# Using 1H MR spectroscopy to evaluate the neurochemical effect of transgenic tauP301L suppression by doxycycline in rTg4510 mouse

Dewen Yang[1], David Caouette[1], Zhiyong Xie[1], Carol Hicks[1], Anthony Milici[1], and Thomas Bocan[1]
[1]*Pfizer Inc., Groton, CT, United States*

**Introduction** The high levels of transgenic $tau_{P301L}$ expression achieved in the Tet-Off rTg4510 strain induces age-dependent development of three major pathological hallmarks of human tauopathy: memory impairment, neurofibrillary tangle (NFT), and neuron loss[1]. Our previous high resolution 3D T2-weighted MRI study made possible the measurement of the volume of brain regions of interest in rTg4510 mice[2]. The results demonstrated that *in vivo* MRI/S can sensitively assess regional brain volumetric changes in aged rTg4510 mice. As expression of the P301L Tau transgene begins in early post-natal development, we evaluated the use of different Doxycycline (DOX) treatment protocols to either chronic suppress Tau expression from conception or to induce Tau expression in adult mice, once the brain has completed development. We found that continuous treatment of rTg4510 mice with Doxycycline (DOX) to repress expression of the Tau transgene that suppressed Tau pathology and Tau-associated brain volume loss as measured by volumetric MRI[3]. In addition, if we exposed rTg4510 mice to DOX prenatally and 2.5-month postnatal DOX treatment and followed up another 7.5 month to induce Tau expression, we found that there was an apparent reduction in both age-dependent Tau pathology and associated brain atrophy as assessed by volumetric MRI when compared to untreated animals with a similar duration of Tau expression[3]. In the current study, we designed a localized MRS study to investigate the neurochemical effect of both chronic and intermittent developmental transgenic $tau_{P301L}$ suppression by doxycycline in rTg4510 mouse hippocampus.

**Methods** 72 mice treated since conception with DOX (36 rTg4510 and 36 wt) were divided to four subgroups: 18 rTg4510 mice were continuously fed with DOX- mixed chow (Tg w dox); 18 rTg4510 mice were fed with DOX-mixed chow until 2.5 months of age and then switched to standard chow (Tg wd dox); 18 wild type mice were continuously fed with DOX- mixed chow (wt w dox); 18 wild type mice were fed with DOX-mixed chow until 2.5 months of age and then switched to standard chow (wt wd dox). The MRI measurements were conducted on a 4.7T magnet with a horizontal bore. All mice were anesthetized with 1.5-2% isoflurane in oxygen air delivered via a mouth piece. $^1$H MRS was performed using a 72-mm volume coil as the RF transmitter and a mouse brain quadrature surface coil as the receiver. After finishing scout images in the three orthogonal planes, the rectangular volume of interest (VOI) of 5.6mm × 1.5mm × 2.0mm for localized MRS was positioned in the hippocampal areas as illustrated in Fig. 1. The magnetic field homogeneity in this voxel was automatically adjusted to yield a water spectrum line width of 8-15 Hz. To acquire the spectrum, a PRESS sequence with the following parameters was used: TR/TE = 2500ms/11.4ms, and 512 averages. Water suppression performed with variable pulse power and optimized relaxation delays (VAPOR). Outer volume suppression was used to avoid spectral contamination. The acquisition time was 21 m 20s per spectrum. One unsuppressed water spectrum with the same parameters but with 16 averages was also acquired for each animal for water scaling and eddy current baseline correction in post-process. All data analyzed by LCModel. Only metabolites that were fitted with reliability index <10% were qualified for analysis. The data analysis was done using a 1-way ANOVA. Upon study completion, animals were euthanized and their brains were harvested and then processed and embedded in paraffin for immunohistochemistry staining. Experimental protocols were approved by the IACUC in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

**Results** All metabolites data are shown at Table 1. No statistically significant DOX treatment effect was found in Glu/Cr, NAA/Cr, Cho/Cr and mIn/Cr ratios. While moderate to high levels of hyperphosphorylated tau protein by AT8 and AT180 immunohistochemistry was detected in the hippocampus of the Tg wd dox mice, these animals, which were induced at 2.5 months to express P301L Tau for an additional 7.5 months, showed blunted Tau pathology when compared to 5-month-old rTg4510 female mice. In the pyramidal cells of the CA1 region of the hippocampus and the dentate gyrus (DG) of Tg wd dox mice, a minimal reduction in neuronal cellularity was observed relative to untreated 5 month old rTg4510 mice, and no observable difference existed between the Tg wd dox, Tg w dox, wt wd dox or wt w dox groups. Mild levels hyperphosphorylated tau protein was detected in ~ half of the Tg w dox, suggesting the DOX treatment did not fully repress Tau expression. No AT8 or AT180 staining was detected in age-matched wt w/wd dox.

**Discussion** Brief (6-8 weeks) and chronic doxycycline treatment of rTg4510 mice at different ages reduced the total levels of tauP301L mRNA to ~15% units of the maximum level, which decreased the amount of tauP301L mRNA relative to endogenous murine tau mRNA from ~15 units to ~2.5 units[4]. This level of reduction was sufficient to reverse and prevent Tau-associated pathology, behavioral deficits and neuronal loss. The MRS data presented here is consistent with this report. We find that mice chronically treated with DOX (Tg w dox) do not develop brain volume loss, MRS changes and Tau pathology reported in younger untreated rTg4510 mice[2]. In addition, we find that delayed inititaion of Tau expression leads to a blunted phenotype that is more similar to the hTau model, than to untreated rTg4510 mice aged to express Tau for a similar duration[5]. Despite induction of P301L Tau for over 7 months, the neuronal density within the CA1 and DG layers in the hippocampus and the degree of hyperphosphorylated Tau is far less then observed in untreated 5-month-old rTg4510 mice. MRS data reported here also shows no metabolites alterations after 7.5 months of Tau induction in adult mice, despite significant transgene-dependent differences occuring in younger untreated rTg4510 mice. Our data indicate that the environment in the early developing brain is necessary for full expression of the reported rTg4510 phenotype. However, the current data cannot distinguish between effects on Tau expression or on early developmental processes (such as neurogenesis) as a possible contributing factor.

**Conclusion** The present study demonstrated that prenatal combined with short-term postnatal DOX treatment to induce Tau expression within the adults brain diminishes hyperphosphorylated tau formation and significantly delays changes in brain metabolites. Across studies, the current MRS results agreed with the associated histological and immunohistopathological results as well as our previous volumetric MRI findings. The study further demonstrated that localized MRS is a helpful tool to assess brain metabolites in rTg4510 mouse.

**Table 1. Average relative concentration of metabolites (Mean±SD)**

| | Female mice (10 months old; n=18 per group) | | | |
|---|---|---|---|---|
| | Tg wd dox | Tg w dox | wt wd dox | wt w dox |
| NAA/Cr | 1.18±0.08 | 1.15±0.10 | 1.15±0.10 | 1.18±0.11 |
| Glu/Cr | 1.21±0.11 | 1.22±0.13 | 1.18±0.11 | 1.19±0.12 |
| mIn/Cr | 0.72±0.10 | 0.69±0.08 | 0.69±0.07 | 0.71±0.08 |
| Cho/Cr | 0.21±0.04 | 0.21±0.02 | 0.20±0.02 | 0.20±0.02 |

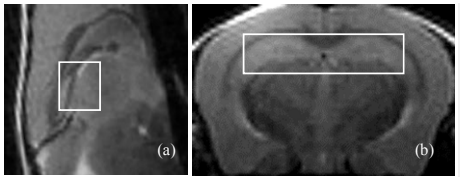


**Figure 1.** Voxel position for localized MRS in hippocampus on sagittal image (a) and coronal image (b).

**References** **1**.Ramsden M, et al (2005). Age-dependent neurofibrillary tangle formation, neuron loss, and memory impairment in a mouse model of human Tauopathy (P301L). The Journal of Neuroscience 25:10637–10647. **2**. Yang D, et al (2011). Volumetric MRI and MRS provide sensitive measures of Alzheimer's disease neuropathology in inducible Tau transgenic mice (rTg4510). Neuroimage 54: 2652-2658. **3**. Yang D, et al. Longitudinal MRI study to monitor brain changes of rTg4510 mice related tauopathy suppressed with/without Doxycycline. ISMRM 2011 Montréal, Canada. **4**. SantaCruz K, et al (2005). Tau suppression in a neurodegenerative mouse model improves memory function. Science 309: 476-481. **5**. Andorfer C, et al (2003). Hyperphosphorylation and aggregation of tau in mice expressing normal human tau i*soforms. Journal of Neurochemistry 86, 582–590.*