Effect of granulocyte-colony stimulating factor on expression of selected proteins involved in regulation of apoptosis in the brain of newborn piglets after cardiopulmonary bypass and deep hypothermic circulatory arrest

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Objective: The study objective was to investigate the effect of granulocyte-colony stimulating factor on the expression of proteins that regulate apoptosis in newborn piglet brain after cardiopulmonary bypass and deep hypothermic circulatory arrest.

Methods: The newborn piglets were assigned to 3 groups: (1) deep hypothermic circulatory arrest (30 minutes of deep hypothermic circulatory arrest, 1 hour of low-flow cardiopulmonary bypass); (2) deep hypothermic circulatory arrest with prior injection of granulocyte-colony stimulating factor (17 μg/kg 2 hours before cardiopulmonary bypass); and (3) sham-operated. After 2 hours of post-bypass recovery, the frontal cortex, striatum, and hippocampus were dissected. The expression of proteins was measured by gel electrophoresis or protein arrays. Data are presented in arbitrary units. Statistical analysis was performed using 1-way analysis of variance.

Results: In the frontal cortex, only Fas ligand expression was significantly lower in the granulocyte-colony stimulating factor group when compared with the deep hypothermic circulatory arrest group. In the hippocampus, granulocyte-colony stimulating factor increased Bcl-2 (54.3 $\pm$ 6.4 vs 32.3 $\pm$ 2.2, $P = .001$) and serine/threonine-specific protein kinase (141.4 $\pm$ 19 vs 95.9 $\pm$ 21.1, $P = .047$) when compared with deep hypothermic circulatory arrest group. Caspase-3, Bax, Fas, Fas ligand, death receptor 6, and Janus protein tyrosine kinase 2 levels were unchanged. The Bcl-2/Bax ratio was 0.33 for deep hypothermic circulatory arrest group and 0.93 for the granulocyte-colony stimulating factor group ($P = .02$). In the striatum, when compared with the deep hypothermic circulatory arrest group, the granulocyte-colony stimulating factor group had higher levels of Bcl-2 (50.3 $\pm$ 7.4 vs 31.8 $\pm$ 3.8, $P = .01$), serine/threonine-specific protein kinase (132.7 $\pm$ 12.3 vs 14 $\pm$ 1.34, $P = 2.3 \times 10^6$), and Janus protein tyrosine kinase 2 (126 $\pm$ 17.4 vs 77.9 $\pm$ 13.6, $P = .011$), and lower levels of caspase-3 (12.8 $\pm$ 5.0 vs 32.2 $\pm$ 11.5, $P = .033$), Fas (390 $\pm$ 31 vs 581 $\pm$ 74, $P = .038$), Fas ligand (20.5 $\pm$ 11.5 vs 57.8 $\pm$ 15.6, $P = .04$), and death receptor 6 (57.4 $\pm$ 4.4 vs 108.8 $\pm$ 13.4, $P = .007$). The Bcl-2/Bax ratio was 0.25 for deep hypothermic circulatory arrest and 0.44 for the granulocyte-colony stimulating factor groups ($P = .046$).

Conclusions: In the piglet model of hypoxic brain injury, granulocyte-colony stimulating factor decreases proapoptotic signaling, particularly in the striatum. (J Thorac Cardiovasc Surg 2012;143:1436-42)

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Congenital heart disease (CHD) is the most common birth defect, affecting 8 per 1000 live births. Many of these neonates will require early surgical intervention. Today, because of improved perfusion techniques, pharmacology, and perioperative care, even the most complex surgical repairs in this patient population are performed with low operative mortality.

Better survival in infants and children with CHD requiring surgical correction has led to a shift in focus to the long-term outcomes, particularly neurodevelopmental progress and quality of life. Recent studies have demonstrated neurodevelopmental dysfunction in patients with complex CHD. The prevalence of this impaired neurologic function seems to vary with the specific cardiac diagnosis.[1,2] Long-term follow-up studies in these children have revealed distinctive patterns of neurodevelopmental dysfunction characterized by cognitive impairment, impaired executive function, expressive speech and language abnormalities, impaired visual-spatial and visual-motor skills, attention deficit/hyperactivity disorder, motor delays, and other learning disabilities.[3,4]

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Funding: The research was supported by Grant HL-58669 from the National Institutes of Health.

Disclosures: Authors have nothing to disclose with regard to commercial support.

Received for publication Jan 14, 2011; revisions received Sept 8, 2011; accepted for publication Jan 4, 2012; available ahead of print Feb 3, 2012.

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0022-5223/$36.00

doi:10.1016/j.jtcvs.2012.01.018

Abbreviations and Acronyms

CHD = congenital heart disease
CPB = cardiopulmonary bypass
DHCA = deep hypothermic circulatory arrest
DR6 = death receptor 6
Fas-L = Fas ligand
G-CSF = granulocyte-colony stimulating factor
LFCPB = low-flow cardiopulmonary bypass
pAkt = serine/threonine-specific protein kinase
pJAK2 = Janus protein tyrosine kinase 2
SEM = standard error of the mean
STAT = signal transducer and activator of transcription

Multiple causative factors for neurodevelopmental dysfunction in patients with complex CHD have been cited.[5] Patient factors have included genetic abnormalities, congenital syndromes, and in utero and postnatal abnormal cerebral blood flow patterns leading to an underdeveloped central nervous system and microcephaly. Putative surgical factors, such as deep hypothermic circulatory arrest (DHCA) and low-flow cardiopulmonary bypass (LFCPB), have been proposed.[4] Early postoperative factors such as low cardiac output, hypoxemia and hyperthermia also may contribute. Genetic abnormalities and other patient factors clearly cannot be altered. However, factors related to DHCA, LFCPB execution, and early postoperative management are modifiable.

Advances in the treatment of cerebral injury associated with hypoxic/ischemic insult depend on thorough understanding of the critical neuropathologic processes involved in neuronal survival and death. Apoptosis, a programmed cell death, seems to be the primary mechanism responsible for cell death in the newborn brain. We have previously used a piglet model of cardiopulmonary bypass (CPB) to delineate the mechanisms of brain injury associated with prolonged DHCA and LFCPB.[6] Marked alterations in expression of selected proteins that play well-established roles in regulation of apoptosis were observed, and we have identified strategies that can limit this neuropathology.[7]

The goal of the present study was to determine whether granulocyte-colony stimulating factor (G-CSF) may be an effective neuroprotective agent, as tested in our CPB/DHCA model of hypoxic cerebral injury. G-CSF, a member of the cytokine family of growth factors, is a glycoprotein broadly present within the central nervous system. G-CSF exerts its effect via a specific receptor present on hematopoietic, neuronal, and glial cells. Numerous studies have reported that ischemia upregulates the production of G-CSF and its receptors. Exogenous administration of G-CSF has been shown to be neuroprotective in a variety of stroke models.[8-10] It has potent anti-inflammatory[11] and anti-excitatory properties.[8] However, its most important function may be as a strong antiapoptotic factor.[8,12,13] Available studies suggest that G-CSF is capable of permeating the intact blood–brain barrier and is safe for use in humans.[8]

We hypothesized that proapoptotic proteins increase and antiapoptotic proteins decrease after CPB-DHCA and that pretreatment of the piglets with G-CSF can suppress these changes in signaling. To test these hypotheses, we have determined the effect of G-CSF treatment on the expression of selected regulatory proteins that play significant role in either initiation (Bax, caspase-3, Fas, Fas ligand (Fas-L), death receptor 6 [DR6]) or inhibition (Bcl-2, serine/threonine-specific protein kinase [pAkt], and Janus protein tyrosine kinase 2 [pJAK2]) of apoptotic signaling.

MATERIALS AND METHODS

Animal Model

Eighteen newborn piglets, 3 to 5 days old (2.0–3.0 kg), were anesthetized with 4% isoflurane (Novaplus, Hospira Inc, Lake Forest, Ill). Pulse oximetry, electrocardiogram, and temperature measurements were begun immediately after induction of anesthesia. A 1.5% lidocaine-HCl was used as a local anesthetic. After tracheotomy, pancuronium (1.5 mg/kg) was used for neuromuscular blockade to allow mechanical ventilation. Fentanyl-citrate (30 μg/kg) was injected intravenously, and the animals were mechanically ventilated with a mixture of oxygen (F_{IO_2} 21%) and 0.5% isoflurane. The femoral artery and vein were then cannulated, and anesthesia was maintained with 0.5% isoflurane and boluses of pancuronium (1 mg/kg/h). After a 2-hour period, CPB was initiated. After bypass, the animals were recovered for 2 hours under anesthesia and then euthanized with saturated KCl.

All animal procedures were in strict accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals: and have been approved by the local Animal Care and Use Committee.

Cardiopulmonary Bypass Technique

The circuit was primed with Plasma-Lyte A (Baxter Healthcare Corp, Deerfield, Ill) and 25% albumin. Donor whole blood was then added to maintain a hematocrit of 25% to 30%. Heparin (1000 units), fentanyl (50 μg), pancuronium (1 mg), $CaCl_2$ (500 mg), methylprednisolone (60 mg), cefazolin (100 mg), furosemide (2 mg), and $NaHCO_3$ (25 meq) were then added to the pump prime. A membrane oxygenator (Lilliput; COBE Cardiovascular, Inc, Arvada, Colo), a roller pump system (COBE), and an arterial filter (Capiox; Terumo Cardiovascular Systems, Corp, Ann Arbor, Mich) were used. A median sternotomy was performed, and after 500 units of heparin were administered intravenously, the ascending aorta and the right atrial appendage were cannulated. Full CPB flow rate was set at 150 mL/kg/min. The pH-stat blood gas management was maintained in all experiments.

After cooling to 18°C, the piglets were introduced to 30 minutes of DHCA followed by 1 hour of LFCPB at 20 mL/kg/min. All animals were then rewarmed for 30 minutes at full flow (150 mL/kg/min), separated from CPB and recovered for 120 minutes under anesthesia, and finally euthanized with saturated KCl. The control animals did not undergo CPB but were anesthetized and underwent a sham operation. After euthanasia, the frontal cortex, striatum, and hippocampus were immediately dissected from the brain and frozen at –80°C for later analysis.

The animals were randomly assigned to 1 of 3 groups: 1) CPB with circulatory arrest (n = 6, DHCA group), 2) DHCA with prior injection of

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