Longitudinal MRI study to monitor brain changes of rTg4510 mice related tauopathy suppressed with/without Doxycycline

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Objectives

Tau is the chief component of neurofibrillary tangles of Alzheimer's disease. Transgenic mouse models have enabled the study of mechanisms and therapeutic strategies for AD as well as validation of potential translational biomarkers. Recently, a mouse model of tauopathy was developed that reversibly expresses human tau with the dementia associated P301L mutation (rTg4510)[1]. The paper demonstrated that the mutant tau in rTg4510 mice could be suppressed by Doxycycline. Our recent *in vivo* MRI study proved that high resolution MRI was a sensitive tool to depict brain atrophy in 5-month-old rTg4510 female mice compared with age matched wild-type (wt) littermates[2]. To investigate the effect of Doxycycline in rTg4510 mice brains, we designed a longitudinal MRI study to follow up brain volume changes. We also compared the morphological MRI results with histopathological findings in the same animals.

Methods

48 female mice (24 rTg4510 and 24 wt) were treated with Doxycycline from the prenatal period until 2.5 months after birth. The 48 mice were divided to four subgroups: 12 rTg4510 mice were fed with Doxycycline mixed chow; 12 rTg4510 mice were fed with Doxycycline mixed chow and then switched to standard chow at 2.5 months of age; 12 wt mice were fed with Doxycycline mixed chow; 12 wt mice were fed with Doxycycline mixed chow and then switched to standard chow at 2.5 months of age. The MRI measurements were conducted on a 4.7T magnet with a 40-cm horizontal bore at 4 and 11 months of age. All mice were anesthetized with 2.5% isoflurane and maintained (1.4-1.7% isoflurane) in oxygen air delivered via a mouth piece. The mice were positioned in prone position with heads fixed to a plastic nose cone with the aid of a tooth bar and ear pins. Body temperature was maintained at 37.0±0.8ºC by a water heated bed and a water heated blanket. MRI was performed using a 72-mm volume coil as the RF transmitter and a mouse brain quadrature surface coil as the receiver. T2-weighted 3D RARE (FOV= 16×16×19.2 mm^3, matrix dimensions= 128×128×64, spatial resolution= 125×125×300 μm^3, TR/TE= 2600/23 ms, RARE factor= 16, total imaging time= 44m 22s 400ms) MRI scans were performed. Whole brains were extracted from T2-weighted images with a semi-automated image segmentation tool ITK-SNAP2. Hippocampus, cortex, lateral ventricle and cerebellum were delineated by an atlas-based segmentation system. An atlas was created based on a T2-weighted MR image (template) from a wild-type mouse. The template image was registered to the brain image of each animal using a deformable registration system[3]. With computed transformation, the label of the template was mapped to each subject. Computer-generated segmentation was then reviewed by a trained expert who was blinded to the group assignment and manual correction was made if necessary. The volumes of whole brain, hippocampus, cortex, lateral ventricle and cerebellum were measured. The analysis was done using SAS Version 8.02. The analysis was done using a 3-way ANOVA with interactions in the Proc Mixed procedure. Significance was decided by a 2-sided significance value of α=0.038 to account for multiple comparisons of correlated segmented brain sub-regions. Upon study completion, animals were sacrificed and their brains were harvested and fixed overnight in 4% paraformaldehyde. Brains were then processed and embedded in paraffin for sectioning.

Results

Table 1 summarized computed volumes of hippocampus, cerebellum, lateral ventricle and cerebral cortex for each animal. A significant genotype effect (rTg4510 vs wt) was observed only with the lateral ventricle volume ($p< 0.0001$). A significant Doxycycline condition effect was observed with cortex, cerebellum and ventricle regions ($p<0.038$). Genotype vs. Doxycycline interaction for hippocampus and ventricle were significant. Hyperphosphorylated Tau protein (AT8 and AT180) was detected by immunohistochemistry at mild to moderate levels throughout the hippocampus and the cerebral cortex in the rTg4510 mice switched to standard chow group at 11 months of age. The lateral ventricle volume correlated well with the degree of hyperphosphorylated Tau staining. No AT8 or AT180 staining was detected in age-matched wt controls fed with/without Doxycycline or in rTg4510 mice remained on Doxycycline.

Discussion

In our previous study, both the volume of the hippocampus and cerebral cortex in the rTg4510 female mice were significantly decreased (>20% changes), and the volume of the lateral ventricles in the rTg4510 mice were significantly increased (>26% changes) compared with that of wt mice, with no significant changes in the volume of the cerebellum (4% changes). In the current study, the rTg4510 mice fed with Doxycycline mixed chow or switched to standard chow groups didn't show clearly volume reduction in the hippocampus and cerebral cortex regions compared with that in the age matched wt mice since the volume changes were less than previous reported cerebellum volume changes (4%). The absence of effect with prenatal Doxycycline treatment observed in this study is consistent with the observed reduction in Tau pathology relative to untreated rTg4510 mice. However, we were able to detect a significant increase in the lateral ventricle volumes in the 11-month old rTg4510 groups when compared with that in 4-month old rTg4510 and wt groups. Combined with immunohistochemistry results, the volumetric MRI findings clearly indicated that the use of Doxycycline suppressed the P301L gene and delayed the progression of Tau pathology once Doxycycline was removed.

Conclusion

The current study further validated that the use of volumetric MRI as translational imaging strategies for Tau-based therapies in rTg4510 mice with the ultimate goal of development of therapies for Alzheimer's disease. The increased lateral ventricle volume was early indicator for Tauopathy progression in rTg4510 mice treated with Doxycycline.

Table 1. Comparisons of sub-regional brain volumes (Mean±SEM)

	Age	Volumes (mm^3) (n=12 per group)			
		wt w Dox	wt wo Dox	Tg w Dox	Tg wo Dox
Hippocampus	4-m	28.23±0.31	28.43±0.21	27.88±0.46	28.27±0.34
	11-m	28.79±0.30	28.58±0.23	29.14±0.37	28.59±0.81
Cerebral Cortex	4-m	129.87±1.01	132.11±1.03	127.15±1.19	131.63±0.21
	11-m	128.67±0.96	130.31±0.96	127.70±0.98	127.94±2.19
Cerebellum	4-m	68.62±0.59	69.30±0.53	68.36±0.37	68.98±0.39
	11-m	69.92±0.64	71.23±0.56	70.75±0.60	72.06±0.64
Lateral ventricle	4-m	0.72±0.06	0.69±0.05	0.73±0.06	0.97±0.17
	11-m	0.83±0.07	0.79±0.08	1.06±0.11	1.86±0.42*

SEM= standard error; Dox= Doxycycline; m= month; w/wo= with/without; * $p< 0.0001$

References:

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