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SCIENCE DIRECT

Experimental Neurology 192 (2005) 109–116

Experimental Neurology

www.elsevier.com/locate/yexnr

Brief Communication

A novel therapeutic derived from apolipoprotein E reduces brain inflammation and improves outcome after closed head injury

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Received 5 August 2004; revised 5 October 2004; accepted 10 November 2004

Abstract

Although apolipoprotein E4 (APOE4) was initially identified as a susceptibility gene for the development of Alzheimer's disease, the presence of the APOE4 allele is also associated with poor outcome after acute brain injury. One mechanism by which apoE may influence neurological outcome is by downregulating the neuroinflammatory response. Because it does not readily cross the blood–brain barrier, the apoE holoprotein has limited therapeutic potential. We demonstrate that a single intravenous injection of a small peptide derived from the apoE receptor binding region crosses the blood–brain barrier and significantly improves histological and functional outcomes after traumatic brain injury (TBI). The development of an apoE-based intervention represents a novel therapeutic strategy in the management of acute brain injury.

Keywords: Apolipoprotein E; Brain inflammation; Closed head injury

Introduction

Traumatic brain injury (TBI) is a common and devastating health problem, with 500,000 cases annually in the United States (Narayan et al., 2002). After the initial trauma, secondary neuronal injury is associated with a neuroinflammatory response characterized by microglial and astrocytic activation, resulting in the release of reactive oxygen species and inflammatory cytokines. In particular, TNFα plays an important role in mediating blood–brain barrier breakdown and the development of cerebral edema. At present, there are no effective therapeutic interventions targeting the central nervous system (CNS) inflammatory response following TBI.

Apolipoprotein E (ApoE) is the primary apolipoprotein synthesized within the CNS, where it is upregulated after injury (Schauwecker et al., 1998). There are three common human apoE polymorphisms, designated E2, E3, and E4, which differ by single amino acid interchanges at residues 112 and 158 (Weisgraber, 1994). The presence of the

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0014-4886/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2004.11.014

apoE4 isoform has been demonstrated to predict poor outcome after head injury (Chen et al., 1997; Friedman et al., 1999; Teasdale et al., 1997). Although the mechanisms by which this occurs remain unclear, apoE has been demonstrated to reduce glial activation and the CNS inflammatory response in vitro and in vivo in an isoform-specific fashion (Barger and Harmon, 1997; Laskowitz et al., 1997, 2001; Lynch et al., 2001). These effects are isoform-specific, with the apoE4 isoform less effective than apoE3 at downregulating inflammatory cytokines in the peripheral circulation as well as in the brain (Laskowitz et al., 2001; Lynch et al., 2003).

Due to its large size, apoE does not readily cross the blood–brain barrier, and the peripheral pool of apoE is distinct from that synthesized in the CNS compartment (Linton et al., 1991). This renders administration of the intact protein an impractical therapeutic strategy for CNS disease. A small peptide apoE(133–149) created from the receptor binding region retains the ability of the intact protein to downregulate glial activation (Laskowitz et al., 2001), protect neurons from excitotoxic injury (Aono et al., 2003), suppress the release of the inflammatory cytokines TNFα and IL-6 after LPS injection (Lynch et al., 2003), and directly compete with apoE for binding to high affinity cell surface receptors (Misra et al., 2001). To test the potential therapeutic role for this apoE-mimetic peptide in closed head injury, we administered a single intravenous dose of peptide after experimental traumatic brain injury (Lynch et al., 2002) and assessed functional outcome. Additional mice were injured and treated in order to explore the hypothesis that apoE improves outcome by downregulating the CNS inflammatory response.

Methods

Closed head injury model

This study was approved by the Duke University Animal Care and Use Committee. This murine injury model (Lynch et al., 2002) was adapted from a previously described model of closed cranial trauma for the rat (Foda and Marmarou, 1994; Marmarou et al., 1994). Twelve- to twenty-week-old C57Bl/6J male mice (Jackson Laboratories, Bar Harbor, ME) were used in these experiments. Mice were intubated and mechanically ventilated with 1.6% isoflurane in 30% O_2/balance N_2. Rectal temperature was maintained at 36.5°C. The scalp was incised and the skull exposed. A concave 3-mm metallic disc was secured to the skull. A pneumatic piston (Air-Power, Inc. High Point, NC) was used to deliver a single midline impact to the skull surface, immediately caudal to bregma. The piston was discharged at 6.8 ± 0.2 m/s with a head displacement of 3 mm. Mice with depressed skull fractures were excluded. After impact, the animals were allowed to recover spontaneous ventilation prior to extubation.

Synthesis and administration of peptides

Peptides were synthesized by the University of North Carolina (Chapel Hill, NC) peptide facility to a purity of 95%. The parent 17 amino acid peptide was derived from apoE residues 133–149 (the receptor binding region; Ac-LRVRLASHLRKLRKRLL-amide). A scrambled control peptide of identical size, amino acid composition, and purity was also created. For behavioral studies, mice were injected via tail vein with high dose peptide (406 μg/kg), low dose peptide (203 μg/kg), or scrambled control peptide in vehicle (100 μL of isotonic sterile PBS) 30 min after head injury. For mechanistic studies, high dose (406 μg/kg) peptide was used.

Quantification of peptide in plasma and brain by liquid chromatography/mass spectrometry (LC/MS)

Injured (30 min post-closed head injury) and uninjured male C57/Bl6 mice were administered apoE(133–149) intravenously at a dose 4 mg/kg via tail vein injection (n = 3 mice/group). Mice were sacrificed at 5 min postinjection, and blood was obtained via cardiac puncture with heparinized syringe. Mice were perfused with saline and brains harvested. Plasma samples were obtained by centrifuging the blood samples at 13,000 rpm for 2 min. The perfused brains were removed and homogenized (0.1 g wet weight of brain/ml extraction buffer) in ice-cooled acetonitrile (ACN)/trifluroacetic acid extraction buffer containing an internal standard. A calibration curve was constructed by spiking brain homogenate obtained from untreated mice with known quantities of apoE(133–149).

Calibration samples were prepared by spiking plasma and brain homogenates from untreated mice with apoE(133–149) (0.005–25 μg/ml and 0.025–25 μg/0.1 g tissue for plasma and brain, respectively). Plasma standards and samples were extracted with ice-cooled PBS/acetonitrile (ACN)/trifluoroacetate (TFA) (50%:50%:1%) extraction buffer containing 3 μg/ml of an internal standard (IS) peptide (LRVRLASHLRKL*A*KRLL; an analog of apoE(133–149) containing a single R→*A* substitution). Brain standards and samples were homogenized in ice-cooled PBS/CAN/TFA extraction buffer (20%: 80%: 1%) containing 5 μg/ml IS. All samples were vortexed, incubated at −20°C for 5 min, and centrifuged at 11,000 rpm for 10 min at 4°C. Supernatants were transferred to LC/MS vials containing polypropylene inserts and were stored at −20°C until directly injected onto the LC/MS. Quantitation is based on the ratio of analyte: IS peak areas.

Liquid chromatography was performed on an Agilent Zorbax 300 SBC_{18} 2.1 mm × 75 mm × 5 μm column with a flow rate of 0.5 ml/min. Injection volume was 5 μl for plasma; 10 μL for brain. Mass spectrometry was performed detecting the $[M + 3H]^{+2}$ ion for apoE(133–149) at m/z