Prolonged White Matter Inflammation After Cardiopulmonary Bypass and Circulatory Arrest in a Juvenile Porcine Model

Ludmila Korotcova, MD, Sonali Kumar, BS, Kota Agematsu, MD, Paul D. Morton, PhD, Richard A. Jonas, MD,* and Nobuyuki Ishibashi, MD*

Children's National Heart Institute, and Center for Neuroscience Research, Children's National Medical Center, and George Washington University School of Medicine and Health Science, Washington, DC

Background. **White matter (WM) injury is common after neonatal cardiopulmonary bypass (CPB). We have demonstrated that the inflammatory response to CPB is an important mechanism of WM injury. Microglia are brain-specific immune cells that respond to inflammation and can exacerbate injury. We hypothesized that microglia activation contributes to WM injury caused by CPB.**

Methods. **Juvenile piglets were randomly assigned to 1 of 3 CPB-induced brain insults (1, no-CPB; 2, full-flow CPB; 3, CPB and circulatory arrest). Neurobehavioral tests were performed. Animals were sacrificed 3 days or 4 weeks postoperatively. Microglia and proliferating cells were immunohistologically identified. Seven analyzed WM regions were further categorized into 3 fiber connections (1, commissural; 2, projection; 3, association fibers).**

Results. **Microglia numbers significantly increased on day 3 after CPB and circulatory arrest, but not after full-flow CPB. Fiber categories did not affect these changes. On post-CPB week 4, proliferating cell number, blood leukocyte number, interleukin (IL)-6 levels, and neurologic scores had normalized. However, both full-flow CPB and CPB and circulatory arrest displayed significant increases in the microglia number compared with control. Thus brain-specific inflammation after CPB persists despite no changes in systemic biomarkers. Microglia number was significantly different among fiber categories, being highest in association and lowest in commissural connections. Thus there was a WM fiber-dependent microglia reaction to CPB.**

Conclusions. **This study demonstrates prolonged microglia activation in WM after CPB. This brain-specific inflammatory response is systemically silent. It is connection fiber-dependent which may impact specific connectivity deficits observed after CPB. Controlling microglia activation after CPB is a potential therapeutic intervention to limit neurologic deficits after CPB.**

(Ann Thorac Surg 2015;100:1030–8)

The hospital mortality of children with severe and complex congenital disease (CHD) has been dramatically reduced in the last 2 decades from more than 50% to less than 10% [1]. However, many of these children suffer some form of neurologic impairment [2, 3]. The most common neurologic deficits seen in infants after CHD repair are fine and gross motor deficits [4, 5]. These symptoms are consistent with diffuse white matter (WM)-injury [6]. Recent magnetic resonance imaging (MRI) studies have demonstrated a high incidence of WM injury after surgery (25% to 55%) in the neonate and infant with CHD [7–10]. Impairment of neural connectivity due to WM injury causes a wide range of neurologic dysfunction including attention deficit, hyperactivity, executive dysfunction, impairment of working memory, and verbal dysfunction [11–13]. These impairments are remarkably similar to deficits observed in children with severe and complex CHD [2, 14, 15]. Altered WM microstructure has also been identified in adolescents with CHD [16] in whom deficits of cognitive or behavioral functions have been documented [14]. Furthermore, it is well known that following premature birth alterations of the WM microstructure persist into later life [17]. In summary, the extent of abnormal WM development early in life likely accounts for the type and degree of neurologic deficits observed in CHD.

Causes of neurologic morbidity associated with cardiopulmonary bypass (CPB) include an exaggerated systemic inflammatory response syndrome (SIRS) as well as risk of ischemia-reperfusion and reoxygenation injury (I-R injury) [18, 19]. Our previous studies have shown that reduction of systemic inflammation is important to minimize the risk of WM injury [20]. On the other hand, the brain is an organ known to be "immune privileged." The blood-brain barrier of the cerebrovascular endothelium and its participation in the complex structure of the neurovascular unit restrict access of immune cells and

Accepted for publication April 1, 2015.

*Drs Jonas and Ishibashi are joint senior authors.

Presented at the Poster Session of the Fifty-first Annual Meeting of The Society of Thoracic Surgeons, San Diego, CA, Jan 24-28, 2015.

Address correspondence to Dr Ishibashi, Children's National Medical Center, 111 Michigan Ave, NW, Washington, DC 20010-2970; e-mail: nishibas@childrensnational.org.

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2015.04.017

immune mediators to the central nervous system [21]. Microglia are the resident central nervous system immune cells and active surveyors of the extracellular environment, playing a central role in neural immune function in reacting to a wide variety of insults [22]. In addition, recent studies have demonstrated that in the developing brain microglia regulate neuronal circuit development by triggering programmed cell death and stripping off synapses in an activity-dependent fashion [23]. Importantly, activation of microglia by cerebral inflammation causes substantial loss of synapses which results in sustained deficits in synaptic connectivity [24, 25]. These new findings highlight the importance of microglia for central nervous system development and function. The observations led our hypothesis that microglia activation is an important component of WM injury caused by CPB. The aim of the present study is to define the acute and long-term cellular response of microglia to CPB in defined WM regions using a porcine CPB survival model.

Material and Methods

Experimental Model

A total of 34 three-week-old female Yorkshire piglets were involved in this study. To investigate the effects of CPB we distinguished CPB-induced brain insults into SIRS and I-R injury. Animals were randomly assigned to 1 of 3 CPB-induced brain insults: (1) no-CPB (control, no-insult n = 10); (2) 34°C full-flow CPB for 60 minutes (mild-CPB, SIRS n = 12); and (3) 25°C circulatory arrest for 60 minutes (severe-CPB, SIRS with I-R injury n = 12). After initial normothermic perfusion, animals were cooled and then circulatory arrest or full-flow bypass was chosen according to the protocol (Fig 1A). The heterologous blood was used to maintain the hematocrit level of 30% (Table 1). For cooling and rewarming, pH-stat strategy was performed to employ the current standard CPB technique. Cerebral tissue oxygen index (TOI) was measured by near-infrared spectroscopy (NIRO-300) and used to determine ischemic status. The SIRS was identified by the leukocyte number and plasma IL-6 concentration. 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. The details of the experimental model have been described previously [20].

Neurologic Outcome

Neurologic and behavioral evaluations were performed at 24-hour intervals. In the system a score of 100 is assigned to each of 4 general components. A total score of 400 indicates brain death while a score of 0 is considered normal.

Cellular Analysis

The brain was harvested after transarterial infusion of 2.0 L normal saline followed by 2.0 L 4%

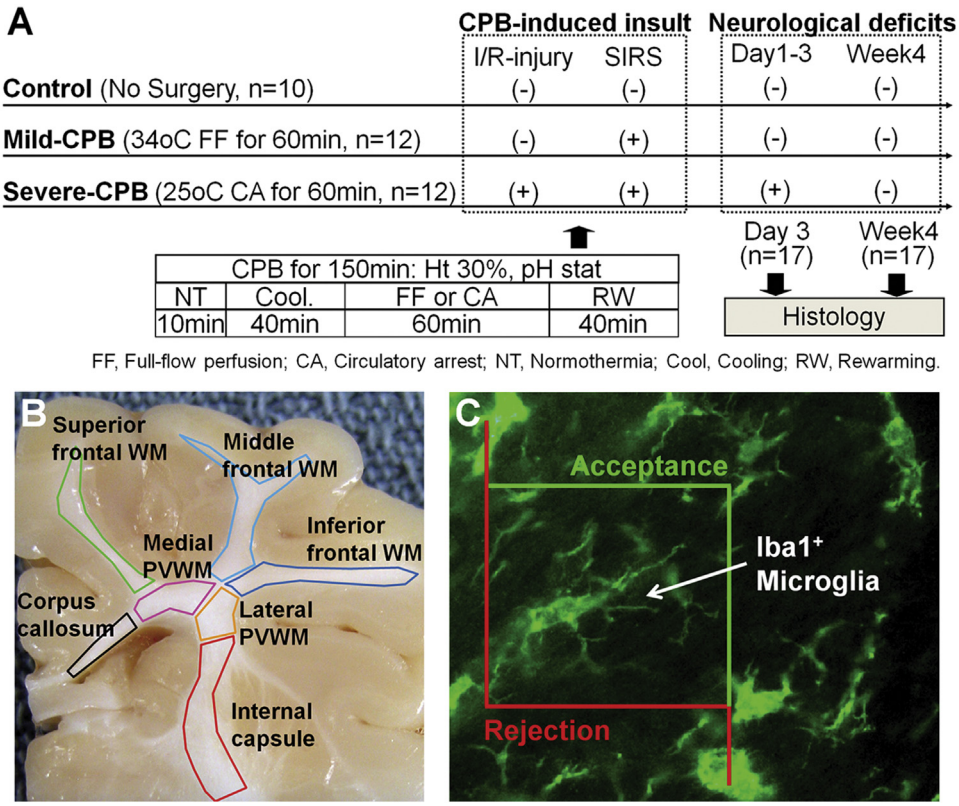

Fig 1. (A) Study design. (B) Subdivision of cerebral white matter (WM). (C) Iba1^{+} microglia cell and the counting frame for analysis. Microglia is counted if it lies entirely within the counting frame or if it touches an acceptance line without touching a rejection line. (CA = circulatory arrest; CPB = cardiopulmonary bypass; FF = full-flow perfusion; I/R = ischemia-reperfusion and reoxygenation; NT = normothermia; PVWM = peri-ventricular white matter; RW = rewarming; SIRS = systemic inflammatory response syndrome.)