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BRAIN RESEARCH

Research Report

Activated protein C reduces endotoxin-induced white matter injury in the developing rat brain

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ABSTRACT

Periventricular leukomalacia (PVL), the dominant form of brain injury in premature infants, is characterized by white matter injury (WMI) and is associated with cerebral palsy. The pathogenesis of PVL is complex and likely involves ischemia/reperfusion, free radical formation, excitotoxicity, impaired regulation of cerebral blood flow, a procoagulant state, and inflammatory mechanisms associated with maternal and/or fetal infection. Using an established animal model of human PVL, we investigated whether activated protein C (APC), an anti-coagulant factor with anti-inflammatory, anti-apoptotic, anti-oxidant, and cytoprotective activities, could reduce endotoxin-induced WMI in the developing rat brain. Intraperitoneal injections of lipopolysaccharide (LPS) (0.5 mg/kg body weight) were given at embryonic days 18 (E18) and 19 (E19) to pregnant Sprague–Dawley rats; control rats were injected with sterile saline. A single intravenous injection of recombinant human (rh) APC (0.2 mg /kg body weight) was given to pregnant rats following the second LPS dose on embryonic day 19 (E19). Reduced cell death in white matter and hypomyelination were shown on TUNEL and myelin basic protein (MBP) staining, respectively, on late postnatal days (P7) in APC-treated groups. There were significantly fewer TUNEL+nuclei in the periventricular WM in the APC+LPS group than in the untreated LPS group. Compared to the APC+LPS and control group, MBP expression was weak in the LPS group on P7, indicating endotoxin-induced hypomyelination in the developing rat brain. APC attenuated the LPS-induced protein expression of inflammatory cytokines, tumor necrosis factor-alpha, and interleukin-6, as evaluated by ELISA in neonatal rat brains. A single intraperitoneal injection of rhAPC (0.2 mg/kg body weight) to neonatal rats on P1 also had similar protective and anti-inflammatory effects against maternally administered LPS. Collectively, these data support the hypothesis that APC may provide protection against an endotoxin-evoked inflammatory response and WMI in the developing rat brain. Moreover, our results suggest that the possible use of APC in treatment of preterm infants and pregnant women with maternal or placental infection may minimize the risk of PVL and cerebral palsy.

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1. Introduction

Perinatal brain injury in the premature infant is a particularly important issue. Approximately 10% of newborns are born prematurely. Of these children, more than 10% have brain injuries that lead to adverse neurological outcomes including motor, cognitive, and behavioral deficits (Wozniak et al., 2004). Periventricular leukomalacia (PVL) has emerged as the dominant neuropathological form of brain injury that occurs in the premature infant (Back and Rivkees, 2004; Perlman, 1998; Wozniak et al., 2004). PVL is a pathological process that occurs within the white matter that is characterized by the loss of oligodendrocytes; PVL is the major antecedent of cerebral palsy. The pathogenesis of PVL is complex and multifactorial. Inflammatory mechanisms associated with maternal and/or fetal infection (Adams-Chapman and Stoll, 2006; Grether and Nelson, 1997; Leviton and Dammann, 2004), hypoxia/ischemia, impaired regulation of cerebral blood flow (Fukuda et al., 2006), formation of free radicals (Haynes et al., 2005; Inder et al., 2002), impaired myelination due to oligodendrocyte loss (Cai et al., 2000; Inder and Volpe, 2000; Kinney, 2005), apoptotic cell death (Kadhim et al., 2006), microglial activation (Volpe, 2001), coagulation abnormalities (Leclerc et al., 2000; Leviton et al., 1999), and glutamate excitotoxicity (Follett et al., 2004) have all been implicated as pathogenetic mechanisms (Boggess, 2005). Treatment strategies that target the above mechanisms could provide protection against WMI in the developing brain (Gressens and Spedding, 2004). However, up to date, no promising therapies for PVL have been found.

The protein C pathway is an important regulator of the coagulation system (Dahlback and Villoutreix, 2005; Esmon, 2003). Activated protein C (APC) is an important natural anticoagulant protein that is converted from protein C by the action of the thrombin–thrombomodulin complex on endothelial cells. APC is a serine protease that regulates the coagulation system by a proteolytic inactivation of the activated forms of coagulation factors V and VIII. It has been shown that the administration of drotrecogin alfa (activated) (recombinant human APC) to severe sepsis patients improved survival; this finding has provided new insights into the protein C pathway (Bernard et al., 2001; Haley et al., 2004). APC is the first effective biological therapy approved for the treatment of severe sepsis (O'Brien et al., 2006). Although APC is well defined as a physiological anticoagulant, emerging data suggest that it also has cytoprotective, anti-inflammatory and antiapoptotic properties (Esmon, 2000, 2001). APC has been shown to provide neuroprotection in ischemic brain and spinal cord injury (Cheng et al., 2003; Griffin et al., 2004). The anti-inflammatory, cytoprotective, and anti-apoptotic effects of APC mediate its neuroprotective action. It has been shown that APC protects neurons against N-methyl-D-aspartate (NMDA) toxicity both in vitro and in vivo (Guo et al., 2004). The cytoprotective effects of APC has also been reported for other types of cells including endothelial cells, keratinocytes, monocytes, pancreatic beta cells and gastric epithelial cells (Contreras et al., 2004; Isobe et al., 2004; Yoon et al., 1997; Xue et al., 2004). APC attenuates ischemia/reperfusion injury in various organs such as brain, kidneys and skeletal muscle (Dillon et al., 2005; Levi et al., 2004; Mizutani et al., 2000). The ameliorating effects of APC on endotoxin-induced inflammatory responses and coagulation abnormalities have been demonstrated using both in vitro and in vivo models (Hoffmann et al., 2004; Iba et al., 2005). The use of APC to treat white matter damage in the adult brain or the developing brain has been proposed (Leviton et al., 1999). However, it is unknown whether antennal maternal or postnatal systemically administration of APC protects against endotoxin induced injury in an animal model of white matter damage in vivo.

In the present study, we investigated (i) whether APC has cytoprotective effects against endotoxin-mediated perinatal WMI; (ii) whether APC decreases the endotoxin-induced proinflammatory cytokine response in the developing rat brain; and (iii) whether APC decreases hypomyelination induced by endotoxin in an animal model of maternal intraperitoneal administration of lipopolysaccharide (LPS).

2. Results

2.1. Physiologic parameters

In this study, we evaluated body and brain weights at P7. A significant loss of brain weight occurred in the LPS group compared with controls (Table 1).

2.2. Activated protein C reduces white matter injury induced by LPS in the developing rat brain

In the present study, LPS-induced brain injury was reproduced as previously reported (Cai et al., 2000). We observed a significant increase in TUNEL-positive cells per field at P7 in

Table 1 – The effect of systemic APC treatment on brain weights, apoptotic cell and MBP staining in the periventricular WM

Treatment	Brain/body weight	Apoptotic cells/field P7 [a]	MBP staining, grading: 0~3 P7 [a]
1 – Control ($n=7$)	0.70/13.2	6.02±0.33	2.57±0.53
2 – LPS ($n=7$)	0.62/11.2	22.6±1.5	1.00±0.00
3 – LPS+ Prenatal APC ($n=7$)	0.67/12.5	13.3±1.5	3±0.00
4 – LPS+ Postnatal APC ($n=7$)	0.62/11.8	18.66±1.5	2.33±0.57
p values			
1 vs. 2	0.001	0.001	0.001
2 vs. 3	0.001	0.001	0.001
2 vs. 4	0.001	0.036	0.018

[a] The values are presented as mean±SD (min–max).

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