

Rapamycin Restores Cerebral Blood Flow and Blood Brain Barrier Integrity in APOE4 Carriers

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Target Audience: The study will benefit general audience, clinicians and researchers who are interested in knowing the MRI-based evaluation of rapamycin treatment efficacy on cerebrovascular dysfunction in APOE4 carriers, who have high risk for Alzheimer's Disease.

Purpose: The ε4 allele of the apolipoprotein E gene (APOE4) is the major genetic risk factor for Alzheimer's Disease (AD)¹. Cross sectional studies in healthy APOE4 carriers show that cerebrovascular dysfunction was observed in carriers years before any clinical changes in memory occur, suggesting that cerebrovascular dysfunction is an initial event in AD². Cerebrovascular dysfunctions include cerebral blood flow (CBF) reduction and blood brain barrier (BBB) impairment, which lead to alteration in neuronal activity, production of proinflammatory cytokines, beta-amyloid deposition and loss of memory—pathological and psychiatric symptoms of AD. We have recently showed that rapamycin, an anti-aging intervention, can restore vascular function and increase CBF in rodents modeling human AD, and that the elevation in CBF was highly associated with enhanced memory³. In the study, we will determine whether rapamycin administered early in disease progression will restore cerebrovascular dysfunctions in the transgenic (Tg) APOE4 mice.

Methods: One month old of female wild-type (WT, C57BL/6, N= 6) and Tg APOE4 mice (N= 12) were purchased from the Jackson Laboratory. WT and six of the APOE4 mice were fed with control diet, whereas the other six APOE4 mice were fed with 14 ppm rapamycin (APOE4-Rapa). Diet was given for 6 months. MR experiments were performed on a Bruker 7T magnet. Mice were imaged under 1.0-1.2% isoflurane. Respiration rate (90-130 bpm) and rectal temperature (37 ± 0.5°C) were continuously monitored. CBF MRI was acquired using the arterial spin labeling (ASL) technique and was measured longitudinally on each mouse after 0 (baseline), 1, 3, and 6 month of feeding. Paired images were acquired with FOV = 12.8x12.8 mm², matrix = 64x64, slice thickness = 1 mm, 12 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. We used manganese (Mn)-enhanced MRI to determine BBB integrity at final time point (6 mo) as Mn would not penetrate BBB in normal condition. Manganese (II) chloride tetrahydrate from Sigma-Aldrich was dissolved in saline and injected intraperitoneally in the volume of 0.25 ml and dosage of 70 mg/kg. The animals were then released back to their cage with free access to water and food. Imaging was acquired after 5-6 hours of Mn injection using RARE: FOV = 12.8x12.8 mm², matrix = 128x128, slice thickness = 1 mm, 0.5 mm gap; 20 slices, TR = 170 ms, and TE = 5 ms. BBB integrity was determined by comparing the impaired and normal area (ratio) of certain brain region. We used one-way, repeated measures ANOVA to determine the difference of the measured indices between the three groups. Post-hoc testing was performed by Newman-Keuls test.

Results: One month old of APOE4 mice showed significant reduction (20%) in CBF compared to the WT (baseline measurement, Fig. 1), consistent with previous findings². With rapamycin treatment, however, APOE4 mice began to show restored CBF in one month (Fig. 2, a-b). The treatment efficacy was more prominent over time - the CBF in APOE4-Rapa were significantly higher than those of APOE4 group, but had no difference compared to that of WT after 6 months of treatment (Fig. 2b). Without rapamycin, APOE4 mice showed BBB impairment in temporal lobe, including ectothal (memory), perirhinal (memory) and piriform (smell) cortices, which were areas highly associated with AD pathology (Fig. 3). The BBB integrity has also been restored by rapamycin, with 45% reduction of impairment area relative to the non-treated group.

Discussion: Our results were consistent with previous findings that Tg APOE4 mice had significant vascular defects at very young age (as early as 2 weeks old)², particularly with phenotype of CBF reduction and BBB impairment. Here we showed that rapamycin was able to restore the cerebrovascular functions in the APOE4 mice, which is also consistent with our previous observation³. After 6 months of treatment, the CBF level and BBB integrity in the APOE4 mice resembled those of the WT mice. We hypothesize that rapamycin would enhance vascular function by activating the endothelial nitric oxide synthase and by inhibiting cyclophilin signaling pathways. Future studies are needed to identify possible mechanistic pathways of rapamycin on vascular integrity. As rapamycin is FDA-approved and the treatment efficacy of rapamycin in humans can be monitored over time using the same non-invasive CBF MRI methods used in this study, this research will have tremendous translation potential.

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Conclusion: We used multi-metric MRI methods to demonstrate that rapamycin can restore vascular integrity in mice expressing the human APOE4 gene. Rapamycin shows promise for future treatment and prevention of AD, vascular dementia and potentially other neurodegenerative disorders.

References: 1. Liu, C.C., *et al.* Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol* 9, 106-118 (2013); 2. Bell, R.D., *et al.* Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. *Nature* 485, 512-516 (2012); 3. Lin, A.L., *et al.* Chronic rapamycin restores brain vascular integrity and function through NO synthase activation and improves memory in symptomatic mice modeling Alzheimer's disease. *J Cereb Blood Flow Metab* 33, 1412-1421 (2013).

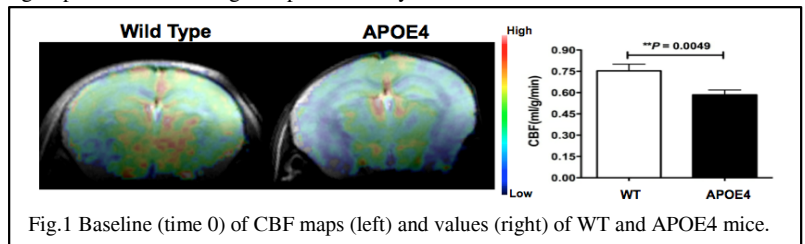


Fig.1 Baseline (time 0) of CBF maps (left) and values (right) of WT and APOE4 mice.

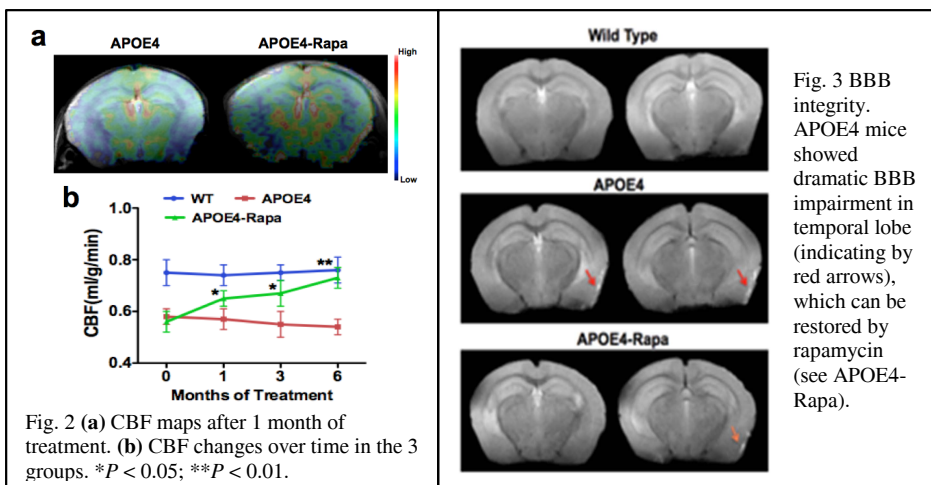


Fig. 2 (a) CBF maps after 1 month of treatment. (b) CBF changes over time in the 3 groups. **P* < 0.05; ***P* < 0.01.

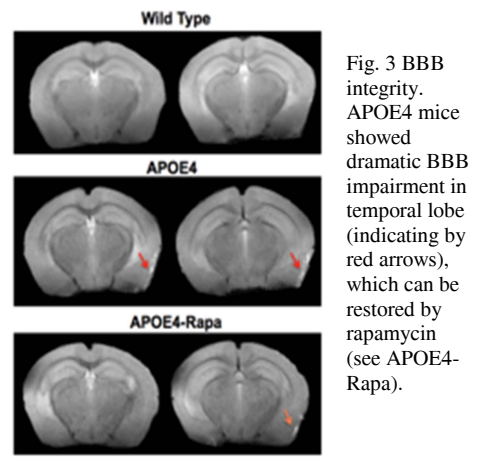


Fig. 3 BBB integrity. APOE4 mice showed dramatic BBB impairment in temporal lobe (indicating by red arrows), which can be restored by rapamycin (see APOE4-Rapa).