**PERSPECTIVE**

**Pathophysiology of periventricular leukomalacia: what we learned from animal models**

Periventricular leukomalacia (PVL), a white matter injury (WMI) affecting the premature infant's brain is commonly associated with cerebrospinal fluid (CSF). Among premature infants <1,500 g, approximately 7,000 develop CP yearly and 20,000–30,000 exhibit major cognitive deficits yearly (Volpe, 2009). PVL results from hypoxia-ischemia (HI) with or without infection and is characterized by white matter necrotic lesions, hypomyelination, microglial activation, astroglialis, and neuronal death. Risk factors for the development of PVL include: prematurity associated with immature cerebrovascular development, HI insults with lack of appropriate last compensatory response rather than blood flow, free radical production, energy deprivation, intrauterine infection and chorioamnionitis. Affected infants show definitive signs of cerebral palsy such as spastic diplegia, seizures, developmental delay, visual and hearing impairment, scoliosis and incontinence by 6–9 months of age. PVL can also occur in term infants with certain congenital cyanotic heart disease which will not be our focus here (Volpe, 2001).

**Timing of PVL and developmental sequelae:** PVL is most common between 23–30 weeks gestation. After 24 weeks, there is axon and dendrite formation, differentiation, synaptogenesis, myelination, and synaptic pruning and development of circuitry. This developmental process persists up to 2 years after birth. Therefore, any injury in this stage will lead to developmental disturbance (Volpe, 2009).

**Etiology of PVL:** There are two major etiologies of PVL: HI and infection/inflammation causing a fetal inflammatory response. Many factors play a pivotal role in the pathogenesis of PVL and increase preterm neonate susceptibility to develop HI, including arterial end and border zones in the periventricular white matter, pressure-passive circulation without autoregulation and the susceptibility of premyelinated oligodendrocytes (pre-OLs) to damage. This event is characterized by reactive oxidative species (ROS) and nitric oxide (NO) causing glutamate release in the white matter and edema (Volpe, 2009). Intrauterine infections and perinatal hypoxia-ischemia affect the premature infant’s brain is commonly associated with cell death and inflammation. Histological evaluation of human autopsy brains has shown the presence of diffuse white matter gliosis caused by astrogliosis and microgliosis. Diffuse white matter gliosis is more prevalent in premature infants with IVH and worse outcomes (Volpe, 2009). The development of PVL is characterized by white matter necrosis cystic (>5 mm), focal necrosis non-cystic (2–3 mm) and diffuse microscopic necrosis (Volpe, 2017). This is a result of hypoxic/ischemic injury leading to a decrease in white matter thickness leading to ventricularomegaly. Diffuse type is also associated with astroglialis and microgliosis. Anatomically, diffuse lesions occur in the distributions of the border zones between the long penetrating arteries and the end zones of the short penetrating arteries. Both types of lesions have an increase in pro-inflammatory cytokines mainly tumor necrosis factor-a, interleukin-1β, interleukin 6, interleukin-8 and interleukin-9 (Volpe, 2009).

**Diagnosis and management of WMI:** PVL can be diagnosed by US and MRI. US can easily diagnose focal cystic, occasionally diagnose non-cystic lesions while maybe unable to detect diffuse lesions. High-resolution MRI with diffusion tensor imaging (DTI) can easily diagnose diffuse PVL. They can even diagnose the mildest form of WMI which is diffuse white matter gliosis caused by astroglialis and microgliosis. No current therapy can reverse or ameliorate PVL. Only preventative measures are available as antenatal steroids, treating hypotension, hypoxia and infections. Supportive therapy including early intervention, physical, occupational therapy and access to tertiary centers for the management of disabilities is of utmost importance. Life expectancy is variable ranging from only a few months to a full life expectancy. This is dependent on number of key disabilities and their severity, as well as mobility, feeding, respiratory and cognitive functioning.

**Spread of WMI:** WMI in infants with PVL can be widespread to the periventricular, subcortical and callosal white matters, and internal capsule. Corpus callosum containing commissural myelinated fibers is impaired in PVL. Injury to the corticospinal tracts in PVL leads to motor deficits, including CP. Axonal injury is frequently seen as axons are susceptible to HI and due to failure of axonal ensheathment by the pre-OLs. Injury to the grey matter areas like thalamus and basal ganglia can also be seen. Selective subplate neuronal injury/death, leads to abnormal cortical development and impaired plasticity. Later migrating GABAergic neurons undergo apoptosis and HI leading to a decrease in subcortical and upper cortical neurons (Volpe, 2009).

**Microglial role:** Microglia plays a key role during brain development, involving apoptosis, synaptic pruning, vascularization, axonal development, and myelination. In utero, microglial number peaks in the white matter in the third trimester. Microglia activation causes the release of pro-inflammatory cytokines and free radicals leading to pre-OLs loss and maturation arrest. Pre-OLs are vulnerable to free radical, whereas the mature OLs are more resistant. Free radicals are both a cause and a result of inflammation. Iron is a main source of free radicals. There is active acquisition of iron during OL differentiation. This mechanism explains why PVL is commonly seen with intraventricular hemorrhage (IVH) (Tahraoui et al., 2001).

**Regeneration after WMI:** It is believed that persistent inflammation and possibly epigenetic modifications including phosphorylation, ubiquitination and acetylation of histones and methylation of DNA and RNA can last for years after the initial injury. These modifications cause added injury, prevent regeneration and affect plasticity. On the other hand, neuronal replenishment in the cerebral white matter in PVL has been shown in animal models evidenced by increased migration of immature neurons (Volpe, 2009). In the sub-ventricular zone (SVZ) to distant sites of injury with neurogenesis persisting for months after the initial insult. In human autopsy brains, doublecortin (DCC), a marker of post mitotic migrating neurons; was significantly increased in PVL cases compared to controls. It is believed that neuronal progenitors in the human SVZ respond to injury by differentiation and migration outward but without proliferation (Haynes et al., 2011).

**Modeling PVL:** Currently, there is no available treatment for this devastating injury. All animal models must show a convincing relevance to human lesion. There are 3 main categories of animal models than can induce PVL: Antenatal or postnatal HI surgery, induction of systemic inflammatory response through administration of bacteria or LPS and lastly using N-methyl-D-aspartate (NMDA) and non-NMDA receptor agonists causing excitotoxicity (Choi et al., 2011).

**HI model of PVL:** Rice-Vannucci HI model (in which 7-day-old rat pups undergo unilateral ligation of the common carotid artery followed...
by exposure to 8% oxygen) is a model of HI which contributed significantly to studying brain injury in neonates. Many modifications to this model have been made to produce selective grey or white matter injury to HI model in the term and the preterm infant. Our HI injury mouse model (which involves temporary bilateral carotid artery ligation at postnatal day (P) 5 followed by 20 minutes of hypoxia 8%, (P5 corresponds to gestational age (GA) 24–30 for white matter maturation), that mimics human PVL in several important ways, including hind or lower limb paresis, incoordination and initial failure to thrive due to lack of adequate feeding. Our model also mimics human PVL histologically in the form of ventriculomegaly due to white matter loss from decreased OLs numbers as well as OLs maturational arrest. We showed, in our model, a significant astrogliosis and microgliosis in the periventricular white matter similar to human PVL pathological changes. At the molecular level, we showed in our model that activation of nuclear factor kappa B pathway; along with a significant increase of pro-inflammatory cytokines; leads to an increase in the number of activated microglia (M1 phenotype) and a reduction of repair microglia (M2 phenotype). Nitric oxide produced by activated microglia causes direct injury to the pre-OLs along with brain hypoperfusion (Zaghloul et al., 2017). Ability to use this HI mouse model in transgenic animals can lead to a deeper understanding of the pathophysiology of PVL and provide numerous preventive/therapeutic options which are enhanced by the long term survival of this model.

**Conclusion:** PVL is a complex combination of destructive and developmental disturbances (Figure 1). Pre-OL injury appears to be the first step in PVL. Currently, MRI/IMRI is successfully used to delineate the extent of the injury and predict the prognosis. Clinically relevant models will enable us to test and provide promising preventive/therapeutic strategies in rodent which eventually can be tested in our neonates with a goal to improve outcome and decrease or alleviate the morbidity of this devastating disease.

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