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Maturation-Dependent Vulnerability of Perinatal White Matter in Premature Birth

Stephen A. Back, MD, PhD; Art Riddle, BS; Melissa M. McClure, PhD

Abstract—Survivors of premature birth have a predilection for perinatal brain injury, especially to periventricular cerebral white matter. Periventricular white matter injury (PWMI) is now the most common cause of brain injury in preterm infants and the leading cause of chronic neurological morbidity. The spectrum of chronic PWMI includes focal cystic necrotic lesions (periventricular leukomalacia) and diffuse myelination disturbances. Recent neuroimaging studies support that the incidence of periventricular leukomalacia is declining, whereas focal or diffuse noncystic injury is emerging as the predominant lesion. In a significant number of infants, PWMI appears to be initiated by perturbations in cerebral blood flow that reflect anatomic and physiological immaturity of the vasculature. Ischemic cerebral white matter is susceptible to pronounced free radical-mediated injury that particularly targets immature stages of the oligodendrocyte lineage. Emerging experimental data supports that pronounced ischemia in the periventricular white matter is necessary but not sufficient to generate the initial injury that leads to PWMI. The developmental predilection for PWMI to occur during prematurity appears to be related to both the timing of appearance and regional distribution of susceptible oligodendrocyte progenitors. Injury to oligodendrocyte progenitors may contribute to the pathogenesis of PWMI by disrupting the maturation of myelin-forming oligodendrocytes. There has been substantial recent progress in the understanding of the cellular and molecular pathogenesis of PWMI. The oligodendrocyte progenitor is a key target for preventive strategies to reduce ischemic cerebral white matter injury in premature infants. (*Stroke*. 2007;38[part 2]:724-730.)

Key Words: hypoxia-ischemia ■ oligodendrocyte ■ prematurity

Periventricular white matter injury (PWMI) is the major form of brain injury and the leading cause of chronic neurological disability in survivors of premature birth.^{1,2} Although major advances in the care of premature infants have resulted in striking improvements in the survival of very-low birth weight infants (<1.5 kg), when compared with the 1980s, improved survival has been accompanied by a significant increase in the number of preterm survivors with long-term neurological deficits.³ In up to 25% of preterm survivors, the major consequence of PWMI is permanent motor impairment (ie, “cerebral palsy”) ranging from mild to profound spastic motor deficits.^{4,5} By school age, 25% to 50% of children with PWMI manifest a broad spectrum of cognitive and learning disabilities.⁶

The period of highest risk for PWMI is between approximately 23 and 32 weeks postconceptional age. Premature infants with PWMI are at markedly increased risk for several others forms of brain injury, notably intraventricular hemorrhage and intraparenchymal hemorrhage.¹ Whereas medical interventions have resulted in a pronounced decrease in the incidence of intraventricular hemorrhage,^{7,8} the incidence of PWMI is not decreasing.⁹ Thus, PWMI is now the major neurological problem that affects very-low birth weight infants.

Pathological Features of PWMI

PWMI includes a spectrum of cerebral injury that ranges from focal cystic necrotic lesions (periventricular leukomalacia [PVL]) to diffuse myelination disturbances (diffuse PWMI).^{8,10} Commonly, PWMI presents as symmetric lesions localized adjacent to both lateral ventricles, with regions of particular predilection for injury being anterior or lateral to the anterior horns and lateral to the trigone and posterior horns. PVL may be accompanied by diffuse PWMI or occur as an isolated lesion.^{11–16} Whereas PVL was more common a decade or more ago, with advances in neonatal care, such lesions are now rarely encountered. In fact, recent neuroimaging studies support that the incidence of PVL is markedly declining, whereas focal or diffuse noncystic white-matter injury is emerging as the predominant lesion.^{17–20} In these recent series, cystic PVL lesions accounted for <5% of cases.

In the early stages of injury, PVL is characterized by foci of coagulation necrosis in the subventricular zone and periventricular white matter (PVWM) that results in degeneration of all cellular elements (ie, blood vessels, axons, astrocytes and oligodendrocytes). Early lesions are distinguished by reactive hypertrophied vascular endothelium, reactive microglia and foci of swollen axons (spheroids). As

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injury progresses, hypertrophic lipid-laden macrophages accumulate. Organizing lesions are distinguished by mineralized axons and prominent reactive astrogliosis. Advanced lesions progress either to cavitation or to a solid glial scar and may be accompanied by reduction in white matter volume, secondary cyst formation or ventricular enlargement.

In the early stages of injury, diffuse PWMI is distinguished by the presence of numerous reactive microglia in the periventricular white matter.¹⁰ Reactive astrocytes are not a prominent feature of early lesions. The boundaries of diffuse lesions are characterized by a transition to regions enriched in resting/minimally reactive microglia. The primary population of degenerating cells within diffuse lesions are premyelinating oligodendroglia (ie, late oligodendrocyte progenitors [preOLs]) and immature oligodendrocytes.¹⁶ In contrast to PVL, diffuse PWMI is characterized by a marked depletion of premyelinating oligodendroglia that ranges from about 50% to 90%. Microglia, astrocytes and axons appear to be more resistant to injury. At later stages, diffuse PWMI lesions contain numerous reactive astrocytes (ie, diffuse gliosis).^{14,15} The injured cell types that provoke this gliosis remain unresolved, but one potential candidate is degenerating oligodendroglia. Consistent with this notion, advanced diffuse PWMI lesions are characterized by extensive myelin pallor and a reduction in immunohistochemical staining for myelin basic protein. Diffuse PWMI, thus, appears to be a milder form of injury than PVL that is initiated through targeted injury to the OL lineage with relative sparing of other glial and axonal elements.

Pathogenetic Mechanisms of Acute PWMI-Maturational Factors

Immature Autoregulation in Human PWMI

A complex interplay of factors related to cerebrovascular immaturity appears to predispose preterm human periventricular white matter to injury from ischemia. Ample evidence supports that the propensity for the premature neonate to exhibit a pressure-passive circulation is related to disturbances of cerebral autoregulation.^{21–23} Basal cerebral blood flow in healthy preterm neonates is markedly lower than in-term infants or adults.^{24–27} Basal flow to cerebral white matter was estimated to be <20% of gray matter.²⁸ However, direct experimental evidence that human periventricular white matter is selectively susceptible to ischemia is lacking. Current measures of global cerebral blood flow lack the spatial resolution to define cerebral hemodynamics in human periventricular white matter. By near infrared spectroscopy, impaired cerebrovascular autoregulation correlated with the development of PVL and germinal matrix-intraventricular hemorrhage.²⁹ With the advent of more sensitive modalities to identify the at-risk preterm neonate, it is clear that an understanding of the vascular basis of preterm white matter injury is needed and will require the application of new technologies in appropriate animal models (see below) to measure blood flow to defined regions of vulnerable white matter.

The Vascular End-Zone Hypothesis

The vascular-anatomic mechanisms that underlie the spectrum of pathology seen in PWMI is a central unresolved question. One attractive hypothesis relates to the presence of apparent vascular end zones in the periventricular white matter that are supplied by long or short penetrating arteries.^{30,31} Volpe proposed that, in the setting of a pressure passive circulation, these vascular end zones may be particularly susceptible to ischemia.⁸ This hypothesis proposes that deep-seated focal cystic-necrotic lesions of PVL arise from severe or persistent ischemia in vascular end zones of long penetrating arteries. The occurrence of less severe or briefer episodes of ischemia in the territory of more superficially situated short penetrating arteries may account for the more extensive myelination disturbances commonly associated with diffuse PWMI.

However, current measures of global cerebral blood flow lack the spatial resolution to define cerebral hemodynamics in human periventricular white matter. Prior studies have not established a relationship between PWMI and perturbations in periventricular blood flow. Small fetal and neonatal animal models have been uninformative because of a paucity of cerebral white matter, a propensity for mixed gray and white matter injury and the technical limitations of invasive blood flow measurements.³² Further, a broad spectrum of injury is seen after uniform ischemic insults within-species as well as differences in histopathology between-species in response to similar experimental conditions.

In effort to circumvent these limitations, we developed methods to quantify fetal cerebral blood flow in utero in the immature sheep fetus (0.65 gestation). The ovine fetus is a widely studied preparation that displays cerebral hemodynamics similar to humans and permits repeated physiological measurements in utero in the unanesthetized state.^{33,34} The immature ovine brain is also similar to preterm humans between 24 to 28 weeks in terms of the completion of neurogenesis, the onset of cerebral sulcation, the detection of the cortical component of the auditory and somatosensory evoked potentials, and an increased predilection for white matter injury.^{35–38} Thus, the cephalically hypotensive immature ovine fetus appears to be particularly susceptible to hypoxia-ischemia, but prior studies provided no data regarding blood flow to periventricular white matter.

We used a well-established global cerebral ischemia-reperfusion preparation in the instrumented 0.65 gestation fetal sheep where cerebral flow was interrupted by bilateral carotid occlusion.³⁶ We found that the duration of cerebral ischemia was a critical factor required to generate a graded spectrum of periventricular white matter injury.³⁴ Ischemia of 30- or 37-minute duration generated selective graded injury to frontal and parietal PVWM, 2 regions of predilection for human PWMI. When the duration of ischemia was increased to 45 minutes, white matter injury was accompanied by extensive damage to cortical and subcortical gray matter. Hence, selective white matter injury was lost with more prolonged ischemia. In fact, prolonged ischemia (>45 minutes) was associated with extensive cystic necrotic encephalomalacia, as seen in severe human PVL.^{39,40} Hence, the neuropathological similarities of our model to human PWMI

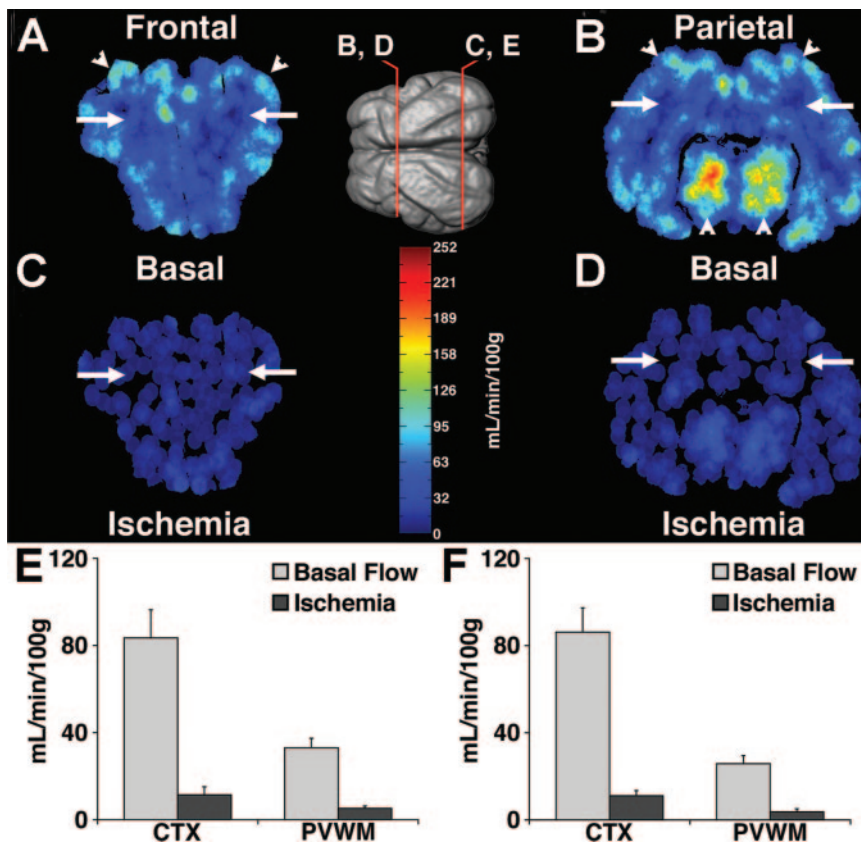


Figure 1. Quantification of fetal cerebral blood flow in situ under conditions of basal cerebral blood flow and global ischemia. A through D, The center image represents a 3-D surface reconstruction of magnetic resonance images of a 0.65 gestation ovine control brain that indicates the frontal and parietal levels at which the blood flow analysis was done in A through D. A and B, Representative pseudocolor basal flow images show higher blood flow (light blue) in cortical gray matter areas (arrowheads) and lower flow (dark blue) in the PVWM (arrows). The pons (B, double arrowheads) had higher basal flow rates than any region of the cerebrum. C and D, During global ischemia, blood flow was dramatically reduced in all regions and approached zero (dark blue/black) in the PVWM (arrows). E and F, Quantification of the change in blood flow (mL/min per 100 g) in the cortex (CTX) relative to the PVWM in both the frontal (E) and parietal (F) levels. Modified from Riddle et al.³⁰

supports the notion that human PVWM may be particularly vulnerable to global cerebral hypoperfusion.

To begin to address the vascular end-zone hypothesis, we asked whether gradients of ischemia could be detected between the cerebral cortex and the periventricular white matter. We developed a method to make repeated measurements of fetal cerebral blood flow in utero in the unanesthetized fetus.³⁴ We used fluorescently labeled microspheres to resolve the spatial heterogeneity of flow in situ in 3-dimensional space (Figure 1). Basal blood flow in both frontal (Figure 1A and 1E)) and parietal (Figure 1B and 1F) PVWM was $\approx 60\%$ to 70% lower than in the overlying cerebral cortex. During global cerebral ischemia (Figure 1C and 1D), induced by carotid occlusion, flow to all regions was reduced by nearly 90% . However, the absolute flow during ischemia (Figure 1E and 1F) was $\approx 50\%$ lower in frontal and parietal PVWM (5 ± 1 mL/min per 100 g) than in the corresponding cerebral cortex (10 ± 2 mL/min per 100 g). Hence, flow to the PVWM under ischemic conditions reaches a nadir that is lower than to cerebral cortex, despite a proportional fall in flow to both regions. As discussed below, cellular maturational factors related to susceptible oligodendrocyte progenitors likely play a greater role than vascular factors in defining the topography of injury.

Selective Vulnerability of PVWM: Cellular Factors

Maturation-Dependent Vulnerability of the Oligodendrocyte Lineage to Oxidative Stress

The last decade has seen considerable progress in the identification of the cellular targets that degenerate in PWMI

lesions in response to oxidative damage. Given that the major period of vulnerability for PWMI occurs before the onset of myelination, Volpe first proposed that the myelination disturbances of PWMI might arise from targeted death of OL progenitors that are the source of mature OLs.⁴¹ This hypothesis proposes that the predilection for PWMI is related to a developmentally regulated susceptibility of more immature stages of the OL lineage to oxidative stress, a well-established sequela of hypoxia-ischemia.⁴²

OLs develop according to a well-established lineage, defined by stage-specific antibodies specific for sequentially expressed OL cell-surface and myelin-specific epitopes^{43,44} (Figure 2). The successive OL stages are distinguished by a progressively more complex morphology. The OL progenitor is identified by staining for the platelet-derived growth factor- α receptor but not the O4 antibody. The preOL is a simple multipolar, mitotically active late OL progenitor identified with the O4 but not the O1 monoclonal antibodies. The immature OL is a postmitotic complex multipolar cell identified by the O1 antibody that binds to galactocerebroside. The mature OL is identified by myelin-associated markers that include myelin basic protein. It is, thus, feasible to precisely define the timing and features of OL lineage progression both in vitro and in vivo.

We found that the developmental window of highest risk for PWMI (ie, about 23 to 32 weeks postconceptional age) corresponded to a period in human white matter development that preceded the onset of myelination.⁴³ This period coincides with the presence of one major population of preOLs in cerebral white matter and identified the preOL as a potential target for injury in

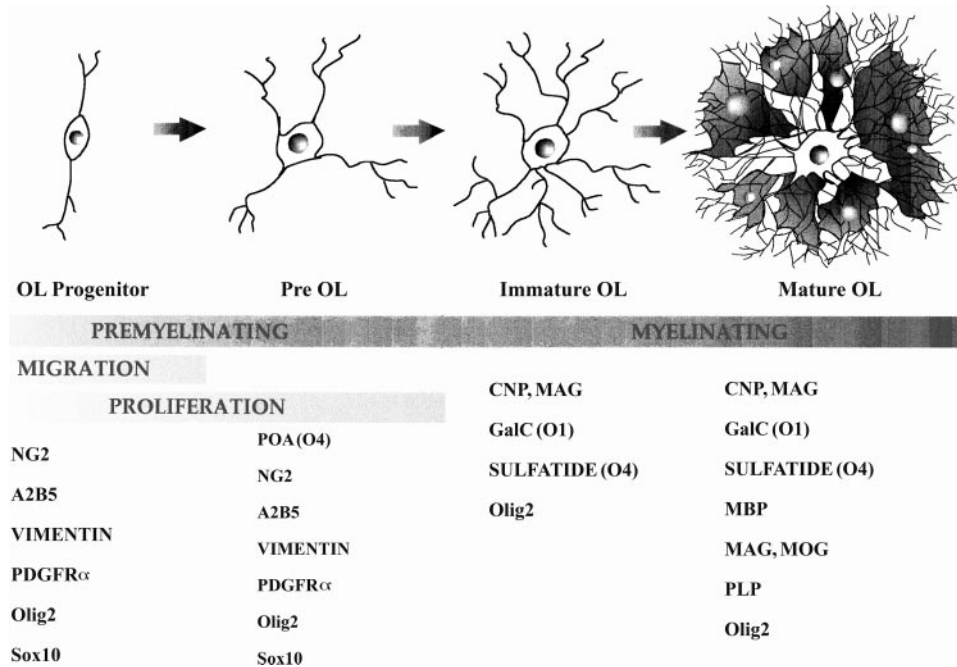


Figure 2. Maturation of the OL lineage. Four principal stages of OL lineage progression are depicted together with their corresponding morphological features and capacity for myelination, migration and proliferation. Each stage is uniquely defined by a combination of marker genes or antibodies. A2B5, O4, O1 refer to mouse monoclonal antibodies. *Olig2* and *Sox10* are genes that are highly enriched in premyelinating OLs. *Olig2* is also expressed at later stage in the OL lineage. CNP indicates CNPase, 2',3'-cyclic nucleotide-3'-phosphodiesterase; GalC, galactocerebroside; MAG, myelin-associated glycoprotein; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; NG2, chondroitin sulfate proteoglycan 4; PDGFR α , platelet-derived growth factor- α ; PLP, proteolipid protein.

PWMI. The decline in risk for PWMI coincides with the onset of a wave of differentiation of preOLs to immature OLs that first initiate myelination of periventricular white matter.⁴⁴

The concept that preOLs are selectively targeted by oxidative stress derives from several studies that have identified maturation-dependent mechanisms of free radical-mediated injury to the OL lineage both in vitro and in vivo.⁴⁵⁻⁵⁰ Many of these studies compared the susceptibility of successive stages in the OL lineage to oxidative stress. We initially demonstrated that preOLs (Figure 3A) are markedly more susceptible than mature OLs (Figure 3B) to intrinsic and extrinsic sources of oxidative stress (Figure 3C).⁵⁰ We defined an oxidative stress pathway in which intracellular depletion of glutathione triggered a downstream rise in reactive oxygen species that lead to preOL death. Several recent in vitro studies found that caspase-mediated death of OL progenitors occurs after oxidative stress in vitro.^{48,51,52} Interestingly, the E₂-isoprostanes, a lipid peroxidation product, are particularly toxic to OL progenitors in vitro, but not mature OLs, which suggests that specific compounds generated endogenously from oxidative stress might be a potential mechanism for OL degeneration in PWMI.⁵³ In early human PWMI cases, significant preOL degeneration was detected in white matter lesions that showed elevated levels of F₂-isoprostanes, a sensitive stable marker of oxidative damage.¹⁶

Role of OL Lineage Susceptibility in Hypoxic-Ischemic White Matter Injury

The timing of appearance and spatial distribution of susceptible OL lineage cells appears to explain the magnitude and distribution of ischemic white matter injury in several exper-

imental models of PWMI. This concept evolved from studies where we compared the relative susceptibility of the cerebral white matter of 2- and 7-day-old rat pups to a hypoxic-ischemic insult of similar severity.⁴⁶ We made the paradoxical observation that the white matter of the 7-day-old rat was markedly more resistant to hypoxia-ischemia than the 2-day-old rat. A major developmental difference at these 2 ages was the extent of differentiation of the OL lineage cells.⁵⁴ The white matter of the 2-day-old rat contains predominantly preOLs and, thus, resembles human periventricular white matter during the high-risk period for PWMI. By contrast, at 7 days of age, the white matter is populated mainly by a more differentiated population of OLs, and thus resembles near-term human. PreOLs were found to be highly susceptible to hypoxia-ischemia, whereas earlier and later OL stages were markedly more resistant.⁴⁶ The enhanced susceptibility of preOLs was, thus, a stage-specific property that was independent of the postnatal age of the animal or the location of these cells in the forebrain. The increasing developmental resistance of the cerebral white matter to hypoxia-ischemia was related to the onset of preOL differentiation to premyelinating oligodendrocytes (immature OLs). The immature OL, thus, displays reduced susceptibility to hypoxia-ischemia and a striking reactive response. Although reduced myelination has been reported after hypoxia-ischemia in the 7-day-old rat, the mechanism is unclear, because these studies did not determine the extent of preOL and OL degeneration.^{55,56}

Relative Contributions of Ischemia and OL Lineage Immaturity to White Matter Damage

We recently reported that the 0.65 gestation fetal sheep displays heterogeneous OL lineage maturation in frontal

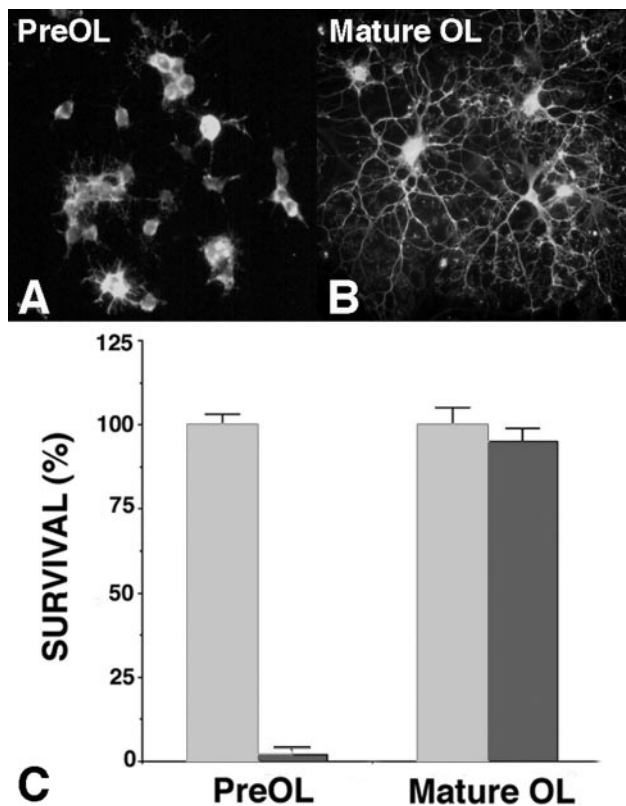


Figure 3. Maturation-dependent vulnerability of the OL lineage to oxidative stress. Fluorescent photomicrographs show that O4-antibody-labeled preOLs (A) in vitro display a simple multipolar morphology which is in striking contrast to mature OLs (B), stained for myelin basic protein, that display a complex arbor of processes. C, When subjected to conditions of oxidative stress that markedly depletes glutathione, preOLs are completely killed within 24 hours, whereas mature OLs are markedly more resistant. Light gray bars, control; dark gray bars, glutathione depletion induced by culture in medium that was depleted of cystine. Modified from Back et al.⁵⁰

periventricular white matter.³⁴ The heterogeneity of the frontal PVWM allowed us to study medial and lateral PVWM to define the relative contributions of oligodendroglial maturational factors and vascular factors on the pathogenesis of PVWM injury. Maturation of the OL lineage in the medial PVWM and parietal PVWM of the 0.65 gestation fetal sheep was similar to human preterm PVWM (≈ 23 to 28 weeks gestation) in that preOLs were the predominant OL stage present. By contrast, the lateral PVWM was more differentiated and mostly contained premyelinating and early myelinating immature OLs. Surprisingly, we found that severe ischemia did not uniformly damage the PVWM. We observed that the medial PVWM relative to the lateral PVWM contained nearly 3 times the number of acutely degenerating O4-labeled cells (Figure 4A) and nearly twice the depletion of O4-labeled cells 24 hours after moderate ischemia of 37 minutes duration that generates selective white matter injury (Figure 4B). Hence, the greater susceptibility of the medial PVWM was related to the predominance of susceptible preOLs in this region.

We then applied our in utero cerebral blood flow technique to the medial and lateral PVWM and found no difference in

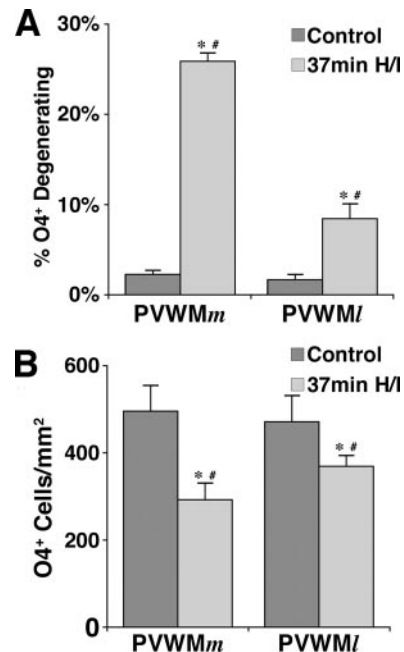


Figure 4. Differential injury in 2 adjacent regions of the ischemic PVWM, the medial PVWM (PVWM_m) and lateral PVWM (PVWM_l), coincides with the extent of preOL degeneration. A, The percentage of total OLs degenerating at 24 hours after ischemia. PVWM_m had a markedly higher ($26 \pm 1\%$) percentage of cells that degenerated than the PVWM_l ($8 \pm 2\%$). B, The total density of O4-labeled cells at 24 hours after ischemia relative to control in the PVWM_m and PVWM_l. A significant loss of O4-labeled cells of $\approx 40\%$ occurred in the PVWM_m, whereas the number of cells in the PVWM_l decreased by only $\approx 20\%$. Modified from Riddle et al.³⁰

blood flow before, during or at 2 time-points after occlusion.³⁴ The medial and lateral PVWM thus sustained differing degrees of acute injury even though they sustained a similar degree of low flow during a prolonged severe ischemia-reperfusion insult. Hence, whereas global ischemia was necessary for white matter injury, no regional differences in blood flow were found within the PVWM under any conditions to account for the disparate rates of cellular degeneration in medial and lateral PVWM.

Rather, differences in the topography of PVWM injury were related primarily to the maturational state of the OL lineage rather than to heterogeneity of PVWM flow. The predilection for PVWM injury closely correlated with the distribution of vulnerable preOLs. In fact, almost all cell degeneration seen in the PVWM lesions was accounted for by OL lineage degeneration. Interestingly, in regions of preOL degeneration, other neural cell types (astrocytes, microglia and axons) were markedly more resistant to injury.

Taken together, these findings suggest that perturbations in cerebral blood flow are necessary but not sufficient to damage the periventricular white matter. The developmental predilection for PWMI to occur during prematurity appears to be related to both the timing of appearance and regional distribution of susceptible preOLs. These findings predict that some near-term infants with delayed OL differentiation and myelination also could be more susceptible to PWMI. Interestingly, a more variable degree of white matter injury was

detected in near-term sheep after several insults.^{35,37,38,57–59} Hence, it appears that targeted death of preOLs could contribute to the pathogenesis of PWMI across a broad range of gestational ages and in multiple susceptible regions.

Conclusions

Considerable progress has been made in identifying maturation-dependent cellular and molecular mechanisms of PWMI that underlie the increased predilection of the developing cerebral white matter to injury. The propensity for PWMI to occur during prematurity appears to be related to both the timing of appearance and regional distribution of susceptible oligodendrocyte progenitors. The identification of these cells as susceptible targets in ischemic white matter provides a rationale for the development of preventive therapies aimed at promoting the survival and maturation of oligodendrocyte progenitors. As our understanding of PWMI pathogenesis improves, it is anticipated that new strategies for directly preventing brain injury in premature infants will evolve.

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Disclosures

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