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PRENATAL MATERNAL LIPOPOLYSACCHARIDE ADMINISTRATION LEADS TO AGE- AND REGION-SPECIFIC OXIDATIVE STRESS IN THE EARLY DEVELOPMENTAL STAGE IN OFFSPRING

M. M. AL-AMIN,[a,b]* T. ALAM,[a] S. M. N. HASAN,[a] A. T. HASAN[a] AND A. H. M. R. QUDDUS[c]

[a] *Department of Pharmaceutical Sciences, North South University, Bashundhara, Dhaka, Bangladesh*

[b] *The Queensland Brain Institute, The University of Queensland, QBI Building 79, St Lucia, QLD 4072, Australia*

[c] *National University, Gazipur, Bangladesh*

Abstract—Prenatal exposure to lipopolysaccharide (LPS) has been exploited to simulate brain disorder in animal model. Prenatal LPS-exposure has shown elevated levels of pro-inflammatory cytokines in the early stages of the postnatal period. This study determines the effect of prenatal LPS-exposure on oxidative stress (OS) in the distinct brain regions in the early postnatal stages. LPS (50 μg/kg, i.p.) and water for injection (100 μl, i.p.) were given to the experimental ($n = 5$) and control ($n = 5$) group of pregnant *Swiss albino* mice respectively on gestational day (GD)-16 and 17. Animals were decapitated on postnatal day (PnD) – 1, 7, 14 and 21 to assay levels of oxidative markers from 6 distinct brain regions. When compared with the control, prenatal LPS-exposure alters levels of OS markers: (i) on PnD-1, glutathione (GSH) level is raised and superoxide dismutase (SOD) activity is dropped, (ii) on PnD-7, advanced oxidation of protein product (AOPP) level is elevated, (iii) on PnD-14, lipid peroxidation (MDA) and activity of catalase (CAT) are enhanced, (iv) on PnD-21, increased MDA continued. The hippocampus (HC) and cerebellum (CB) were mostly susceptible to OS in the early postnatal development. Levels of MDA and activity of CAT enzyme were increased on PnD-14 in the cortex, HC and CB. Except MDA, all OS markers recovered and returned to the level of control animals on PnD-21. Taken together, these results suggest that prenatal LPS-exposure induces age- and region-specific OS in the early postnatal stage.

*Correspondence to: Md. M. Al-Amin, Plot 15, Block B, Bashundhara, Dhaka 1229, Bangladesh. Tel: +880-2-55668200x1959; fax: +880-2-55668202.

E-mail address: mamun.al-amin@northsouth.edu (M. M. Al-Amin).

Abbreviations: ANOVA, analysis of variance; AOPP, advanced oxidation of protein product; CAT, catalase; CB, cerebellum; EDTA, Ethylenediaminetetraacetic acid; GD, gestational day; GSH, glutathione; HC, hippocampus; HT, hypothalamus; IL, interleukin; LPS, lipopolysaccharide; MDA, lipid peroxidation; NO, nitric oxide; OS, oxidative stress; PBS, phosphate-buffered saline; PCX, parietal cortex; PFC, prefrontal cortex; PnD, postnatal day; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; ST, striatum; TBA, 2-thiobarbituric acid; TNF, tumor necrosis factor.

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INTRODUCTION

The brain is highly susceptible to the reactive oxygen and nitrogen species (ROS and RNS) (Reynolds et al., 2007; Friedman, 2011). ROS and RNS are the major players in the pathogenesis of neurodegenerative disorders: Alzheimer (Christen, 2000), Parkinson (Hald and Lotharius, 2005); neuropsychiatric disorders: schizophrenia (Fendri et al., 2005; Bitanihirwe and Woo, 2011), depression (Sarandol et al., 2007; Wolkowitz et al., 2011), and bipolar disorder (Berk et al., 2011). Evidence suggest that immune dysfunction, oxidative and nitrosative stress pathways play a vital role in the pathophysiology of anxiety disorder (Masood et al., 2008; Rammal et al., 2008; Bouayed et al., 2009), autism, and memory impairment (Cui et al., 2006; Al-Amin et al., 2014). Prenatal exposure to lipopolysaccharide (LPS) has been exploited to develop brain disorders in the offspring: autism (Zimmerman et al., 2005; Meyer et al., 2011), schizophrenia (Meyer et al., 2011), depression and anxiety (Depino, 2015), and cognitive deficits (Hao et al., 2010). LPS exposure during a specific time window during pregnancy leads to fetal neurodevelopmental disorders (Meyer et al., 2006; Patterson, 2011).

Prenatal exposure to LPS activates astrocyte and microglial cell which in turn produces oxidative stress (OS) (Cai et al., 2000; Hu and El-Fakahany, 1995). LPS binds to membrane CD14 and is transferred to TLR-4 on glial cells (Carpentier et al., 2005). Binding of LPS to TLR-4 (Hoshino et al., 1999; Zhang and Ghosh, 2001) activates intracellular signaling, translocation of proteins to the nucleus via NF-κB signaling pathway (Chow et al., 1999), and transcription of genes associated with inflammation (Beutler, 2000). Activated NF-κB causes the formation of inflammatory cytokines: interleukin-1 (IL-1), tumor necrosis factor (TNF-α), interleukin-6 (IL-6) and chemokines (MCP-1, RANTES) (Rivest, 2003; Glezer et al., 2007). These inflammatory cytokines cross the placenta and enter into the fetus (Ashdown et al., 2006). IL-6 and TNF-α have been documented to cross the weak BBB of trophoblast (Fidel et al., 1994) and subsequently activates astrocyte and microglia (Cai et al., 2000). Evidence suggests activated microglia release ROS and RNS via phagocyte oxidase and inducible nitric

oxide synthase. Production of ROS and RNS during the neurodevelopmental stages is critical since it may damage neurons.

Inflammation in the prenatal stage is associated with the level of OS. For example, prenatal LPS causes lipid peroxidation and GSH (glutathione) depletion (Chen et al., 2005; Xu et al., 2007). In the animal study of LPS-model, ROS controls target gene transcription which down-regulates nuclear receptor (PXR), (Chen et al., 2005). LPS (50 μg/kg, i.p.) on GD (gestational day)-13, induces OS through the production of lipid peroxidation in the mouse placenta (Ejima et al., 2000). Prenatal LPS-exposure also reduces oligodendrocyte in the developing fetus through the ROS generation, GSH depletion and peroxisomal dysfunction (Paintlia et al., 2008).

The association between prenatal inflammation and the level of OS in the postnatal stage has been reported by a limited numbers of studies. For example, prenatal LPS exposure results in the altered level of OS in the postnatal day (PnD)-24 (Ginsberg et al., 2012). The administration of LPS 2-days before delivery leads to the accelerated production of stress markers (Lante et al., 2007) on PnD-28. The authors have demonstrated that OS occurred by observing an increase in protein carbonylation, decrease in alpha-tocopherol levels and an imbalance in the ratio of reduced/oxidized forms of GSH in the hippocampus (HC) of 28-day-old offspring (Lante et al., 2007, 2008). Previous study showed levels of oxidative markers in PnD-24 and - 28 day of animals that were prenatally LPS-exposed. Therefore, a research gap remains to discover the status of early stages of OS in the animals that are LPS-exposed at the prenatal stage.

Prenatal LPS-exposure on GD-16 to -17 (Stigger et al., 2011) may lead to the accelerated production of ROS and the depletion of antioxidants. To date, a number of studies have shown that ROS interrupts the developing brain of the fetus (Lante et al., 2007; Paintlia et al., 2008). However, inflammation-related study reports, prenatal LPS-exposure leads to age- and region-specific changes in inflammatory cytokines during the developmental stages in offspring (Garay et al., 2013). To our knowledge, the effects of prenatal LPS-exposure on the age- and region-specific six OS markers is undetermined in a single study.

The specific objective of this study is to expose animals to LPS on GD-16 and -17. Then, we assay the OS markers in the early postnatal stages. We collect tissue sample from six distinct brain regions: such as prefrontal cortex (PFC), striatum (ST), parietal cortex (PCX), hypothalamus (HT), HC and cerebellum (CB). These brain regions are responsible for executing indifferent brain functions and varied susceptibility to the inflammatory cytokines. Therefore, region-specific study would provide a clear view on OS susceptibility. In addition, this study aims to assay time-dependent changes in the OS markers in the early postnatal period: on PnD-1, -7, -14 and -21 day. We maintain a consistent interval while observing time-dependent changes of OS markers in the early developmental stages of life.

EXPERIMENTAL PROCEDURES

Animals

In this experiment, pregnant *Swiss albino* mice ($n = 5$) mouse used and kept in 12:12-h light and dark cycle. Pregnant mice were divided into 2 groups on GD-16. LPS at a dose of 50 μg/kg, i.p. administered to the experimental groups ($n = 5$) and water for injection (100 μl, i.p.) given to the control mice on GD-16 and 17 (Fig. 1). A varied range of LPS dose had been used to develop the impaired brain and behavioral model in rodents. LPS at a low dose (50 μg/kg) to a high dose (2.5 mg/kg) has been used in the past (Boksa, 2010). However, high dose of LPS (300 μg/kg, i.p.) on GD-15 has shown to cause preterm delivery and 89.3% of fetal death, whereas a lower dose results in intrauterine growth restriction (IUGR) (Zhao et al., 2013). Therefore, a low dose (50 μg/kg, i.p.) is used for this study (Liu et al., 2014; Wang et al., 2014; Toyama et al., 2015) that will not cause preterm delivery or fetal death. Pups from the experimental and control groups were decapitated on postnatal days-1, 7, 14 and 21. Postnatal pups were coded as PnD-1 ($n = 6$), PnD-7 ($n = 6$), PnD-14 ($n = 6$) and PnD-21 ($n = 6$). The experimental procedure was reviewed and approved by the local ethics committee at the North South University, Dhaka, Bangladesh (NSU/PHA/2015/133-046). Animals were handled in accordance with the international principles guiding the usage and handling of experimental animals (United States National Institute for Health Publication, 1985). Usually, animal were handled by the experimenter each day.

Tissue processing

Mice were anesthetized with ketamine (100 mg/kg, body weight, 0.1 ml) and perfused through the heart with cold 0.9% sodium chloride to wash blood from the brain tissue. Then animals were killed by decapitation. The entire brain was rapidly removed cautiously and kept in a Petri dish placed over ice. Six brain regions were dissected in the following order; PFC, ST, HT, PCX, CB, and HC. Brain tissue was dissected according to the previous study (Al-Amin et al., 2015). At first, for PFC dissection, the olfactory bulb was removed, and cut into coronal slices of 4 mm below the olfactory bulb. Then, the ST was isolated by removing the cortex, CB was dissected from the hindbrain region. The remaining part of the brain was turned upside down to dissect HT. For HIP dissection, HT was removed, sagittal incision was done to separate the two hemispheres, noncortical forebrain and meningeal tissues were removed to isolate the HC. Homogenates of various brain regions, 10% (w/v) were prepared in phosphate-buffered saline (PBS) (10 mmol/l, pH 7.0) using an Ultra-Turrax T25 (USA) homogenizer. Homogenized tissue samples were sonicated at a 5-s cycle for 150 s using an ultrasonic processor and centrifuged at 10,000 RPM (7960 g) for 10 min. Then, upper clear supernatants were collected for the biochemical analysis.