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Immuno-modulator inter-alpha inhibitor proteins ameliorate complex auditory processing deficits in rats with neonatal hypoxic-ischemic brain injury

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ABSTRACT

Hypoxic-ischemic (HI) brain injury is recognized as a significant problem in the perinatal period, contributing to life-long language-learning and other cognitive impairments. Central auditory processing deficits are common in infants with hypoxic-ischemic encephalopathy and have been shown to predict language learning deficits in other at risk infant populations. Inter-alpha inhibitor proteins (IAIPs) are a family of structurally related plasma proteins that modulate the systemic inflammatory response to infection and have been shown to attenuate cell death and improve learning outcomes after neonatal brain injury in rats. Here, we show that systemic administration of IAIPs during the early HI injury cascade ameliorates complex auditory discrimination deficits as compared to untreated HI injured subjects, despite reductions in brain weight. These findings have significant clinical implications for improving central auditory processing deficits linked to language learning in neonates with HI related brain injury.

1. Introduction

Perinatal hypoxic-ischemic (HI) brain injury, prematurity, very-low-birth-weight (VLBW <1200 g) and infectious disorders often place neonates at high risk for developmental language delays (Vohr, 2014, 2016). Central auditory processing deficits have been reported to contribute to later language delays similar to delays observed in children with language learning impairments of unknown origin (Ortiz-Mantilla et al., 2008). Previous work has consistently reported an association between complex and/or rapid auditory processing impairments and later language outcomes in young children (Benasich and Tallal, 2002). Further, infant auditory temporal processing thresholds for the discrimination of rapidly presented tone-pairs are highly predictive of the age at which language commences in both typical and at risk infants (Benasich and Tallal, 2002; Cantiani et al., 2016). Likewise, experimental models in neonatal rodents after exposure to unilateral HI brain injury exhibit complex central auditory processing deficits and injury profiles similar to those observed in human full term and premature infants with brain injury (Downie et al., 2002; McClure et al., 2006a,b; McClure et al., 2007).

Currently, hypothermia is the only approved therapy to attenuate brain damage in infants, but is only partially protective and can only be used in the presence of HI encephalopathy (HIE) in full term infants (Gunn et al., 1997; Shankaran et al., 2005; Gluckman et al., 2006). Despite efforts to develop additional therapeutic strategies, there are currently no pharmacological agents available to treat human neonates at risk for brain injury (McClure et al., 2006a,b; McClure et al., 2007; Xiong et al., 2011). Nonetheless, recent work has suggested a critical role for inflammation and immune reactivity in regulating the injury cascade after exposure to HI in neonates (Dammann and Leviton, 1997; Becker, 1998; Ferriero, 2004; Iadecola and Anrather, 2011).

Inter-alpha inhibitor proteins (IAIPs) are a family of structurally related serine protease inhibitors with immune modulating capabilities that have been shown to down-regulate pro-inflammatory cytokines, Interleukin (IL)-1β and Tumor Necrosis Factor (TNF)-α and upregulate anti-inflammatory cytokine IL-10, leading to improved outcomes in several different models of systemic inflammatory disorders (Baek et al., 2003; Wakahara et al., 2005; Chen et al., 2016). The major forms if IAIPs are inter-alpha

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inhibitor (IαI), which consists of two heavy chairs (H1 & H2), and a single light chain (LC), and pre-alpha inhibitor (PαI) consisting of one heavy (H3) chain and one light chain (LC) (Fries and Blom, 2000). Recently, blood plasma levels of this protein have been shown to accurately predict the development of sepsis in premature infants, as levels decrease during systemic inflammation (Chaaban et al., 2009). Furthermore, hepatic IAIP synthesis is down regulated during severe inflammation and levels of IAIPs have been shown to decrease during sepsis, (Chaaban et al., 2009) which is associated with an increased incidence of brain damage in premature infants (Stoll et al., 2004; Shah et al., 2008). Recently, our group has shown that systemic IAIPs administration increases neocortical cell survival, prevents hippocampal and cortical tissue loss, and improves spatial and non-spatial learning and working memory performance in rats after exposure to neonatal HI brain injury (Threlkeld et al., 2014; Gaudet et al., 2016). Given the potential for IAIPs to attenuate neonatal brain injury, and the importance of delayed language development in infants with premature delivery and related brain injury (Vohr, 2014, 2016), it is important to examine the effects of IAIPs on complex auditory processing in model species with neonatal HI injury.

In addition, investigations in humans and rodent models of neonatal brain injury have shown that deficits in learning and in auditory processing are increasingly detected with progressive demand of any given task (Threlkeld et al., 2006, 2014; Barde et al., 2012; Gaudet et al., 2016). We have previously shown that as the demand of a specific task increases, the deficits become more apparent in juvenile and adult rodents exposed to neonatal HI brain injury relative to sham subjects, irrespective of the quantity of HI injury (Threlkeld et al., 2009a,b; Gaudet et al., 2016). Therefore, given the above considerations, the present study sought to determine the efficacy of IAIPs to improve auditory processing using a modified acoustic startle paradigm with increasing levels of cue complexity (i.e., task demand; simple normal single tone detection, gap detection in white noise and complex oddball tone-pair discrimination) in adult rats exposed to neonatal HI.

2. Methods

2.1. Subjects and surgical procedures

Subjects were 34 male Wistar rats born to 7 time-mated dams (Charles River Laboratories; Wilmington, MA) at Rhode Island College. Dams arrived at the college on gestational day 7. Animals were housed using a 12-h light/dark cycle with food and water available *ad libitum*. On postnatal day one (P1), pups were separated into litters of eight males and two females to control for litter size and sex ratio. Weighting for males produced five litters that were maintained up until weaning (P21), when subjects were pair housed. Prior to surgery on postnatal day (P) 7, male subjects, within each litter, were randomly assigned to one of three groups: Sham + vehicle (n = 12), hypoxia-ischemic vehicle treated (HI + Vehicle, n = 13), and hypoxia-ischemic IAIP treated (HI + IAIP, n = 9). This distributed treatment assignment was designed to control for litter effects and reflected an even distribution of treatment groups for each dam. Male subjects were assessed given that rodent and human epidemiological data indicate greater behavioral deficits in males as compared to females with neonatal brain injury (Raz et al., 1995; Abe et al., 1996; Peiffer et al., 2004; Hill et al., 2011) – findings that are similar to the higher diagnostic rates of neurodevelopmental disorders including dyslexia, epilepsy, autism and intellectual disabilities that are detected in human males compared with females (Raz et al., 1995; Rutter et al., 2003; Liederman and Flannery, 2005).

Subjects were weighed and anesthetized using 3–4% isoflurane and maintained with 1% during the surgical procedure. Methods for HI brain injury in neonatal rodents have been extensively described elsewhere (Rice et al., 1981; McClure et al., 2006a,b; Threlkeld et al., 2014). Following a 1 cm midline incision of the neck, the right common carotid artery (RCCA) was located and completely cauterized (McClure et al., 2006a,b). The incision was sutured and labeled with dermal paw India Ink (Higgins) injections (10 μl) for later identification. Sham subjects underwent identical surgical procedures without cauterization of the RCCA. Body temperature was maintained at 37 °C preoperatively, during surgery and during postoperative recovery. After surgery, the pups were returned to their dams and allowed to feed for 2–3 h before exposure to hypoxia.

After recovery from surgery, as described above, subjects were removed from their home cage and received an intraperitoneal (IP) injection of either 30 mg/kg of human IAIP (HI + IAIPs, Pro Thera biologics, Providence, RI) or placebo (0.9% NaCl vehicle; sham and HI). The dose of IAIPs was selected based upon studies showing that the same dose of IAIPs reduced the incidence of death from sepsis in neonatal and adult rats and increased cortical neuronal survival after neonatal HI (Lim et al., 2003; Singh et al., 2010; Threlkeld et al., 2014). After the IP injections, the HI groups were exposed to humidified 8% O_2 and 92% N_2 for 120 min. Body temperature was maintained for all groups during hypoxia or room air exposure using isothermal heating pads (Braintree Scientific, Braintree, MA). Sham subjects received identical treatment but were maintained in a separate container exposed to room air for 120 min and received 0.9% NaCl vehicle injections. A second identical dose of IAIPs or vehicle was administered 24 h after hypoxia. The 12-h half-life of IAIPs was hypothesized to ensure that protein levels would remain high between the two dosing windows prior to clearance by the kidneys (Fries and Blom, 2000). Prior to auditory assessment subjects were tested for spatial and non-spatial learning as previously reported (see Fig. 1 for study timeline) (Threlkeld et al., 2014). All procedures were performed according to the National Institutes of Health guide for the care and use of laboratory animals and approved by the Rhode Island College Institutional Animal Care and Use Committee.

2.2. Production and purification of IAIPs

The methods for IAIPs extraction and purification have been described previously (Threlkeld et al., 2014). Briefly, A monolithic anion-exchange chromatographic method was used to extract IAIPs from frozen human plasma (Rhode Island Blood Center, RI; US Patent #7,932,365,2011; (Opal et al., 2011; Spasova et al., 2014). After binding, the column was sequentially washed with buffer containing 200nM NaCl and 200nM acetate buffer, with pH 3.0. IAIPs were eluted from the column using a buffer containing 759 mM NaCl, and concentrated and buffer exchanged using a tangential flow filtration system (Labscale, Millipore). Purity analysis was performed by SDS-Page, Western immunoblot, competitive IAIP ELISA and standardized in vitro trypsin inhibition assay ***(Lim et al., 2003; Opal et al., 2011; Spasova et al., 2014). As previously reported, the biological activity is based on the ability of IAIPs to inhibit the hydrolysis of the substrate N-benzoyl-L-argi nine-p-nitroaniline HCl (BAPNA, Sigma, St. Louis, MO) by trypsin.

2.3. Brain analyses

At the end of auditory testing, subjects were weighed, anesthetized with pentobarbital (Sleepaway, Fort Dodge, IA), and transcardially perfused with saline followed by 10% phosphate buffered formalin. Brains were extracted and weighed (see Fig. 2). Tissue was then sectioned, mounted on glass slides and