Life Sciences 94 (2014) 164–171

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Perinatal inflammation results in decreased oligodendrocyte numbers in adulthood

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ARTICLE INFO

Article history:
Received 5 September 2013
Accepted 12 November 2013

Keywords:
Lipopolysaccharide
Inflammation
Hyperoxia
Oligodendrocyte
Microglia
White matter injury
Premature brain injury
Development
Periventricular leukomalacia

ABSTRACT

Aims: Maternal inflammation is a risk factor for preterm birth, and premature infants are often exposed to supplemental oxygen as a life-sustaining therapy. While more immature neonates are surviving, rates of neurodevelopmental impairment are not improving. We developed a novel mouse model with clinically relevant exposures to test the hypothesis that systemic maternal inflammation with transient neonatal hyperoxia exposure will induce a phenotype similar to diffuse periventricular leukomalacia (PVL) like that observed in premature human infants.

Main methods: Timed-pregnant C3H/HeN mice received intraperitoneal injections of lipopolysaccharide (LPS) or saline on embryonic day 16. Newborn pups were placed in room air (RA) or 85% oxygen (O_2) for 14 days, followed by 14 days in RA recovery. Oligodendroglial and microglial populations were evaluated at 14 and 28 days.

Key findings: Brain weight to body weight ratios were lower in mice exposed to LPS. Oligodendrocyte numbers were decreased significantly in the cerebral cortex and hippocampus in groups exposed to LPS or LPS/O_2 at 14 days, and persisted in the cerebral cortex at 28 days for LPS/O_2 mice. At day 14, cleaved caspase 3 was increased and numbers of microglia were elevated in the cerebral cortex and hippocampus of LPS/O_2 animals.

Significance: These data indicate that combining systemic maternal LPS and neonatal hyperoxic exposure impairs myelination, and suggests that this novel mouse model may represent a subtle, diffuse form of periventricular white matter injury that could provide a clinically relevant platform for further study of perinatal brain injury.

Introduction

Advances in neonatal care have resulted in smaller and more immature babies surviving the perinatal period. However, adverse neurologic outcomes remain a significant consequence of premature birth, with as many as 25–50% of very low birth weight (VLBW) babies, born weighing less than 1500 g, experiencing some degree of neurodevelopmental impairment (Robertson et al., 2007; Volpe, 2009a; Allen, 2008). Historically, cerebral palsy was a major defining factor of adverse neurologic outcomes from the perinatal period. However, it is increasingly recognized that long term impairments related to brain injury in preterm neonates represent a spectrum of type and severity, involving mechanisms outside of classic intraventricular hemorrhage or cystic periventricular leukomalacia (PVL) (Back, 2006; Volpe, 2009b). This shift is encapsulated by descriptions of non-cystic PVL, characterized by diffuse periventricular white matter injury in the cerebrum involving a constellation of damage to vulnerable premyelinating oligodendrocytes, microglial activation, astrogliosis, and neuronal injury (Back, 2006; Volpe et al., 2011; Volpe, 2011).

Translational science including investigation of animal models is necessary to understand the mechanisms and develop preventive and treatment strategies for perinatal brain injury in preterm infants (Kinney and Volpe, 2012). Such animal models need to show brain pathology consistent with that seen in human neonates and should be induced by clinically relevant stimuli. While existing animal models have studied the impact of neonatal hypoxia–ischemia, chorioamnionitis, neonatal infection, and neonatal hyperoxia on brain development and injury (Burd et al., 2012; Boksa, 2010; Back et al., 2002; Hagberg et al., 2002), the effects of combined exposures have been reported only recently (Brehmer et al., 2012).

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0024-3205/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.lfs.2013.11.015

Maternal inflammation and infection are risk factors for both preterm birth and subsequent cerebral palsy (Burd et al., 2012; Horvath et al., 2012; Soraisham et al., 2013). Systemic maternal inflammation in the absence of chorioamnionitis or other infection is commonly seen in mothers with chronic disease, obesity, preeclampsia, and diabetes (Schmatz et al., 2010). Animal studies have demonstrated that multiple infectious and inflammatory stimuli may induce a range of effects on the developing brain (Rousset et al., 2006, 2008; Debillon et al., 2000; Normann et al., 2009). Premature neonates commonly require ventilator support and supplemental oxygen therapy at a time when decreased antioxidant capacities make them most vulnerable to oxidative stress. Injurious effects of hyperoxic conditions on the developing brain have been reported in existing animal studies (Gerstner et al., 2008; Yis et al., 2008; Ramani et al., 2013; Zaghloul et al., 2012; Sifringer et al., 2012).

In this study, we investigated a novel mouse model of maternal systemic lipopolysaccharide (LPS) administration followed by transient neonatal hyperoxia exposure. Prior work in our laboratory indicates that this model is well suited to examining the effects of neonatal inflammation on developing systems. We have observed an inflammatory lung phenotype similar to severe bronchopulmonary dysplasia (Velten et al., 2010, 2012; Rogers et al., 2009) as well as significant functional and structural alterations in cardiac development that persist into adulthood (Velten et al., 2011).

The combination of systemic maternal inflammation and neonatal hyperoxia has overwhelming potential for injury in the neonatal brain during a critical developmental period. However, the neuropathology after these combined exposures has not been elucidated, even though it is one of the most common courses experienced by VLBW infants in Neonatal Intensive Care Units worldwide. Our hypothesis was that the combination of systemic maternal inflammation and transient neonatal hyperoxia exposure would result in patterns of injury consistent with pathologic changes observed in preterm infants.

Materials and methods

Animal model

Animal study protocols were approved by the Institutional Animal Care and Use Committee at the Research Institute at Nationwide Children's Hospital, Columbus, OH. Adult C3H/HeN mice (8–10 weeks old) were purchased from Harlan Sprague Dawley (Indianapolis, IN). Mice were housed in our facility for at least 7 days prior to breeding. Male and females were paired, and the presence of a vaginal plug was designated as embryonic day 1 (E1). On E16, dams received intraperitoneal injections of LPS (serotype 0111:B4, catalog no. 437627; Calbiochem, Gibbstown, NJ) in saline (approximately 0.1 mL), or saline only. The LPS dose (80 μg/kg) was chosen based on preliminary studies that consistently resulted in a viable litter (Velten et al., 2010). Newborn mice from saline- or LPS-injected dams were pooled and redistributed randomly (yielding mixed litters of 6 to 7 pups) to the two dams in separate cages within 24 h of birth. One litter of pups was exposed to 85% oxygen (O_2) for 14 days in a Plexiglas chamber which was regularly calibrated (chamber calibrated every other day with an Oxygen Analyzer, Hudson RCI, model 5577) while the litter of pups from the corresponding maternal E16 treatment was maintained in room air (RA) for the same period. Day 1 was defined as the first 24 h of O_2 or RA exposure. Litters were either sacrificed at day 14 (after hyperoxia exposure) or maintained in RA for 14 days of recovery and sacrificed at day 28. In this fashion, four treatment groups were created: saline/RA, saline/O_2, LPS/RA, and LPS/O_2. To avoid oxygen toxicity in the dams and reduce confounding maternal effects between groups, the nursing dams from the same E16 treatment groups were rotated between their RA and O_2 litters every 24 h.

Histology processing

At days 14 and 28, two animals from each litter were anesthetized with intraperitoneal administration of ketamine/xylazine (150 mg/kg:15 mg/kg, respectively). Whole-body perfusion was performed with ice cold saline followed by freshly prepared ice cold neutral buffered 4% paraformaldehyde. Brains were removed immediately and immersed in fresh fixative for 24 h, then washed 3× in phosphate buffered saline (PBS) and transferred into PBS until processed and embedded into paraffin blocks. Coronal sections cut serially at 4 μm were placed on positively charged slides and then either stained with hematoxylin and eosin to evaluate general tissue architecture or labeled by indirect immunohistochemistry to examine the distribution of selected cell populations (Table 1). All immunohistochemical methods were visualized with a polymer detection system and the chromogen 3,3′-diaminobenzidene (DAB; kit DS9800; Leica Microsystems, Buffalo Grove, IL). Sections were counterstained with hematoxylin.

Glial cell counts

For both days 14 and 28, oligodendrocyte and microglial counts were performed on selected brain regions (cerebral cortex, hippocampus) of the middle cerebrum at the level of the internal capsule and thalamus. Intracortical oligodendrocytes, defined by their immunoreactivity with 2′, 3′ cyclic nucleotide 3′ phosphodiesterase (CNPase), and microglia, identified by their immunoreactivity with ionizing calcium-binding adaptor molecule 1 (Iba1), were counted between the cingulum and rhinal fissure in four non-overlapping high-power fields (i.e., 100×). Additionally, tissue counts were performed on hippocampal neurons within the dentate gyrus in two non-overlapping high-power fields. Microglial cell bodies were also quantified in four non-overlapping high-power fields in the cerebral cortex and hippocampus. All counts were performed by a single investigator (oligodendrocytes by KMH, microglia by AEG) for consistency.

Caspase 3 expression

Whole brains were extracted from randomly selected pups (2 per litter) at postnatal days 3, 7, and 14. Whole brains were selected as the sample due to the small size of the brains at the early time points,

Table 1
Antibodies for immunohistochemistry analyses.

Antibody	Clone	Dilution	Antigen retrieval	Catalog #	Supplier
CNPase (oligodendrocytes)	11-5B mouse anti-human	1:800	Citrate	ab6319	Abcam, Cambridge, MA
MBP (oligodendrocytes, myelin)	7H11 mouse anti-human	1:200	Citrate	NCL-MBP	Leica Microsystems, Buffalo Grove IL
Iba-1 (microglia, macrophages)	Rabbit anti-human polyclonal	1:1000	EDTA	PP290-AA	Biocare Medical, Concord, CA
GFAP (reactive astrocytes)	Rabbit anti-guinea pig polyclonal	Predilute	None	RB-087-R7	Thermo LabVision, Kalamazoo MI

All antibodies were diluted with Antibody Diluent, catalog# S3022, Dako, Carpentaria CA. Abbreviations: CNPase—2′, 3′ cyclic nucleotide 3′ phosphodiesterase; MBP—myelin basic protein; Iba-1—ionizing calcium-binding adaptor molecule 1; GFAP—glial fibrillary acidic protein.