NeuroImage: Clinical 4 (2014) 711–717

Contents lists available at ScienceDirect

NeuroImage: Clinical

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

In vivo axonal transport deficits in a mouse model of fronto-temporal dementia

Tabassum Majid [a,b], Yousuf O. Ali [c,d], Deepa V. Venkitaramani [e], Ming-Kuei Jang [e], Hui-Chen Lu [c,d,f,g], Robia G. Pautler [a,b,g,*]

[a] Interdepartmental Program in Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, TX, USA
[b] Department of Molecular Physiology and Biophysics, Baylor College of Medicine, USA
[c] Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA
[d] The Cain Foundation Laboratories, Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, Houston, TX, USA
[e] Institute for Applied Cancer Science, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA
[f] Developmental Biology Program, Baylor College of Medicine, Houston, TX, USA
[g] Department of Neuroscience, Baylor College of Medicine, Houston, TX, USA

ARTICLE INFO

Article history:
Received 12 October 2013
Received in revised form 12 February 2014
Accepted 14 February 2014
Available online 31 March 2014

Keywords:
MEMRI
MRI
Axonal transport
Tau
Alzheimer's disease
Fronto-temporal dementia

ABSTRACT

Background: Axonal transport is vital for neurons and deficits in this process have been previously reported in a few mouse models of Alzheimer's disease prior to the appearance of plaques and tangles. However, it remains to be determined whether axonal transport is defective prior to the onset of neurodegeneration. The rTg4510 mouse, a fronto-temporal dementia and parkinsonism-17 (FTDP-17) tauopathy model, over-express tau-P301L mutation found in familial forms of FTDP-17, in the forebrain driven by the calcium–calmodulin kinase II promoter. This mouse model exhibits tau pathology, neurodegeneration in the forebrain, and associated behavioral deficits beginning at 4–5 months of age.

Animal model: rTg4510 transgenic mice were used in these studies. Mice were given 2 μL of $MnCl_2$ in each nostril 1 h prior to Magnetic Resonance Imaging (MRI). Following $MnCl_2$ nasal lavage, mice were imaged using Manganese enhanced Magnetic Resonance Imaging (MEMRI) Protocol with TE = 8.5 ms, TR = 504 ms, FOV = 3.0 cm, matrix size = 128 × 128 × 128, number of cycles = 15 with each cycle taking approximately 2 min, 9 s, and 24 ms using Paravision software (BrukerBioSpin, Billerica, MA). During imaging, body temperature was maintained at 37.0 °C using an animal heating system (SA Instruments, Stony Brook, NY).

Data analysis: Resulting images were analyzed using Paravision software. Regions of interest (ROI) within the olfactory neuronal layer (ONL) and the water phantom consisting of one pixel (ONL) and 9 pixels (water) were selected and copied across each of the 15 cycles. Signal intensities (SI) of ONL and water phantom ROIs were measured. SI values obtained for ONL were then normalized the water phantom SI values. The correlation between normalized signal intensity in the ONL and time were assessed using Prism (GraphPad Software, San Diego, CA).

Results: Using the MEMRI technique on 1.5, 3, 5, and 10-month old rTg4510 mice and littermate controls, we found significant axonal transport deficits present in the rTg4510 mice beginning at 3 months of age in an age-dependent manner. Using linear regression analysis, we measured rates of axonal transport at 1.5, 3, 5, and 10 months of age in rTg4510 and WT mice. Axonal transport rates were observed in rTg4510 mice at 48% of WT levels at 3 months, 40% of WT levels at 5 months, and 30% of WT levels at 10 months of age. In order to determine the point at which tau appears in the cortex, we probed for phosphorylated tau levels, and found that pSer262 is present at 3 months of age, not earlier at 1.5 months of age, but observed no pathological tau species until 6 months of age, months after the onset of the transport deficits. In addition, we saw localization of tau in the ONL at 6 months of age.

Discussion: In our study, we identified the presence of age-dependent axonal transport deficits beginning at 3 months of age in rTg4510 mice. We correlated these deficits at 3 months to the presence of hyperphosphorylated tau in the brain and the presence within the olfactory epithelium. We observed tau pathology not only in the soma of these neurons but also within the axons and processes of these neurons. Our characterization of axonal transport in this tauopathy model provides a functional time point that can be used for future therapeutic interventions.

* Corresponding author at: One Baylor Plaza, MC:335, Houston, TX 77030, USA.

http://dx.doi.org/10.1016/j.nicl.2014.02.005

1. Introduction

Fronto-temporal dementia (FTD) is a neurodegenerative disorder that accounts for approximately 20% of all pre-senile dementia cases (Wszolek et al., 2006). It is characterized by changes in reasoning, movement, speech, and language and primarily affects the frontal and anterior temporal lobes in the brain (Ingram and Spillantini, 2002). Diagnosis of FTD is difficult due to the fact that patients can display a wide variation in the severity of symptoms. Over the past quarter century, a proportion of FTD cases have been discovered to have a strong familial component. One of these includes FTD with parkinsonism (FTDP-17) (Wszolek et al., 2006). This form of FTD is associated with tau tangle pathology due to mutations in the microtubule associated protein tau (MAPT) gene on chromosome 17 (Wszolek et al., 2006). Current therapies have focused on delaying disease progression, but have been limited in their success. Part of the challenge in this area is identifying an optimal time point for intervention.

Autopsies from FTDP-17 patients have revealed neurofibrillary tau tangle pathology in the frontal and temporal lobes of the brain (Wszolek et al., 2006); (Snowden et al., 2002); (Goedert et al., 2012). The tau mutations in FTDP-17 have been linked to two major pathological outcomes (Combs and Gamblin, 2012). The first of these is disruption in alternative splicing that leads to a higher ratio of 4R tau rather than 3R tau. The second being the disruption of tau binding to microtubules in neurons, causing the accelerated accumulation of aggregate forms of tau as well as an increase in the formation of tau filaments (Wszolek et al., 2006). One of the more predominant mutations in the tau gene that is associated with FTDP-17 is the P301L mutation (Bird et al., 1999). This mutation results in a phenotype where the average age of onset is between 41 and 50 years, and is more associated with a personality change rather than with parkinsonism (Wszolek et al., 2006). However, most familial mutations causing FTDP-17 have been linked to tau's role in microtubule stabilization and assembly in neurons due to their presence in the coding regions of the tau gene. Finally, in FTDP-17 autopsy samples, tau oligomers have been identified and also have been demonstrated to propagate endogenous tau pathology in the brain (Lasagna-Reeves et al., 2012); (Goedert and Spillantini, 2011); (Karch et al., 2012).

The protein-coding region of tau encodes genes involved in axonal transport, or the transport of organelles and vesicles from the soma to the synapse of neurons. Organelles such as mitochondria and lysosomes are transported *via* both anterograde and retrograde axonal transport (Chevalier-Larsen and Holzbaur, 2006; Seamster et al., 2012). In addition, the role of axonal transport has been studied in models of neurodegenerative diseases such as ALS and Alzheimer's disease (Bearer, 2012); (Seamster et al., 2012; Smith et al., 2007; Zhang et al., 2004). For example, several studies in patient cell lines (Schulz et al., 2012; Trimmer and Borland, 2005) as well as *in vivo* models of neurodegenerative diseases have demonstrated deficits in this process prior to the onset of overt pathology (Kim et al., 2011); (Chevalier-Larsen and Holzbaur, 2006; Smith et al., 2010; Trimmer and Borland, 2005). Measuring axonal transport *in vivo* is very challenging. Manganese-enhanced MRI (MEMRI) is one of the few unique methods for axonal transport assessments in a non-invasive manner (Massaad et al., 2010; Smith et al., 2010); (Silva et al., 2004). MEMRI utilizes the presence of Ca^{2+} channels in neurons in order to enter cells and be transported down their axons (Inoue et al., 2011). MEMRI has many applications and has been used to measure rates of transport in models of both hyperglycemia as well as Alzheimer's disease (Kim et al., 2009) (Fu-Hua Wang, 2012). MEMRI can also be used to measure synaptic strength *in vivo* (Serrano et al., 2008). For example, in the amyloid overproducing Tg2576 mouse model, axonal transport deficits were detected using MEMRI prior to the onset of amyloid plaque deposits in the brains of these mice, indicating that the method reliably and sensitively can detect such deficits *in vivo* and such deficits more importantly precede disease pathology (Smith et al., 2007); (Massaad et al., 2010). In addition, a study demonstrated that WT human tau in a model lacking APP was enough to significantly worsen *in vivo* axonal transport deficits (Smith et al., 2010). Other studies have also demonstrated that axonal transport rates are unaffected by tau deletion or over-expression (Perez et al., 2013; Yuan et al., 2008). Furthermore, other studies using mouse models of tauopathy have implicated *ex vivo* deficits in axonal transport at late stages in disease progression post tau pathology (Zhang et al., 2004); (Zhang et al., 2010).

In this study, we characterized axonal transport deficits in the rTg4510 transgenic line, a mouse model of FTDP-17. This well characterized model carry the human P301L mutation found in several FTDP-17 cases (Combs and Gamblin, 2012). The expression of P301L tau in the forebrain is driven by the calmodulin kinase II (CaMKII) promoter. It has also been demonstrated that the CaMKII promoter has been shown to drive expression in the olfactory bulb (Liu, 2000). The phenotype of this mouse has also been used to test biomarker serum levels for tau spinal fluid levels, as well as several potential antioxidant biomarkers (Kopeikina et al., 2011); (Berger et al., 2007). Additionally, several learning and memory assays have been used to characterize the model, and these indicate a progressive, age-related decline in both short and long term memory beginning at 4 months of age (Yue et al., 2011); (Ludvigson et al., 2011). Anatomical MRI studies have also been conducted in this mouse model at critical points in the disease process, demonstrating that neurodegeneration is isolated to the forebrain (Kopeikina et al., 2011). A recent study using the MEMRI technique has indicated a correlation between neuronal transport and tangle pathology in an alternate model (JNPL3) of tauopathy (Bertrand et al., 2013). In this study, the authors longitudinally characterized JNPL3 mice for their pathology and correlated peak values of transport rates. However, to date, a characterization of axonal transport with extensive tau isoform characterizations at each age point has not been conducted in a well-characterized FTDP-17 model such as the rTg4510 mouse model of tauopathy.

Here, we characterized axonal transport rates in the rTg4510 mouse model of tauopathy and correlated these rates to the onset of tau tangle pathology in the mouse. Our data show that axonal transport deficits precede the deposition of tau aggregates and the formation of tau fibrillary tangles that are typically associated with cognitive deficits.

2. Materials and methods

2.1. Mice and genotyping

For this study, we used mice that over-express the P301L mutation in 4R0N human tau associated with FTDP-17. The generation of rTg (tau_{P301L}) 4510 mice (abbreviated as rTg4510 mice) has been described previously (Berger et al., 2007; Ludvigson et al., 2011). These transgenic mice were generated by F1 crossing of responder mice carrying tau_{P301L} cDNA with an upstream tetracycline-operon responsive element (abbreviated as P301L) and activator mice containing a trans-activator gene consisting of the tetracycline-off open reading frame placed downstream of the CaMKII promoter (abbreviated as tTA). Both transgenic lines were gifts from Michael Hutton (Mayo Foundation) and Karen Ashe (University of Minnesota Medical School). Littermates lacking both the responder transgenes (P301L) and activator (tTA) were used as wild-type controls (NTg). For all groups, both male and female mice were used, and pilot studies were conducted prior to data collection to confirm that there were no sex differences in these measurements.

Mouse genotypes were determined from tail biopsies using real-time qPCR with specific probes designed for each gene by a commercial service (Transnetyx, Cordova, TN, USA). For accurate genotyping, tail tissues from mice were digested overnight in extraction buffer (50 mM KCl; 10 mM Tris–HCl (pH 9.0); 0.1% Triton X-100; 0.4 mg/mL Proteinase K) at 55 °C to extract genomic DNA. Human tau and CAMKII transgene were detected using the following primers: CamKII tTa forward (5′-CGC TGT GGG GCA TTT TAC TTT-3′); CamKII tTa reverse