

## Rapamycin as a therapy for vascular damage in Alzheimer's disease

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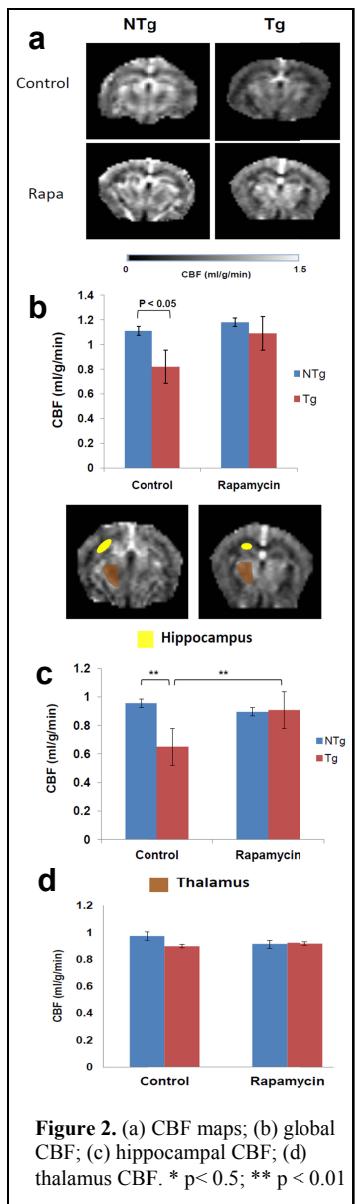
**Introduction:** Rapamycin, a drug originally used to keep the immune system from attacking transplanted organs, has been recently found having significant effects on increased lifespan and delayed aging in mice (1). Our group recently showed that treatment of mice modeling Alzheimer's disease (AD) with rapamycin halts the progression of AD-like memory deficits and reduces A $\beta$  accumulation in mice (2; Fig.1). The purpose of the present study was to use multi-metric neuroimaging systems (MRI and PET) to investigate the effect of rapamycin on hemodynamic, vascular and metabolic functions in brains of the AD transgenic mice. Cerebral blood flow (CBF) and angiography were determined by MRI, whereas cerebral metabolic rate of glucose (CMR<sub>Glc</sub>) was measured by PET.

**Material and Methods:** Alzheimer's transgenic mice (Tg; hAPP (J20)) and age-matched controls (NTg) (F:M = 4:4 for each group) were used in the study. Separate groups of Tg and NTg mice were fed with rapamycin, starting at 7 months of age (earliest detectable cognitive deficits). Imaging studies were conducted at 12 months of age. The study protocol was approved by the UTHSCSA IACUC. Mice were imaged under 1.0-1.2% isoflurane. Respiration rate (90-130 bpm) and rectal temperature ( $37 \pm 0.5^\circ\text{C}$ ) were continuously monitored. MR experiments were performed on a Bruker 7T magnet. CBF MRI was acquired using the arterial spin labeling (ASL) technique. Paired images were acquired in an interleaved fashion with FOV =  $12.8 \times 12.8 \text{ mm}^2$ , matrix =  $64 \times 64$ , slice thickness = 1 mm, 9 slices, labeling duration = 2100 ms, TR = 3000 ms per segment, and TE = 15 ms. ASL image analysis employed codes written in Matlab and STIMULATE software (University of Minnesota). Regional CBF was obtained from thalamus and hippocampus. MR angiography was obtained with 3D FLASH. FOV =  $12.8 \times 12.8 \text{ mm}^2$ , matrix = 128 x 128, TR/TE = 500/6 ms. PET measurements were performed on a Focus 220 MicroPET scanner.  $^{18}\text{FDG}$  of 0.5 mCi was injected through the tail vein. Emission data was acquired for 20 min after 40 min of injection. Glucose uptake was determined using the mean standardized uptake value (SUV<sub>mean</sub>) equation.

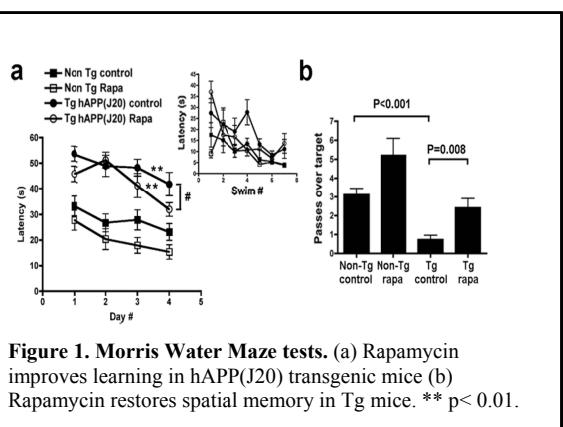
**Results:** Figure 2a shows global CBF maps from each group. AD Tg mice had significantly lower global CBF compared to the NTg littermates ( $p < 0.05$ ; Fig 2b). Rapamycin-treated Tg mice had similar CBF relative to the NTg groups ( $p > 0.5$ ). Rapamycin did not affect CBF in NTg groups ( $p > 0.5$ ). Tg mice also had reduced CBF in hippocampus ( $p < 0.01$ ; Fig 2c), which is involved in memory and learning, but not in the thalamus (Fig 2d). Similarly, rapamycin restored hippocampal CBF in the Tg mice (Fig 2c). Consistent with these observations, control- but not rapamycin-treated AD Tg mice showed reduced vessel density (Fig 3a). No significant differences in CMR<sub>Glc</sub> were found between control- and rapamycin-treated groups ( $p > 0.5$ ; Figs 3b and 3c).

**Discussion and Conclusion:** Our results demonstrate that hAPP(J20) Tg mice have significant vascular dysfunction (relative to metabolic), especially in the regions that have a prominent role in learning and memory (e.g., hippocampus). Rapamycin treatment restored vascular function in hippocampus, which may consequently preserve memory and learning in rapamycin-treated AD Tg mice. In conclusion, using multi-metric neuroimaging methods to determine cerebral hemodynamics and metabolism, we demonstrated that rapamycin can restore vascular integrity, possibly explaining its ability to preserve cognitive function. Rapamycin shows promise for future treatment and prevention of AD and potentially other neurodegenerative disorders.

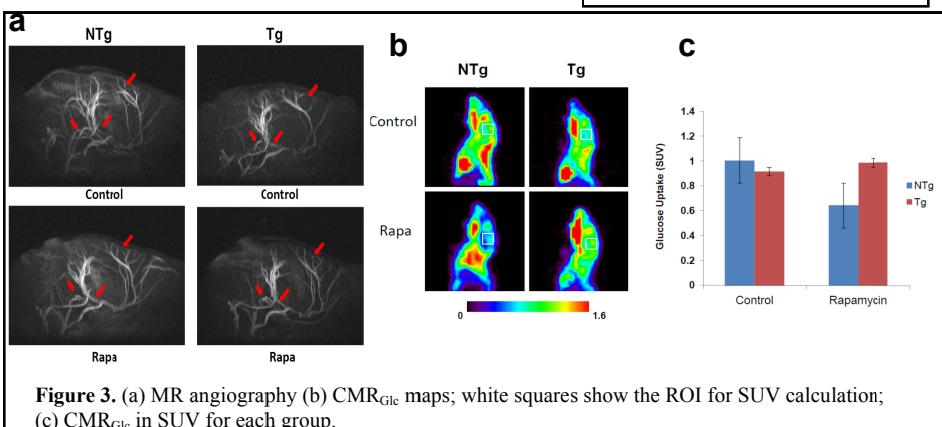
**References** (1) Harrison et al., Nature, 460:392, 2009 (2) Spilman et al PLoS One, 5:e9979. 2010.



**Figure 2.** (a) CBF maps; (b) global CBF; (c) hippocampal CBF; (d) thalamus CBF. \*  $p < 0.5$ ; \*\*  $p < 0.01$



**Figure 1. Morris Water Maze tests.** (a) Rapamycin improves learning in hAPP(J20) transgenic mice (b) Rapamycin restores spatial memory in Tg mice. \*\*  $p < 0.01$ .



**Figure 3.** (a) MR angiography (b) CMR<sub>Glc</sub> maps; white squares show the ROI for SUV calculation; (c) CMR<sub>Glc</sub> in SUV for each group.