Neurobiology of Disease 85 (2016) 174–186

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

TSPO in a murine model of Sandhoff disease: presymptomatic marker of neurodegeneration and disease pathophysiology

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ARTICLE INFO

Article history:
Received 29 September 2015
Accepted 2 November 2015
Available online 3 November 2015

Keywords:
Translocator protein 18 kDa
TSPO
Sandhoff disease
Biomarker
Microglia
Astrocytes
Neurodegeneration

ABSTRACT

Translocator protein (18 kDa), formerly known as the peripheral benzodiazepine receptor (PBR), has been extensively used as a biomarker of active brain disease and neuroinflammation. TSPO expression increases dramatically in glial cells, particularly in microglia and astrocytes, as a result of brain injury, and this phenomenon is a component of the hallmark response of the brain to injury. In this study, we used a mouse model of Sandhoff disease (SD) to assess the longitudinal expression of TSPO as a function of disease progression and its relationship to behavioral and neuropathological endpoints. Focusing on the presymptomatic period of the disease, we used ex vivo [^{3}H]DPA-713 quantitative autoradiography and in vivo [^{125}I]IodoDPA-713 small animal SPECT imaging to show that brain TSPO levels markedly increase prior to physical and behavioral manifestation of disease. We further show that TSPO upregulation coincides with early neuronal GM2 ganglioside aggregation and is associated with ongoing neurodegeneration and activation of both microglia and astrocytes. In brain regions with increased TSPO levels, there is a differential pattern of glial cell activation with astrocytes being activated earlier than microglia during the progression of disease. Immunofluorescent confocal imaging confirmed that TSPO colocalizes with both microglia and astrocyte markers, but the glial source of the TSPO response differs by brain region and age in SD mice. Notably, TSPO colocalization with the astrocyte marker GFAP was greater than with the microglia marker, Mac-1. Taken together, our findings have significant implications for understanding TSPO glial cell biology and for detecting neurodegeneration prior to clinical expression of disease.

1. Introduction

Sandhoff disease (SD) is an autosomal recessive disorder characterized by a deficiency in the lysosomal enzyme β-hexosaminidase, which leads to the accumulation of gangliosides and glycolipids, specifically GM2 and GA2 (Jeyakumar et al., 2002; Mahuran, 1999). Because gangliosides are expressed at high levels in the central nervous system (CNS), the brain is one of the most affected organs (Jeyakumar et al., 2002). Ganglioside accumulation in the brain ultimately leads to progressive and widespread neurodegeneration and motor impairments (Wada et al., 2000). In humans, the onset of the infantile form of SD occurs around 6 months of age with symptoms such as motor weakness, early blindness, macroencephaly, and seizures. Symptoms progress rapidly, and death occurs between 3 to 5 years of age (Maegawa et al., 2006).

β-Hexosaminidase is formed by the dimerization of two subunits, α- and β-subunits to form β-hexosaminidase A (αβ) or two β subunits to form β-hexosaminidase B (ββ). In the mouse model of SD, the gene encoding for the β subunit of β-hexosaminidase (*Hexb*) is disrupted, resulting in the deficiency of both β-hexosaminidase A and B (Yamanaka et al., 1994). This deficiency leads to impaired degradation and subsequent accumulation of GM2 and GA2 gangliosides in neurons (Sango et al., 1995; Jeyakumar et al., 2002; Mahuran, 1999), resulting in severe neurodegeneration in the brain (Wada et al., 2000). Sandhoff (*Hexb* KO) mice exhibit spastic and reduced hind limb movements with progressive motor deficits starting at 3 months of age (Sango et al., 1995). The life span of SD mice is approximately 5 months of age as the mice lose the ability to move and are unable to retrieve food or water (Sango et al., 1995; Tifft and Proia, 1997; Jeyakumar

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Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2015.11.001
0969-9961/

et al., 2002). Histopathological studies have shown that excess storage of glycolipids in lysosomes leads to neuronal apoptosis in the cerebellum, brainstem, spinal cord, trigeminal ganglion, retina, and thalamus in SD mice (Sango et al., 1995; Wada et al., 2000). Tissues from humans diagnosed with SD have also shown neurodegeneration in the spinal cord, cerebral cortex, and thalamus (Huang et al., 1997; Wada et al., 2000). Thus, the mouse model of SD shares many of the clinical symptoms and neuropathology as the human form of the disease.

Previous work has shown that microglia become activated in both the mouse model and in human cases of SD (Wada et al., 2000; Visigalli et al., 2009) and that microglial activation appears to precede neuronal degeneration. Furthermore, cDNA microarray analysis has shown increased TSPO gene expression in the SD mice (Wada et al., 2000), and using PET imaging, uptake of [^{11}C]-PK11195, a TSPO-specific ligand, was higher in SD mice at 4 months of age compared to wildtype (Visigalli et al., 2009). However, a longitudinal assessment of TSPO expression as a function of disease progression using behavioral and neuropathological endpoints has not been investigated. Furthermore, it has been previously reported that [^{11}C]-PK11195 PET imaging shows significant non-specific binding and poor brain uptake (Boutin et al., 2007; Endres et al., 2009), and thus, better ligands are needed for in vivo TSPO PET/SPECT studies.

DPA-713, also known as *N,N*-diethyl-2-[2-(4[methoxy-phenyl)-5,7-dimethyl-pyrazolo[1,5-a]pyrimidin-3-yl]-acetamide, is a pyrazolopyrimidine that is 10-fold less lipophilic than PK11195 but with twice the affinity for TSPO (Wang et al., 2009). [^{11}C]DPA-713 PET imaging has been performed in a rodent model of brain injury and in a human study with higher signal-to-noise ratios than [^{11}C]-PK11195 (Doorduin et al., 2009; Endres et al., 2009). [^{125}I]IodoDPA-713 is a novel TSPO-specific radioligand that has been previously used to detect TSPO in vivo in a mouse model of lung inflammation (Wang et al., 2009), but it is unknown whether [^{125}I]IodoDPA-713 can be used to detect in vivo TSPO binding in the brain. In this study, we used [^{3}H]DPA713 and [^{125}I]IodoDPA-713 to assess the progression of TSPO expression in SD mouse model in a longitudinal fashion. The goal of this study was to examine how early in disease progression TSPO levels increased in relation to: 1) neuronal GM2 ganglioside accumulation, 2) activation of microglia and astrocytes, 3) neurodegenerative changes based on silver staining, and 4) behavioral expression of disease focusing on the presymptomatic phase of the disease.

2. Materials and methods

2.1. Animal care and use statement

All animal studies were reviewed and approved by both Columbia University Medical Center and the Johns Hopkins University Animal Care and Use Committee. Studies were conducted in accordance with the Guide for Care and Use of Laboratory Animals as stated by the United States National Institutes of Health.

2.2. Animal model and tissue preparation

Mice heterozygous for *Hexb* were generously donated by Dr. Richard Proia (National Institute of Kidney & Digestive Diseases, Bethesda, MD). Wildtype (*Hexb* +/+) or SD (*Hexb* −/−) mice were euthanized at 1, 1.5, 2, or 3 months of age by either decapitation to obtain fresh-frozen brain tissue for receptor autoradiography or by transcardiac perfusion for immunohistochemistry. Fresh frozen brains were stored at −80 °C. For transcardiac perfusion, animals were deeply anesthetized with pentobarbital (100 mg/kg body weight) and perfused with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (pH 7.4 at 4 °C). Perfused brains were post-fixed overnight in the same fixative, cryoprotected with 25% sucrose for 48 h, flash-frozen in dry-ice-cooled isopentane, and stored at −80 °C.

2.3. Behavioral assessment

2.3.1. Motor skill

Motor skill was measured using the rotarod apparatus (Columbus Instrument, Columbus, OH). The latency time the mouse remained on the rod at accelerating speeds was recorded. Each mouse was trained for 5 min at a speed of 4 RPM. Training was followed by a 30-min rest period in the home cage. Mice were then placed back on the rotarod for three trials. Each trial began at 4 RPM and accelerated every 30 s by 4 RPM to a maximum of 40 RPM. Trials were separated by a 10-min rest period. Mice were tested for 3 consecutive days.

2.3.2. Locomotor activity

Mice were placed in open field activity chambers with infrared beams (San Diego Instruments Inc., San Diego, CA) for 1 h. During this time, horizontal activity and rearing were automatically recorded.

2.4. Quantitative receptor autoradiography

Fresh frozen brains were sectioned at 20 μm on a freezing cryostat (Leica, Nussloch, Germany) in the horizontal plane. Brain sections were thaw-mounted onto poly-L-lysine-coated slides (Sigma-Aldrich, St. Louis, MO) and stored at −20 °C. [^{3}H]DPA-713 autoradiography was used to assess TSPO levels in multiple brain regions. Adjacent brain sections were used as follows: slides were thawed and warmed on a slide warmer at 37 °C for 30 min and prewashed for 5 min in 50 mM Tris–HCl buffer (pH 7.4) at room temperature. Slides were incubated in a buffer containing 1.0 nM [^{3}H]DPA-713 for 30 min at room temperature. For non-specific binding, adjacent sections were incubated in the presence of 10 μM racemic PK11195. Slides were washed twice for 3 min each in 4 °C buffer and dipped twice in 4 °C deionized water. Sections were apposed to Kodak Bio-Max MR films with tritium microscales for 4 weeks (GE Healthcare, Piscataway, NJ). Images were acquired and quantified using the MCID software (InterFocus Imaging Ltd., Cambridge, England).

2.5. Immunohistochemistry

Brain sections from PFA-perfused animals were sectioned at 40 μm using a freezing microtome (Leica SM2000R; Leica Microsystems, Wetzlar, Germany). Sections were stored in cryoprotectant consisting of 50% glycerol in 0.05 M phosphate buffer at −20 °C. Sections were washed with Tris-buffered saline for 30 min. For GFAP and Mac-1 (CD11b) immunohistochemistry, sections were pre-treated with 0.6% H_2O_2 in TBS for 10 min and blocked with a 5% normal goat serum and 0.2% Triton X-100 solution for 1 h. Sections were incubated with rabbit anti-GFAP antibody (1:1000, Dako Z-0334, Carpinteria, CA) or rat anti-CD11b antibody (1:250, BD PharMingen 553,308, San Diego, CA) at 4 °C overnight. After washing with TBS, sections were incubated with the appropriate biotinylated secondary antibodies (1:200, Vector, Burlingame, CA) for 1 h. This was followed by an incubation in ABC elite, an avidin–biotin–horseradish peroxidase (HRP) complex (Vector) for 30 min. Immunoreactivity was visualized with a 0.25 mg/mL 3,3′-diaminobenzidine (DAB) (Sigma) and 0.03% H_2O_2 solution. Sections were mounted on slides, dehydrated in increasing concentrations of ethanol, and coverslipped using Permount media (Sigma). For double and triple labeling immunofluorescence, sections were washed with TBS for 60 min and then blocked in 5% normal donkey serum with 0.2% Triton X-100 solution for 1 h. Sections were incubated in primary antibodies diluted in blocking solution overnight at 4 °C using the following dilutions: rat anti-Mac-1 (1:250, BD Pharmingen 553308), rabbit anti-TSPO (1:500, Abcam ab109497), mouse anti-GFAP (1:2000, Millipore MAB3402), rabbit anti-GM2 (1:500, Millipore 345759), or mouse anti-NeuN (1:1000, Chemicon MAB377). After washing in TBS, sections were incubated with appropriate secondary antibodies (1:500, AlexaFluor488, AlexaFluor594, AlexaFluor647; Molecular