Utilizing MEMRI to Screen Drug Therapies in Mouse Models: The Effect of an $A\beta_{1-42}$ Lowering Drug, R-Flurbiprofen, on Axonal Transport Rates in an Alzheimer's Disease Mouse Model

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Alzhiemer's Disease (AD) effects approximately 4.5 million people in the United States. This number is expected to increase as current populations across the world continue to live longer. The hallmarks of AD pathology include extracellular amyloid beta (Aβ) plaques and intracellular neurofibrillary tangles comprised of hyper-phosphorylated tau. Recent studies indicate that accumulation of specific isoforms of Aβ such as $A\beta_{1-42}$ may contribute to disease symptoms[1]. Biomarkers that can identify AD symptoms *in vivo* and then monitor treatment responses to pharmacological therapy are desperately needed for improvements in early diagnosis and treatment. Previously, we have measured axonal transport rates *in vivo* utilizing Manganese Enhanced Magnetic Resonance Imaging (MEMRI) in the Tg2576 mouse model of AD and found deficits in axonal transport rates prior to plaque formation that was also evident after plaque formation[2]. It is currently unknown whether pharmacological reduction of $A\beta_{1-42}$ in these animals would recover the axonal transport deficits. In the present study we utilized MEMRI to determine whether treatment with the $A\beta_{1-42}$ lowering drug R-Flurbiprofen (R-F), a non-steroidal anti-inflammatory drug (NSAID), can recover axonal transport rates *in vivo*.

Methods

Homozygous mutant (K670N/M671L) Tg2576 (Tg) and age-matched littermate control mice (50% C57B6, 50%SJL) were obtained from in-house and from Taconic Farms, Inc. The NSAID R-F (Sigma) was administered for 3 days in 5% aqueous sucrose at a dose of 25mg/kg. Mice were imaged 1 week later. This dosing regime has been shown to be effective in other R-F mouse studies[1]. Control groups received only 5% sucrose. Animals were anesthetized with 5% isoflurane in 100% O_2 5 min prior to lavage. A manganese (Mn^{2+}) lavage of 4 μl of 0.75 g/ml $MnCl_2$ was administered 1 hr prior to imaging. Animals recovered prior to imaging and were then induced at 5% isoflurane and maintained with 2% isoflurane in 100% O_2. Images were acquired utilizing a 9.4T, Bruker Avance Biospec Spectrometer, 21 cm bore horizontal scanner with 35 mm volume resonator (Bruker BioSpin, Billerica, MA). The imaging parameters to acquire olfactory multi-spin/multi-echo MEMRI images were as follows: TR = 500 ms; TE = 10.2 ms; FOV = 3.0 cm; slice thickness = 1 mm; matrix = 128 x 128; NEX = 2; number of cycles = 15; each cycle ~2 min. Core temperature was maintained at 37°C during scanning. Data was acquired using Paravision (Bruker BioSpin) and then analyzed using linear regression and two-tailed t-tests with Prism (Graphpad Software, Inc). Region of interest (ROI) was placed on an axial slice 1.5 mm from the posterior of the olfactory bulb (OB). It measured 0.23 X 0.23 mm and was vertically centered on the dorsal olfactory neuronal layer. This ROI was copied for each cycle and each value normalized to un-enhanced muscle within the same slice[2].

Results

Figure 1 shows the measurement of the change in signal intensity (ΔSI) /time (min) as a result of increased Mn^{2+} transport over the course of 32 min in Tg animals treated with sucrose or sucrose plus R-F compared to normalized controls. The first two columns are the measurement of SI rate changes in 7 mth old Tg while the last two columns were obtained from 1yr old Tg animals. The Tg mice treated with sucrose alone exhibited transport rate deficits comparable to what we have previously reported at both age points[1]. The Tg mice treated with sucrose plus R-F exhibited minimal variance from normalized controls indicating recovery in axonal transport at both age groups.

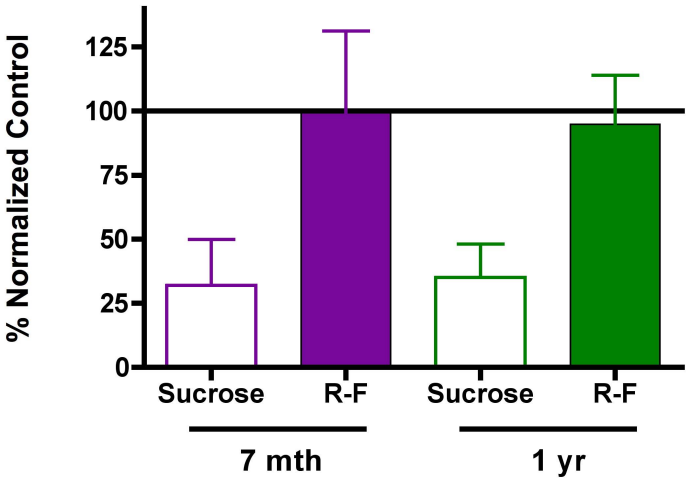

Figure 1. Tg2576 mice treated with either sucrose or sucrose plus R-F at 7 months and 1 year of age as % of Normalized Control. **7mth**: Sucrose N=6; R-F N=3. **1yr**: Sucrose N=3; R-F N=3. 100% line represents the average (ΔSI) /time (min) of control mice given 5% sucrose.

Discussion

The preliminary data reported here indicate that acute treatment with R-F has the ability to improve axonal transport rates in Tg2576 mice during the period of Aβ accumulation that occurs prior to plaque formation (~7 mth group), as well as after plaque formation (1yr group). We will continue to increase the N for an appropriate power analysis. In the near future, we will assess the levels of $A\beta_{1-42}$ in the treated animals to determine whether a decrease in $A\beta_{1-42}$ coincides with the improved axonal transport in this AD model to begin to establish causality.

While it has already been shown that MEMRI can be used to detect changes in the Tg2576 mouse model[2], this work demonstrates that MEMRI may also be utilized to detect improvements and recovery of function. We have found that treatment of the Tg2576 AD mouse model with R-F demonstrates marked improvement of previously reported axonal transport deficits utilizing MEMRI. These data collectively implicate $A\beta_{1-42}$ as having a deleterious effect on axonal transport and support the use of MEMRI as a pharmacological screening tool in mouse models of disease.

References

1. Eriksen, J. L., et al. / J Clin Invest **112**(3): 440-9 (2003)
2. K.D.B. Smith et al. / NeuroImage 35 (2007) 1401-1408