Neuroscience 194 (2011) 195–207

INTRANASAL ADMINISTRATION OF INSULIN-LIKE GROWTH FACTOR-1 PROTECTS AGAINST LIPOPOLYSACCHARIDE-INDUCED INJURY IN THE DEVELOPING RAT BRAIN

Z. CAI,* L.-W. FAN, S. LIN, Y. PANG AND P. G. RHODES

Department of Pediatrics, Division of Newborn Medicine, University of Mississippi Medical Center, Jackson, MS 39216, USA

Abstract—Our previous studies show that insulin-like growth factor-1 (IGF-1) can either protect against or increase lipopolysaccharide (LPS)-induced damage in the developing brain, depending on the dose, when it is co-administered with LPS through intracerebral injection. To further explore effects of IGF-1 on central inflammation associated brain injury, IGF-1 was administered through intranasal infusion in the current study. Postnatal day 5 (P5) rats were exposed to LPS at a dose of 1 μg/g body weight or sterile saline through intracerebral injection. Recombinant human insulin-like growth factor-1 (rhIGF-1) at a dose of 50 μg/pup or vehicle was administered intranasally 1 or 2 h after the LPS injection. Neonatal LPS exposure resulted in oligodendrocyte (OL) and white matter injury in the P6 or P21 rat brain. The damages include dilatation of lateral ventricles, pyknotic cell death, loss of OL progenitor cells and mature OLs in the cingulum area, and impairment of myelination at the corpus callosum area. Neurological dysfunctions were observed in juvenile rats with neonatal LPS exposure. Intranasal IGF-1 treatment at either 1 or 2 h after LPS exposure significantly attenuated LPS-induced brain injury and improved some behavioral deficits. Intranasal IGF-1 treatment also reduced infiltration of polymorphonuclear (PMN) leukocytes and activation of microglia in the rat brain 24 h after LPS exposure, but it did not prevent the elevation in concentrations of interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNFα) in the LPS-exposed rat brain during the first 24 h. This is an indication that direct anti-inflammation might not be the primary mechanism for the protection of IGF-1, and other mechanisms, such as anti-apoptotic effects, are likely involved in its protective effects.

Key words: insulin-like growth factor-1, intranasal administration, LPS, oligodendrocyte, apoptotic cell death, inflammatory cytokine.

Cerebral white matter damage or periventricular leukomalacia (PVL) is one of the most devastating conditions in preterm infants. It is estimated that approximately 60,000 infants (1.5% of the 4 million yearly live births) are born with a birth weight less than 1500 g, and based on MRI data at least 50% of them exhibit some degree of cerebral white matter damage (Volpe, 2003). The pathogenesis of PVL is not completely understood, but investigators believe that hypoxia-ischemia and infection/inflammation are two primary causes (Leviton and Dammann, 2004; Khwaja and Volpe, 2008). Therefore, several animal models have been developed based on these two factors. We previously reported that intracerebral delivery of lipopolysaccharide (LPS) preferentially induces white matter damage, loss of immunoreactivity of immature oligodendrocyte (OL) markers, increased size of lateral ventricles, delayed myelination, and neurological dysfunctions (Cai et al., 2003; Fan et al., 2005b; Pang et al., 2003).

Pre-oligodendrocytes (pre-OLs) or late OL progenitor cells are the major cell type selectively damaged in PVL (McQuillen and Ferriero, 2004; Back et al., 2002). Therefore, protection of pre-OLs could be the primary strategy for PVL treatment. Insulin-like growth factor-1 (IGF-1) appears to be a plausible candidate for such a purpose due to its potent survival effect. IGF-1 has been reported to protect OLs from various insults, including tumor necrosis factor alpha (TNFα) cytotoxicity (Pang et al., 2007), growth factor deprivation, (Cui et al., 2005) and excitotoxicity (Ness et al., 2004). Exogenous IGF-1 has been shown to protect against ischemic brain damage in both the adult (Dempsey et al., 2003; Schäbitz et al., 2001) and newborn animals (Brywe et al., 2005; Cao et al., 2003; Guan et al., 2000; Lin et al., 2005) when injected directly into the brain. However, our *in vivo* study showed that when IGF-1 was co-administered with LPS, it can either protect against or increase LPS-induced damage in the developing brain, depending on the dose (Pang et al., 2010). Since IGF-1 was co-administered with LPS through intracerebral injection in our study, the local concentration of IGF-1 could be high at the injection site and consequently affect the action of IGF-1. We and other investigators have shown that IGF-1 can be delivered to the rat or mouse brain along olfactory and trigeminal pathways with intranasal administration (Thorne et al., 2004; Lin et al., 2009) and that intranasally delivered IGF-1 protects against cerebral hypoxic-ischemic injury (Liu et al., 2001a,b; Lin et al., 2009, 2011) or other neurodegenerative damages (Vig et al., 2006). Therefore, the objective of the current study was to test whether a single dose of IGF-1 delivered through intranasal infusion in the neonatal rat can provide protec-

*Corresponding author. Tel: +1-601-984-2786; fax: +1-601-815-3666.
E-mail address: zcai@umc.edu (Z. Cai).

Abbreviations: APC-CC1, adenomatous polysis coli (clone CC1); BBB, blood brain barrier; FB, front brain; IGF-1, insulin-like growth factor-1; IL-1β, interleukin-1 beta; LPS, lipopolysacharride; MBP, myelin basic protein; OB, olfactory bulb; OL, oligodendrocyte; PB, posterior brain; PFA, paraformaldehyde; PI3K, phophatidylinositol-3 kinase; PMN, polymorphonuclear; pre-OLs, pre-oligodendrocytes; PVL, periventricular leukomalacia; P5, postnatal day 5; rhIGF-1, recombinant human insulin-like growth factor-1; TNFα, tumor necrosis factor alpha; TUNEL, terminal deoxynucleotidyl transferase-mediated uridine 5′-triphosphate-biotin nick end labeling.

0306-4522/11 $ - see front matter
doi:10.1016/j.neuroscience.2011.08.003

tion against LPS-induced OL and white matter injury and improve neurological functions in juvenile animals.

EXPERIMENTAL PROCEDURES

Chemicals

Unless otherwise stated, all chemicals used in this study were purchased from Sigma (St. Louis, MO, USA). Recombinant human IGF-1 (rhIGF-1) was purchased from Cell Sciences (Canton, MA, USA). Monoclonal mouse antibodies against O4 oligodendrocyte or myelin basic protein (MBP, a marker of myelination), as well as the terminal deoxynucleotidyl transferase-mediated uridine 5′-triphosphate-biotin nick end labeling (TUNEL) staining kit were acquired from Millipore (Temecula, CA, USA). Monoclonal mouse antibodies against rat OX42 (a marker of microglia) or CD43 (a marker of polymorphonuclear cells, PMN), and against adenomatous polysis coli (clone CC1) (APC-CC1, a marker of mature OLs) were obtained from Serotec (Raleigh, NC, USA) and Calbiochem (San Diego, CA, USA), respectively. ELISA kits for immunoassay of interleukin-1 beta (IL-1β), TNFα or rhIGF-1 in the rat brain and the Caspase-3 colorimetric assay kit were purchased from R&D Systems (Minneapolis, MN, USA).

Animals and drug treatment

Timed pregnant Sprague–Dawley rats arrived in the laboratory on day 19 of gestation. Animals were maintained in a room with a 12-h light/dark cycle and at constant temperature (22±2 °C). The day of birth was defined as postnatal day 0 (P0). After birth, the litter size was adjusted to 10 pups per litter to minimize the effect of litter size on body weight and brain size. Intracerebral injection of LPS to 5-day old rat pups was performed as described previously (Cai et al., 2003; Fan et al., 2005b; Pang et al., 2003). Under light anesthesia with isoflurane (1.5%), LPS (1 μg/g, from *Escherichia coli*, serotype 055: B5) in sterile saline (total volume of 2 μl) was administered to the rat brain at the location of 1.0 mm posterior and 1.0 mm left to the bregma, and 2.0 mm deep to the scalp at the left hemisphere in a stereotaxic apparatus with a neonatal rat adapter. The injection site was aimed at the area just above the left cingulum. The injection was completed in 5 min and the needle was kept in this position for an additional 2 min and then retrieved slowly out of the brain. The wound was sutured and the pups were placed on a thermal blanket (34 °C–35 °C) for recovery before being returned to their dams. The dose of LPS has been shown to produce reproducible white matter brain injury (Cai et al., 2003; Fan et al., 2005b; Pang et al., 2003). The control rats were injected with the same volume of sterile saline. All animals survived the intracerebral injection. Each dam had the same litter size (10 pups) and equal numbers of LPS-treated and saline-treated rat pups were included in a litter. All procedures for animal care were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center. Every effort was made to minimize the number of animals used and their suffering.

Intranasal administration of rhIGF-1 in the rat pup was performed as previously described (Lin et al., 2009). Briefly, P5 SD rat pups were placed on their backs under light anesthesia with isoflurane (5% for induction and 1.5% for maintenance). After pups were sedated, 50 μg of rhIGF-1 dissolved in 5 μl PBS containing 0.1% BSA was given into the left naris using a fine tip. This dose of IGF-1 has been shown to protect the neonatal rat brain from hypoxic-ischemic injury as demonstrated in our previous studies (Lin et al., 2009, 2011). The pups were then maintained sedated with isoflurane for 5 min to ensure that they stayed on their backs. All pups woke up within 1–2 min upon withdrawal of isoflurane and were returned to their dams. For pups in the control group, 0.1% BSA was given by intranasal administration. In an initial study to determine whether rhIGF-1 administered through intranasal infusion can penetrate into the rat brain, pups were decapitated at 30 min following intranasal infusion of IGF-1 at 0, 1 or 2 h after intracerebral injection of LPS or saline and the brain was collected. Selection of the 30-min duration is based on previous reports that concentration of IGF-1 delivered through intranasal infusion reaches its peak at that time in the rat brain (Thorne et al., 2004). The brain (without the cerebellum) was separated into three parts: olfactory bulbs (OB), frontal brain (FB), and posterior brain (PB). The FB and PB were separated coronally at the bregma level. Brain tissue was stored at −80 °C for determination of brain concentrations of rhIGF-1 using an ELISA kit (R&D Systems, Minneapolis, MN, USA) at a later time. The initial study showed that concentrations of rhIGF-1 in the rat brain were not affected by the time elapsed following LPS exposure (see results and Fig. 1 for details). Because post-treatment is of more therapeutic significance, rhIGF-1 or vehicle was administered at 1 or 2 h after the intracerebral injection of LPS or saline in all our studies.

Brain section preparation and immunohistochemistry

Rat pups were sacrificed by transcardiac perfusion with normal saline followed by PBS-buffered 4% paraformaldehyde (PFA) for brain section preparation or by decapitation for fresh brain tissue (without the cerebellum) collection 24 h (P6) after the LPS exposure or 16 days (P21) later following the completion of behavioral tests. After fixation in PFA overnight at 4 °C, the brain was equilibrated in cryoprotection solution containing 10∼30% sucrose at 4 °C. Frozen coronal brain sections at 10 μm of thickness were prepared in a cryostat. These sections were used for immunohistochemistry except for O4 staining. Free-floating coronal brain sections at 40 μm of thickness were prepared in a freezing sliding microtone and used for Nissl staining and pre-OL surface antigen O4 immunostaining.

For immunohistochemical staining, primary antibodies were used at the following dilution: O4, 1 μg/ml; MBP, 1:500; CD43, OX42, 1:200; or APC-CC1, 1:20. Sections were incubated with primary antibodies at 4 °C overnight, and then incubated with appropriate secondary antibodies conjugated with various fluores-

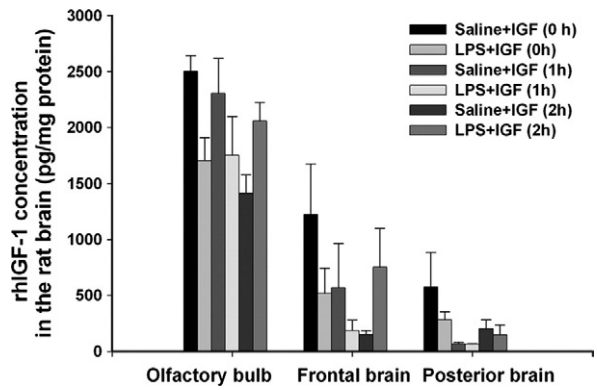

Fig. 1. Concentrations of rhIGF-1 in different parts of the rat brain 30 min after intranasal administration of rhIGF-1 in the initial study. Intranasal infusion of rhIGF-1 was performed at 0, 1 or 2 h after the intraceberal injection of LPS or sterile saline in P5 rats. Each group contained five animals. rhIGF-1 was detected in the brain of rhIGF-1-infused animals, but not in that of the vehicle (0.1% BSA)-infused animals, from either the saline- or the LPS-injected group. rhIGF-1 concentrations in the FB and the PB of saline- or LPS-injected rat brain were lower than that in the OB. No significant differences in rhIGF-1 concentrations were observed among the treatment groups within respective brain region.