Brain, Behavior, and Immunity xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Full-length Article

Postpartum increases in cerebral edema and inflammation in response to placental ischemia during pregnancy

Ahsia M. Clayton [a], Qingmei Shao [a], Nina D. Paauw [b], Ashtin B. Giambrone [a], Joey P. Granger [c], Junie P. Warrington [a,*]

[a] Department of Neurology, University of Mississippi Medical Center, Jackson, MS 39216, United States
[b] Department of Obstetrics, Wilhelmina Children's Hospital Birth Center, University Medical Center Utrecht, Utrecht, The Netherlands
[c] Department of Physiology & Biophysics, University of Mississippi Medical Center, Jackson, MS 39216, United States

ARTICLE INFO

Article history:
Received 27 October 2017
Received in revised form 15 March 2018
Accepted 23 March 2018
Available online xxxx

Keywords:
Cerebral edema
Postpartum
Preeclampsia
Cerebral inflammation
Tight junction proteins
Neuroglia
Astrocyte
Microglia
Blood-brain barrier

ABSTRACT

Reduced placental blood flow results in placental ischemia, an initiating event in the pathophysiology of preeclampsia, a hypertensive pregnancy disorder. While studies show increased mortality risk from Alzheimer's disease, stroke, and cerebrovascular complications in women with a history of preeclampsia, the underlying mechanisms are unknown. During pregnancy, placental ischemia, induced by reducing uterine perfusion pressure (RUPP), leads to cerebral edema and increased blood-brain barrier (BBB) permeability; however whether these complications persist after delivery is not known. Therefore, we tested the hypothesis that placental ischemia contributes to postpartum cerebral edema and neuroinflammation. On gestational day 14, time-pregnant Sprague Dawley rats underwent Sham (n = 10) or RUPP (n = 9) surgery and brain tissue collected 2 months post-delivery. Water content increased in posterior cortex but not hippocampus, striatum, or anterior cerebrum following RUPP. Using a rat cytokine multi-plex kit, posterior cortical IL-17, IL-1α, IL-1β, Leptin, and MIP2 increased while hippocampal IL-4, IL-12(p70) and RANTES increased and IL-18 decreased following RUPP. Western blot analysis showed no changes in astrocyte marker, Glial Fibrillary Acidic Protein (GFAP); however, the microglia marker, ionized calcium binding adaptor molecule (Iba1) tended to increase in hippocampus of RUPP-exposed rats. Immunofluorescence staining revealed reduced number of posterior cortical microglia but increased activated (Type 4) microglia in RUPP. Astrocyte number increased in both regions but area covered by astrocytes increased only in posterior cortex following RUPP. BBB-associated proteins, Claudin-1, Aquaporin-4, and zonular occludens-1 expression were unaltered; however, posterior cortical occludin decreased. These results suggest that 2 months postpartum, neuroinflammation, along with decreased occludin expression, may partly explain posterior cortical edema in rats with history of placental ischemia.

1. Introduction

Preeclampsia, a hypertensive disorder of pregnancy, is often associated with reduced utero-placental perfusion and placental ischemia. Following delivery of the fetus and placenta, blood pressure returns to normal for most patients, and other symptoms resolve, making delivery the only "treatment strategy" for preeclampsia patients. There is strong evidence that a preeclampsia-complicated pregnancy predisposes women to increased cardiovascular and cerebrovascular risk (Brown et al., 2013). Indeed, women with a history of a preeclampsia-complicated pregnancy have increased risk of mortality from Alzheimer's disease, stroke, and cerebrovascular complications (Theilen et al., 2016). While some potential mechanisms contributing to cerebrovascular complication during preeclampsia and placental ischemia have been identified, the mechanisms underlying the increased risk for postpartum cerebrovascular complications later in life are not well understood.

One potential mechanism could be increased cerebral tissue inflammation. During pregnancy, preeclampsia patients present with increased circulating cytokines such as TNFα (LaMarca et al., 2005; Kalantar et al., 2013; Lau et al., 2013; Zhou et al., 2012), IL-17 (Darmochwal-Kolarz et al., 2012; Martínez-García et al., 2011; Toldi et al., 2011), IL-18 (El-Kabarity and Naguib, 2011; Huang et al., 2005; Seol et al., 2009), interferon gamma (Ozkan et al., 2014; Yang et al., 2014), IL-6 (Gadonski et al.,

* Corresponding author at: Department of Neurology, University of Mississippi Medical Center, 2500 N. State Street, Jackson, MS 39216, United States.
E-mail address: jpwarrington@umc.edu (J.P. Warrington).

https://doi.org/10.1016/j.bbi.2018.03.028
0889-1591/

2006), and other cytokines/chemokines, leading to the idea that preeclampsia is an inflammatory disorder (Pinheiro et al., 2013). There is significant evidence that placental ischemia is also associated with increased circulating cytokines (Gadonski et al., 2006; LaMarca et al., 2011; LaMarca et al., 2005), suggesting that the ischemic placenta is the primary source of the circulating cytokines. Our laboratory recently demonstrated that the rat model of placental ischemia displays increased levels of cerebrospinal fluid cytokines during pregnancy (Warrington, 2015); however, whether this increased inflammatory environment persists after delivery and whether cerebral tissue cytokine levels are increased in the postpartum period following exposure to placental ischemia are not known.

Both inflammation and placental ischemia have been shown to contribute to cerebral edema (Warrington et al., 2015; Warrington et al., 2014) during pregnancy, a consequence of impaired cerebrovascular function. Magnetic Resonance Imaging (MRI) and other imaging methods reveal that preeclampsia patients present with abnormalities consistent with edema and blood–brain barrier (BBB) leakage during pregnancy (Matsuda et al., 2005). Moreover, several studies have reported the presence of white matter lesions in previously preeclamptic women (Aukes et al., 2012; Siepmann et al., 2017; Wiegman et al., 2014). Nevertheless, it is not known whether features of edema persist in the postpartum period following placental ischemia or whether cerebral edema occurs along with cerebral tissue inflammation.

We utilize the rat placental ischemia model, a well-characterized rodent model of preeclampsia, sharing numerous characteristics as the preeclampsia patient. Reduced uterine perfusion pressure (RUPP) is induced on gestational day 14, the start of the third trimester in the rat, to mimic the clinical condition of preeclampsia which is diagnosed after the second half of pregnancy. Like preeclampsia patients, RUPP rats develop hypertension, have increased circulating factors such as inflammatory cytokines TNFα (LaMarca et al., 2005) and interleukin-6 (Gadonski et al., 2006) and anti-angiogenic factors soluble endoglin (Gilbert et al., 2009) and soluble Fms-like tyrosine kinase -1 (sFlt) (Gilbert et al., 2007), proteinuria, and cerebrovascular abnormalities towards the end of pregnancy. Thus, the RUPP model is ideal for assessing underlying pathophysiological mechanisms underlying preeclampsia associated with placental ischemia.

While studies have begun to identify potential mechanisms underlying cerebrovascular abnormalities in response to placental ischemia during pregnancy, studies in the postpartum period are lacking. Therefore, we assessed cerebral changes in rats subjected to placental ischemia at two months postpartum for two (2) major reasons. First, a recent study (Paauw et al., 2017), from which the brains used in the current study were obtained, showed that at 2 months postpartum, rats subjected to placental ischemia have reduced renal and cardiac function, suggesting that other organs such as brain may be affected as well. Secondly, several clinical studies report white matter lesions and other subtle cognitive deficits in women with a history of preeclampsia as early as 4–9 years postpartum (Aukes et al., 2012; Postma et al., 2014, 2016; Siepmann et al., 2017), which according to (Sengupta, 2013) is equivalent to 1.3–3 months in the rat.

In this study, we tested the hypothesis that exposure to gestational placental ischemia contributes to increased cytokines/chemokines and water content in specific brain regions during the postpartum period. To test this hypothesis, we used the rat model of placental ischemia and measured changes in water content in the anterior cerebrum, striatum, hippocampus, and posterior cortex at 2 months postpartum. We also measured posterior cortical and hippocampal levels of cytokine/chemokines and determined whether increases in brain water content was associated with changes in expression of tight junction proteins, neuroglia markers, or astrocyte and microglia activation at 2 months postpartum in rats exposed to sham or placental ischemic pregnancy.

2. Materials and methods

2.1. Animals

A subset of Sprague Dawley rats, randomly selected from the larger study used in (Paauw et al., 2017) were used (10 Shams and 9 RUPP). Only rats with brain samples collected for brain water content, protein analysis, and/or immunofluorescence analysis are included in this study. Time-pregnant rats arrived from Harlan Laboratories (Indianapolis, IN) at gestational day 10 or 11 and housed in the Lab Animal Facilities at the University of Mississippi Medical Center and maintained on a 12 h light, 12 h dark cycle with continuous access to food and water. All animal protocols were approved by the Institutional Animal Care and Use Committee at UMMC before experiments were performed.

2.2. Induction of Placental Ischemia (Reduced Uterine Perfusion Pressure, RUPP)

On gestational day (GD)14, pregnant rats were anesthetized using isoflurane (3% induction, 2.5% maintenance). Silver clips were placed on the abdominal aorta (0.203 mm) and on both branches of the uterine arteries between the ovaries and the first pup (0.103 mm). Sham surgery involved the exteriorization of the uterine horn containing the pups with no placement of clips. All rats received Carprofen (5 mg/kg) as a pre-operative analgesic. Following delivery, pups were removed from the dams within 24 h to avoid lactation effects.

2.3. Implantation of Carotid Catheters and Blood pressure recording

At 8 weeks postpartum, rats were instrumented with carotid catheters. The following day, rats were placed in restrainer cages and blood pressure was determined after 60 min acclimation using Lab Chart software and PowerLab pressure transducers (AdInstruments, Colorado Springs, CO). Blood pressure was recorded for 30 min. Two rats (1 from each group) underwent surgery for the placement of carotid catheters on GD18 followed by blood pressure recording on GD19. These 2 rats did not undergo additional surgery for blood pressure monitoring at 8 weeks postpartum.

2.4. Urine collection and measurement of urinary albumin

Twenty four hour urine was collected from rats at 8 weeks postpartum. Albumin concentration was measured using Exocell Nephrat kit (Philadelphia, PA) and multiplied by the volume of urine excreted over 24 h.

2.5. Dissection of Brain Regions for brain water content and homogenization

Brains were removed, cerebellum and brainstem dissected and discarded. The remaining cerebrum was then hemisected. Brains to be used for water content and homogenization (n = 5 per group) had both hemispheres further dissected to obtain the following regions: anterior cerebrum, hippocampus, striatum, and posterior cortex. Fig. 1A depicts the landmarks used for dissection of brain regions. Briefly, each hemisphere was cut along the most lateral portion of the middle cerebral artery to separate into anterior (Fig. 1B) and posterior cerebrum (Fig. 1C). The posterior cerebrum (Fig. 1D) was then further dissected by removing the striatum (Fig. 1E), and carefully peeling off the hippocampus (Fig. 1G). The

Please cite this article in press as: Clayton, A.M., et al. Postpartum increases in cerebral edema and inflammation in response to placental ischemia during pregnancy. Brain Behav. Immun. (2018), https://doi.org/10.1016/j.bbi.2018.03.028