Brain, Behavior, and Immunity 63 (2017) 186–196

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Special Issue on Perinatal Inflammation

Maternal obesity increases inflammation and exacerbates damage following neonatal hypoxic-ischaemic brain injury in rats

Jonathan D. Teo, Margaret J. Morris, Nicole M. Jones *

Department of Pharmacology, School of Medical Sciences, UNSW Australia, New South Wales, Australia

ARTICLE INFO

Article history:
Received 13 May 2016
Received in revised form 11 October 2016
Accepted 12 October 2016
Available online 13 October 2016

Keywords:
Hypoxia-ischaemia
Microglia
Astrocytes
Inflammation
Brain
Behaviour
Neurons
Maternal obesity

ABSTRACT

Objective: In humans, maternal obesity is associated with an increase in the incidence of birth related difficulties. However, the impact of maternal obesity on the severity of brain injury in offspring is not known. Recent studies have found evidence of increased glial response and inflammatory mediators in the brains as a result of obesity in humans and rodents. We hypothesised that hypoxic-ischaemic (HI) brain injury is greater in neonatal offspring from obese rat mothers compared to lean controls.

Methods: Female Sprague Dawley rats were randomly allocated to high fat (HFD, n = 8) or chow (n = 4) diet and mated with lean male rats. On postnatal day 7 (P7), male and female pups were randomly assigned to HI injury or control (C) groups. HI injury was induced by occlusion of the right carotid artery followed by 3 h exposure to 8% oxygen, at 37 °C. Control pups were removed from the mother for the same duration under ambient conditions. Righting behaviour was measured on day 1 and 7 following HI. The extent of brain injury was quantified in brain sections from P14 pups using cresyl violet staining and the difference in volume between brain hemispheres was measured.

Results: Before mating, HFD mothers were 11% heavier than Chow mothers ($p < 0.05$, *t*-test). Righting reflex was delayed in offspring from HFD-fed mothers compared to the Chow mothers. The Chow-HI pups showed a loss in ipsilateral brain tissue, while the HFD-HI group had significantly greater loss. No significant difference was detected in brain volume between the HFD-C and Chow-C pups. When analysed on a per litter basis, the size of the injury was significantly correlated with maternal weight. Similar observations were made with neuronal staining showing a greater loss of neurons in the brain of offspring from HFD-mothers following HI compared to Chow. Astrocytes appeared to more hypertrophic and a greater number of microglia were present in the injured hemisphere in offspring from mothers on HFD. HI caused an increase in the proportion of amoeboid microglia and exposure to maternal HFD exacerbated this response. In the contralateral hemisphere, offspring exposed to maternal HFD displayed a reduced proportion of ramified microglia.

Conclusions: Our data clearly demonstrate that maternal obesity can exacerbate the severity of brain damage caused by HI in neonatal offspring. Given that previous studies have shown enhanced inflammatory responses in offspring of obese mothers, these factors including gliosis and microglial infiltration are likely to contribute to enhanced brain injury.

1. Introduction

Maternal obesity during pregnancy has increased at an alarming rate over the past decades with the current global estimate of overweight and obese women being 40% and 15% of the global female population, respectively (WHO, 2015). There is overwhelming evidence that with greater maternal body mass index (BMI), the risk of foetal death, stillbirth and neonatal death increases (Aune et al., 2014). In a recent cohort study from Sweden, infant mortality rates increased from 2.4/1000 among women of normal BMI to 5.8/1000 in obese women (Johansson et al., 2014). Along with gestational diabetes, pre-eclampsia and stillbirth, many intrapartum complications can arise as a result of maternal obesity, for example birth-related trauma and asphyxia resulting in hypoxic-ischaemic (HI) brain injury (Persson et al., 2014; Usha Kiran et al., 2005). Shoulder dystocia and traumatic labour due to foetal

Abbreviations: BMI, body mass index; HI, hypoxia-ischaemia; HFD, high fat diet; IL, interleukin; TNF-α, tumour necrosis factor-α; BBB, blood-brain barrier; CNS, central nervous system; LPS, lipopolysaccharide; GFAP, glial fibrillary acid protein; CV, cresyl violet; NeuN, neuronal nuclei; Iba-1, ionized calcium binding adaptor molecule 1; PBS, phosphate buffered saline; PFA, paraformaldehyde; c, control.

* Corresponding author at: Department of Pharmacology, School of Medical Sciences, UNSW Australia, NSW 2052, Australia.
E-mail address: n.jones@unsw.edu.au (N.M. Jones).

http://dx.doi.org/10.1016/j.bbi.2016.10.010
0889-1591/

macrosomia are some causes of birth-related complications and these are known to increase in obese women, which is an issue of grave concern (Heslehurst et al., 2008; Hogan et al., 2007). Previous studies also showed a greater risk of low Apgar scores at 1, 5 and 10 min, in offspring of overweight or obese women, which is an indicator of birth asphyxia (Persson et al., 2014; Scott-Pillai et al., 2013; Cedergren, 2006; Ovesen et al., 2011).

Various processes are likely to contribute to the detrimental impact of maternal obesity on offspring. An increase in visceral fat mass is associated with insulin resistance, inflammation and lipotoxicity which can lead to oxidative stress and endothelial dysfunction in human maternal and placental tissues (Saben et al., 2014; Jarvie et al., 2010). These factors link maternal high fat diet (HFD) or obesity to enhanced systemic inflammation which can impact the developing foetal brain. Exposure to HFD prior to and during pregnancy in rats elevated body weight, leptin levels and pro-inflammatory mediators interleukin (IL)-6, IL-1β and tumour necrosis factor-α (TNF-α) in the brains of offspring (Bahari et al., 2013). Rodent studies have also shown that IL-6 and IL-1β can cross the placenta and enter the foetal bloodstream (Girard et al., 2010; Dahlgren et al., 2006). Rat offspring from HFD dams have shown higher levels of brain cytokines and alterations in microglial phenotype which was accompanied by a long-lasting increase in anxiety levels and spatial learning deficits (Bilbo and Tsang, 2010; Sasaki et al., 2013). Thus exposure to maternal HFD may potentiate neonatal brain damage due to increased inflammation in the brains of offspring.

Cytokines are produced by different cell types within the brain in response to injuries (Teo et al., 2015). An increase in brain cytokine levels can also occur via peripheral signals such as those produced by infection which can cross the blood-brain barrier (BBB) into the central nervous system (CNS). Peripheral cytokines and signalling factors can cause changes in brain cell types such as activation of microglia and induce hypertrophy in astrocytes (Town et al., 2005; Henn et al., 2011). In the context of the obesity paradigm and brain injury, increased peripheral cytokines can cross the injury-compromised BBB to exacerbate brain injury responses (Pohl et al., 2014; Rummel et al., 2010).

Microglia are primarily known for mediating brain inflammation in response to infection and injury, with recent studies revealing additional roles in shaping the brain in response to diet. Rather than simply existing in one single state such as: "inactive", "resting" or "activated" microglia move between these diverse phenotypic states of activity and have varied physiological functions. The proportion of microglia in a more activated state was increased in the brains of offspring born to obese rat dams, at one day after birth (Bilbo and Tsang, 2010). Microglial activation was increased in adult hippocampus of rat offspring from obese mothers and became even more reactive following a neuroimmune challenge with lipopolysaccharide (LPS) (Bilbo and Tsang, 2010). In offspring from lean mothers, HI injury can induce phenotypic changes in cortical microglia, displaying amoeboid morphology up to one week after HI (Teo et al., 2015). Additional evidence suggests a strong association between brain inflammation and enhanced oxidative stress after neonatal HI (D'Angelo et al., 2015; Perrone et al., 2010; Hagberg et al., 2015; Juul and Ferriero, 2014; Fatemi et al., 2009). Prolonged microglial activation in a model of excitotoxic injury enhanced neuronal cell death via increasing pro-inflammatory cytokines, neurotoxic compounds and reactive oxygen and nitrogen species (Hagberg et al., 2015; Kichev et al., 2014). Taken together, these studies indicate that maternal HFD could potentially exacerbate brain injury in offspring due to increased oxidative stress and pro-inflammatory responses.

Glial cells play an important role in normal brain function and in response to injury, where astrocytes become more hypertrophic and form a glial scar to seal off the injury (Teo et al., 2015; Parmar and Jones, 2015). Increased markers of oxidative stress were observed in the cortex of HFD-fed adult rats from mothers on pre- and postnatal HFD however, administration of HFD to adult offspring only modestly increased glial hypertrophy, regardless of maternal diet (White et al., 2009). Glial fibrillary acid protein (GFAP) mRNA levels in astrocytes were not different in offspring from dams on HFD compared to low fat diet (Bilbo and Tsang, 2010). In the context of neuroinflammation, astrocytes control infiltration of peripheral pro-inflammatory leukocytes into the CNS (Bush et al., 1999; Myer et al., 2006) and have a complementary role in regulating microglial activation. Impaired astrocyte recruitment resulted in increased microglial activation following a stab wound *in vivo* (Robel et al., 2011). Conversely, increased astrocyte activation in an *in vitro* model of oxygen-glucose deprivation caused increased production of anti-inflammatory mediators and attenuated microglial activation (Kim et al., 2010). These studies highlight the dynamic and interactive roles of astrocytes and microglia in the brain, however their response following neonatal HI in offspring from obese dams remain to be investigated.

To address this gap, in this study, the effects of maternal HFD on brain cell survival in offspring and the response of microglia and astrocytes to HI injury were examined. Overall brain tissue loss was assessed using cresyl violet (CV) staining and a neuron specific marker. Changes in microglia and astrocytes were also examined using specific antibodies. Short-term functional deficits following HI were determined in offspring from HFD and Chow-fed mothers. The hypothesis examined here was that offspring from HFD dams would have an exacerbated injury response to HI compared to offspring from lean mothers.

2. Materials and methods

2.1. General procedures and materials

Experiments were performed in accordance with the ethical code of the National Health and Medical Research Council (Australia) and with the approval of the Animal Care and Ethics Committee of UNSW Australia (14/31A).

2.2. Animals

Twelve female Sprague-Dawley rats (3–4 weeks old) were obtained from Animal Resources Centre (Canning Vale, Western Australia). Rats were given one week to acclimatise to the housing environment and exposed to daily handling before being randomly distributed into Chow (n = 4, Rat and mouse breeder diet, 13 kJ/g, Gordon's speciality stockfeed, NSW Australia) or HFD groups. To account for reduced fertility in obese dams, and the possibility of increased HI-induced mortality with maternal HFD, more rats were placed on the HFD. Rats in the HFD group (n = 8) were fed a combination (~50:50) of normal Chow and HFD pellets (SF04-001, 19 kJ/g, 43% energy from lipids, Specialty Feeds). Dams and pups were housed under standard holding conditions with a 12 h light/-dark cycle and food and water were provided *ad libitum*. Energy intake was assessed by measuring 24 h food intake, once a week.

2.3. Breeding

Once an average weight difference of 10% was observed between the HFD and Chow-fed female rats, they were mated with two Chow-fed male Sprague-Dawley rats (weighing 288 and 305 g at the start of mating). In order to accommodate all of the subsequent procedures which were dependent on pup delivery dates, the mating sessions were staggered. One male rat was housed together with 2 female rats for 7 days to cover the 4–5 day oestrus