Antonawich FJ, Federoff HJ, Davis JN. 1999. BCL-2 transduction, using a herpes simplex virus amplicon, protects hippocampal neurons from transient global ischemia. Exp Neurol 156:130-7.

Bellinger DC, Jonas RA, Rappaport LA, et al. 1995. Developmental and neurologic status of children after heart surgery with hypothermic circulatory arrest or low-flow cardiopulmonary bypass. N Engl J Med 332:549-55.

Di Eusanio M, Schepens MA, Morshuis WJ, et al. 2003. Brain protection using antegrade selective cerebral perfusion: a multicenter study. Ann Thorac Surg 76:1181-9.

Gibson ME, Han BH, Choi J, et al. 2001. Bax contributes to apoptotic-like death following neonatal hypoxia-ischemia: evidence for distinct apoptosis pathways. Mol Med 7:644-55.

Greeley WJ, Kern FH, Ungerleider RM, et al. 1991. The effect of hypothermic cardiopulmonary bypass and total circulatory arrest on cerebral metabolism in neonates, infants, and children. J Thorac Cardiovasc Surg 101:783-94.

Gu CH, Wei XF, Wang YY, et al. 2008. No evidence of infection with porcine endogenous retrovirus in recipients of acellularized porcine aortic valves. Xenotransplantation 15:121-8.

Harris MH, Thompson CB. 2000. The role of the Bcl-2 family in the regulation of outer mitochondrial membrane permeability. Cell Death Differ 7:1182-91.

Hata R, Gillardon F, Michaelids TM, Hossmann KA. 1999. Targeted disruption of the bcl-2 gene in mice exacerbate focal ischemic brain injury. Metab Brain Dis 14:117-24.

Institute of Laboratory Animal Research. 1996. Guide for the care and use of laboratory animals. Commission on Life Sciences, National Research Council. Washington, DC: National Academies Press. NIH publication 86-23.

Jonas RA. 1996. Hypothermia, circulatory arrest, and the pediatric brain. J Cardiothorac Vasc Anesth 10:66-74.

Jurgensmeier JM, Xie Z, Deveraux Q, et al. 1998. Bax directly induces release of cytochrome c from isolated mitochondria. Proc Natl Acad Sci U S A 95;4997-5002.

Kaneda K, Kashii S, Kurosawa T, et al. 1999. Apoptosis DNA fragmentation and upregulation of Bax induced by transient ischemia of the rat retina. Brain Res 815:11-20.

Kazui T, Washiyama N, Muhammad B, Terada H, Yamashita K, Takinami M. 2001. Improved results of atherosclerotic arch aneurysm operations with a refined technique. J Thorac Cardiovasc Surg 121:491-9.

Kurth CD, Priestley M, Golden J, McCann J, Raghupathi R. 1999. Regional patterns of neuronal death after deep hypothermic circulatory arrest in newborn pigs. Thorac Cardiovasc Surg 118:1068-77.

Langley SM, Chai PJ, Miller SE, et al. 1999. Intermittent perfusion protects the brain during deep hypothermic circulatory arrest. Ann Thorac Surg 68:4-13.

Lossi L, Merighi A. 2003. In vivo cellular and molecular mechanisms of neuronal apoptosis in the mammalian CNS. Prog Neurobiol 69:287-312.

Mills NL, Ochsner JL. 1980. Massive air embolism during cardiopulmonary bypass. Causes, prevention, and management. J Thorac Cardiovasc Surg 80:708-17.

Oltvai ZN, Milliman CL, Korsmeyer SJ. 1993. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 74:609-19.

Plesnila N, Zinkel S, Amin-Hanjani S, Qin J, Korsmeyer SJ, Moskowitz MA. 2002. Function of BID—a molecule of the bcl-2 family—in ischemic cell death in the brain. Eur Surg Res 34:37-41.

Strauch JT, Spielvogel D, Haldenwang PL, et al. 2003. Cerebral physiology and outcome after hypothermic circulatory arrest followed by selective cerebral perfusion. Ann Thorac Surg 76:1972-81.

Swain JA, McDonald TJ Jr, Griffith PK, Balaban RS, Clark RE, Ceckler T. 1991. Low-flow hypothermic cardiopulmonary bypass protects the brain. J Thorac Cardiovasc Surg 102:76-84.

Swanton E, Savory P, Cosulich S, Clarke P, Woodman P. 1999. Bcl-2, regulates a caspase-3/caspase-2 apoptotic, cascade, in cytosolic extracts. Oncogene 18:1781-7.

Tseng EE, Brock MV, Lange MS. 1997. Neuronal nitric oxide synthase inhibition reduces neuronal apoptosis after hypothermic circulatory arrest. Ann Thorac Surg 64:1639-47.

Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. 1984. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science 226:1097-9.