ELSEVIER

Neurobiology of Disease

www.elsevier.com/locate/ynbdi
Neurobiology of Disease 26 (2007) 36–46

TNF-α knockout and minocycline treatment attenuates blood–brain barrier leakage in MPTP-treated mice

Chaohui Zhao,[a] Zaodung Ling,[a] Mary B. Newman,[a,b] Ankush Bhatia,[a] and Paul M. Carvey[a,b,*]

[a]*Department of Pharmacology, Rush University Medical Center, 1735 W Harrison St, Cohn Research Building, Suite 406, Chicago, IL 60612, USA*
[b]*Department of Neuroscience, Rush University Medical Center, Chicago, IL, USA*

Received 22 September 2006; revised 14 November 2006; accepted 22 November 2006
Available online 17 January 2007

Following intraparenchymal injection of the dopamine (DA) neurotoxin 6-hydroxydopamine, we previously demonstrated passage of fluoresceinisothiocyanate-labeled albumin (FITC-LA) from blood into the substantia nigra (SN) and striatum suggesting damage to the blood–brain barrier (BBB). The factors contributing to the BBB leakage could have included neuroinflammation, loss of DA neuron control of barrier function, or a combination of both. In order to determine which factor(s) was responsible, we assessed BBB integrity using the FITC-LA technique in wild-type (WT), tumor necrosis factor alpha (TNF-α) knockout (KO), and minocycline (an inhibitor of microglia activation) treated mice 72 h following treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Compared with WT mice, TNF-α KO mice treated with MPTP showed reduced FITC-LA leakage, decreased numbers of activated microglia, and reduced proinflammatory cytokines (TNF-α and interleukin 1β) associated with significant MPTP-induced DA neuron loss. In contrast, minocycline treated animals did not exhibit significant MPTP-induced DA neuron loss although their FITC-LA leakage, numbers of activated microglia, and MPTP-induced cytokines were markedly attenuated. Since both TNF-α KO and minocycline treatment attenuated MPTP-induced BBB dysfunction, microglial activation, and cytokine increases, but had differential effects on DA neuron loss, it appears that neuroinflammation and not DA neuron loss was responsible for disrupting the blood–brain barrier integrity.

Keywords: Parkinson's disease; Neuroinflammation; Microglia; TNF-α; IL-1β; Minocycline; Endothelial cells

Introduction

Parkinson's disease (PD) is marked by the progressive loss of dopamine (DA) neurons in the substantia nigra (SN) (Hastings and Zigmond, 1997). While the etiology of PD remains unclear, both genetic factors and environmental toxins have been proposed in its pathogenesis (Ladeby et al., 2005; McGeer and McGeer, 2004). Regardless of the underlying etiology, neuroinflammation [the some total of cellular changes (e.g., microglial activation) and secreted factors (e.g., proinflammatory cytokines or free radicals) that accompany an inflammatory response within the CNS] is thought to contribute to the loss of DA neurons seen in patients with PD (McGeer and McGeer, 2004; Ladeby et al., 2005). Neuroinflammation is also present in trauma, stroke, multiple sclerosis, epilepsy and bacterial meningitis (Mennicken et al., 1999; Phillis et al., 2006) in which damage to the blood–brain barrier (BBB) has been reported (Huber et al., 2001). More recent studies point to microvascular changes in the SN and alterations in several markers of normal BBB integrity in PD patients (Barcia et al., 2004; Faucheux et al., 1999; Kortekaas et al., 2005). Whether actual changes in permeability, functionality, or physical damage of the BBB occur in PD is currently unknown.

We recently demonstrated that the DA neurotoxin, 6-hydroxydopamine (6-OHDA), compromised BBB integrity producing apparent leakage of both fluoresceinisothiocyanate-labeled albumin (FITC-LA) and horseradish peroxidase into the SN and striatum. This leakage was accompanied by loss of DA neurons, activation of microglia, up-regulation of p-glycoprotein and β-integrin on the endothelial cells that comprise the BBB, and attenuation of a DA-mediated behavior by domperidone, a DA antagonist that normally does not cross the BBB (Carvey et al., 2005). Since neuroinflammation including microglia activation and increased levels of proinflammatory cytokines including tumor necrosis factor-alpha (TNF-α) are present in patients with PD and in animal models of the disease (Aschner, 1998; Hirsch et al., 2005; Nagatsu and Sawada, 2005) and both are well known to affect the integrity of the BBB (Ryu and McLarnon, 2006; Tsao et al., 2001; Yenari et al., 2006), it is quite possible that 6-OHDA-induced neuroinflammation was responsible for the breakdown in barrier integrity. However, neurons containing biogenic amines are found in close proximity to brain capillaries (Rennels and Nelson, 1975; DiCarlo et al., 1984; Kapadia and de Lanerolle, 1984),

* Corresponding author. Department of Pharmacology, Rush University Medical Center, 1735 W Harrison St, Cohn Research Building, Suite 406, Chicago, IL 60612, USA. Fax: +1 312 563 3552.
E-mail address: pcarvey@rush.edu (P.M. Carvey).
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2006.11.012

endothelial cells express both noradrenergic and serotoninergic transporters (Wakayama et al., 2002) and receptors (Wakayama et al., 2002; Kobayashi et al., 1985), and stimulation of the locus coeruleus increases BBB permeability (Raichle et al., 1975) suggesting that neurotransmitters may regulate BBB function as well. The BBB leakage we observed in rats treated with 6-OHDA could be the result of neuroinflammation, DA neuron loss, or a combination of both. Therefore, we designed a set of experiments to determine the relative contribution of each to the leakage of the BBB.

Materials and methods

Animals

A total of 88 male mice, 8 weeks of age and weighing 22–25 g at the start of the study, were used. The TNF-α KO mice (B6;129S6-Tnf^{tmlGkl}/J; $n=22$), WT background control mice (B6;129S6; $n=22$), and C57BL/6 ($n=44$) mice were purchased from Jackson Laboratory (Bar Harbor, ME). All mice were acclimated to the animal facility for at least 2 weeks prior to the start of the study. One day prior to MPTP treatment, the mice were moved to a controlled ventilated room and housed in ventilation chambers until sacrificed. Mice were allowed free access to food and water for the duration of the study. The protocols used in this study were approved by the Rush University Medical Center Institutional Animal Care and Utilization Committee and were compliant with all regulations at the institutional, state, and federal levels. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–HCl (Sigma, St. Louis, MO) handling and safety measures followed methods described by Przedborski et al. (2001).

Study design

Study one

TNF-α KO ($n=22$) and WT ($n=22$) mice were randomly assigned to one of two groups (Saline or MPTP) for a total of 4 groups designated as follows: WT/Sal=WT mice treated with saline ($n=10$); WT/MPTP=WT mice treated with MPTP ($n=12$); TNF-α KO/Sal=KO mice treated with saline ($n=10$); and TNF-α KO/MPTP=KO mice treated with MPTP ($n=12$). TNF-α KO and WT mice were injected (i.p.) with either saline or MPTP and sacrificed 72 h later. MPTP–HCl (10 mg/kg freebase) was injected (i.p.) in a saline vehicle four times at 1-hour intervals for a total of 40 mg/kg over a 4-hour period. Saline treated mice followed the same injection protocol. After their injections, the mice were returned to their home-chambers and sacrificed after 3 days.

Study two

C57BL/6 mice received either saline or minocycline followed by either MPTP or saline injection. Mice ($n=44$) were randomly divided into four groups designated as follows: Sal/Sal=saline injections given in place of minocycline and MPTP ($n=10$); Sal/MPTP=mice treated with saline in place of minocycline, but treated with MPTP ($n=12$); Mino/Sal=mice treated with minocycline and given saline in place of MPTP ($n=10$); and Mino/MPTP=mice treated with minocycline and MPTP ($n=12$). Minocycline (90 mg/kg per day, dissolved in 5% sucrose, Sigma, St. Louis, MO) was injected (i.p) for 3 consecutive days, with the first minocycline dose administered 30 min prior to the first MPTP injection and last dose administered 24 h prior to sacrifice. MPTP–HCl (10 mg/kg per hour) was injected as described above.

In both studies, 5 mice from each treatment group were processed for immunohistochemistry and 5 mice were processed for biochemical analysis. Extra animals were added in all the MPTP groups to account for the anticipated 10% morbidity/mortality that is normally encountered in the MPTP protocol. Only animals that were overtly healthy (no apparent distress, normal appearance, and normal weight) were processed further. The dependent measures in each study were identical and consisted of visualization of BBB leakage throughout the brain, DA and microglia cell counts in the SN, and determination of levels of TNF-α and IL-1β protein in the SN and striatum.

FITC-LA leakage

The leakage of FITC-LA (MW=69–70 kDa, Sigma, St. Louis, MO) from vasculature into brain parenchyma was assessed as described previously (Carvey et al., 2005) to determine BBB integrity. Briefly, 3 days following the last MPTP or saline injections, the mice were anesthetized with pentobarbital (60 mg/kg). Heparin (100 units/kg in Hank's Balanced Salt Solution) was injected intracardially followed immediately by 5 ml FITC-LA (5 mg/ml, in 0.1 M phosphate-buffered saline (PBS) buffer) injected at a rate of 1.5 ml/minute with the right atrium open. After perfusion, the brains were removed immediately and immersed into 4% paraformaldehyde. Three days later, the fixative was replaced with three changes of 30% sucrose in 0.1 M PBS buffer. Each brain was sectioned at 40 μm using a sliding microtome, divided into 6 consecutive free-floating series, and stored in cryoprotectant (0.05 M PBS with 30% sucrose and 30% ethylene glycol). One series of sections from each of the 5 mice from both studies were mounted onto gelatin-coated slides, dehydrated, and cover-slipped for confocal microscopy (Olympus) analysis of FITC-LA leakage.

Immunohistochemistry

A second series of brain sections from each animal in both studies was used for tyrosine hydroxylase (TH, a DA neuron marker) immunohistochemistry as described previously (Carvey et al., 2005). Briefly, sections were washed (6×10 min) in Tris buffered saline (TBS, pH 7.4) and incubated for 1 h in 0.05% TBS Triton-X-100 solution. Sections were then incubated 30 min in TBS solution with 2.13% sodium periodate to block endogenous peroxidase activity and then incubated for 1 h with 3% normal goat serum in TBS. Tissues were incubated overnight with the primary antibody to TH (rabbit:anti-mouse, Chemicon, 1:1,000 dilution). Immunolabeling was continued using biotinylated secondary antibody (goat:anti-rabbit, Vector Laboratories, 1:200) in TBS with 3% goat serum and incubated for 1 h at room temperature. The antibody complex was amplified using an avidin–biotin kit (ABC-Elite kit; Vector) and visualized with 3,3′-diaminobenzidine (DAB) with nickel enhancement.

A third series of sections from each animal of both studies was processed for CD_{11b} immunohistochemistry to reveal activated microglial cells. The procedure for CD_{11b} immunochemistry was similar to the THir staining except that the primary antibody to CD_{11b} originated in rat (rat:anti-mouse CD_{11b} 1:200; Serotec) and the second antibody was goat:anti-rat IgG (1:200; Vector Laboratories).