Hypoxia diminishes the protective function of white-matter astrocytes in the developing brain

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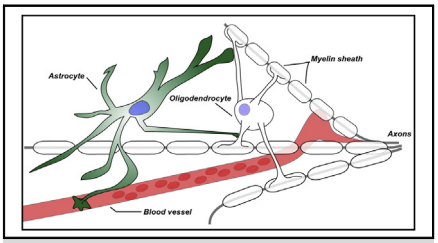
ABSTRACT

Objectives: White-matter injury after surgery is common in neonates with cerebral immaturity secondary to in utero hypoxia. Astrocytes play a central role in brain protection; however, the reaction of astrocytes to hypothermic circulatory arrest (HCA) remains unknown. We investigated the role of astrocytes in white-matter injury after HCA and determined the effects of preoperative hypoxia on this role, using a novel mouse model.

Methods: Mice were exposed to hypoxia from days 3 to 11, which is equivalent to the third trimester in humans (prehypoxia, n = 49). Brain slices were transferred to a chamber perfused by cerebrospinal fluid. Oxygen–glucose deprivation (OGD) was performed to simulate ischemia-reperfusion/reoxygenation resulting from circulatory arrest under hypothermia. Astrocyte reactions were compared with preoperative normoxia (prenormoxia; n = 45).

Results: We observed astrocyte activation after 25°C ischemia-reperfusion/reoxygenation in prenormoxia ($P < .01$). Astrocyte number after OGD correlated with caspase-3$^+$ cells (rho = .77, $P = .001$), confirming that astrogliosis is an important response after HCA. At 3 hours after OGD, astrocytes in prenormoxia had already proliferated and become activated ($P < .05$). Conversely, astrocytes that developed under hypoxia did not display these responses. At 20 hours after ischemia-reperfusion/reoxygenation, astrogliosis was not observed in prehypoxia, demonstrating that hypoxia altered the response of astrocytes to insult. In contrast to prenormoxia, caspase-3$^+$ cells in prehypoxia increased after ischemia reperfusion/reoxygenation, compared with control ($P < .01$). Caspase-3$^+$ cells were more common with prehypoxia than with prenormoxia ($P < .001$), suggesting that lack of astrogliosis permits increased white-matter injury.

Conclusions: Preoperative hypoxia alters the neuroprotective function of astrocytes. Restoring this function before surgery may be a therapeutic option to reduce postoperative white-matter injury in the immature brain. (J Thorac Cardiovasc Surg 2016;151:265-72)



Interaction between astrocytes and oligodendrocytes in the white matter.

Central Message

Astrocyte activation is critical in protecting white matter. Restoration of this function, which is altered by preoperative hypoxia, may reduce postoperative white-matter injury in neonates with CHD.

Perspective

We have demonstrated for the first time that astrocyte activation plays a critical role in white-matter protection. The reaction is altered after hypoxia, and thus contributes to increased white-matter injury after neonatal cardiac surgery. Restoring the neuroprotective function before surgery can be targeted to reduce postoperative white-matter injury in the neonate with CHD.

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Neurodevelopmental delay has become recognized as one of the most important challenges for children with surgically repaired congenital heart disease (CHD).[1] Clinical studies have demonstrated an important incidence of white-matter injury in children with CHD.[2-6] Abnormal white-matter development early in life accounts for the type and degree of the neurologic deficits commonly observed in children with CHD.[7] Thus, elucidating the

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Abbreviations and Acronyms
CHD = congenital heart disease
CPB = cardiopulmonary bypass
GFAP = glial fibrillary acidic protein
GFP = green fluorescent protein
HCA = hypothermic circulatory arrest
OGD = oxygen–glucose deprivation
PBS = phosphate buffered saline

cellular mechanisms underlying CHD-induced white-matter injury is not only a fundamental research endeavor, but also vital for the design of targeted therapies and conditions to improve such disorders.

Although previous clinical trials and laboratory research have focused on white-matter injury that occurs during surgery, and cardiopulmonary bypass (CPB), new evidence from neuroimaging studies has additionally linked CHD with white-matter immaturity in the fetus during the prenatal period.[8,9] The basic structural morphology of the heart is complete by 7 weeks of gestation. Thus, a cardiac anomaly can affect fetal cerebral oxygen saturation for >7 months, a period of critical importance to brain development. Newly developed postoperative white-matter injury has been demonstrated to be significantly associated with preoperative white-matter immaturity.[4,10] However, cellular events secondary to CHD-induced hypoxia, and the impact of cardiac surgery on these events in developing white matter, remain largely unexplored.[7]

Reproducing abnormal fetal cerebral blood flow and oxygen saturation observed in CHD patients is challenging in large laboratory animals. However, a rodent model has been well established, of chronic hypoxia during the period of early postnatal development, which is equivalent to the third trimester in humans.[11-13] Hypothermic circulatory arrest (HCA) and CPB can cause major brain insults during surgery.[2,8] The HCA poses a specific pathologic condition for patients undergoing cardiac surgery: exposure to ischemia-reperfusion/reoxygenation under hypothermia. Using a unique rodent brain-slice model, we have replicated specific brain conditions of CPB and HCA.[14]

To understand the complex pathology of CHD-induced white-matter injury, we have additionally established a combined experimental paradigm of prenatal hypoxia and HCA in a mouse hypoxia and brain-slice model.[15] Using this model, we recently demonstrated that white-matter immaturity caused by preoperative hypoxia causes increased vulnerability of oligodendrocyte lineage cells to hypothermic ischemia-reperfusion/reoxygenation.[15]

Astrocytes are the most numerous cells in the mammalian brain, and they make essential contributions to normal function in the healthy brain, including regulation of blood flow, provision of energy metabolites, and maintenance of the extracellular balance of fluid and neurotransmitters.[16] In addition, astrocytes react to all forms of brain insults, and their response—commonly referred to as reactive astrogliosis—is a hallmark of all central nervous system pathologies.[17] The crucial role of reactive astrogliosis in white-matter injury has been well documented,[18,19] yet little is known regarding the reaction of astrocytes to the multifaceted insults in CHD brains.

Thus, the overall aim of the present study is to determine the response of white-matter astrocytes to brain injury associated with CHD. Using our unique rodent hypoxia and brain-slice model, we first defined the role of astrocytes in reducing white-matter injury associated with CPB and HCA. We assessed the effects of preoperative hypoxia on both the acute reaction of astrocytes against insults, and their protective role.

METHODS

Animals

A total of 83 mice were involved in this study. We used a transgenic human glial fibrillary acidic protein (GFAP)/green-fluorescent protein (GFP) mouse (The Jackson Laboratory, Bar Harbor, Me) to identify white-matter astrocytes (Figure 1, *A*). Astrocytes have been visualized in transgenic mice, in which the GFAP promoter region directs expression of GFP. To investigate the effects of preoperative hypoxia, animals aged 3 days were randomly assigned to 1 of 2 preoperative conditions: (1) prenormoxia (n = 49); and (2) prehypoxia (n = 34).

The effects of brain insults associated with CPB and HCA on white-matter astrocytes were investigated in day-11 mice, which have a white-matter maturation level equivalent to that of the human full-term newborn (Figure 1, *B*).[20] The living brain slices collected from transgenic mice at day 11 were perfused, using artificial cerebrospinal fluid, and assigned to 1 of 4 groups with various levels of brain insult associated with HCA: (1) control (no OGD); (2) 15°C OGD for 60 minutes; (3) 25°C OGD for 60 minutes; and (4) 35°C OGD for 60 minutes (Table E1). Brains in each group were fixed at 3 hours and 20 hours after rewarming, respectively (Figure 1, *C*). We performed all experiments in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The study was approved by the Animal Care and Use Committee of the Children's National Medical Center. More details are described in Appendix E1.

Preoperative Hypoxia

The hypoxic rearing began on day 3 and continued until day 11 in a hypoxic chamber system (BioSpherix, Redfield, NY) to reproduce the preoperative hypoxic state (prehypoxia, 10.5% oxygen) of the CHD fetus (Figure 1, *B*).[13] The maturation stage from day 3 to day 11 in the mouse white matter is developmentally equivalent to the third trimester in humans.[20] A recent clinical magnetic resonance imaging (MRI) study revealed that during the third trimester, a progressive and significant decline occurs in gestational age–adjusted white-matter volume in CHD fetuses, compared with healthy controls.[21] Strain- and age-matched mice that were reared in a setting with normal oxygen levels were used to simulate a preoperative normoxic condition (prenormoxia; 21% oxygen). More details are described in Appendix E1.

Perfusion Protocol

Brains of transgenic mice at day 11 were collected. Living brains were sliced, using a vibratome (Leica 1200 VT; Leica Microsystems, Inc,

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