Age-dependent Neurovascular Changes in C57BL/6 Wild Type Mice Using Contrast Enhanced Micro-MR Angiography

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Introduction: There is increasing evidence that neurovascular dysfunction may be a very important risk factor in the development of Alzheimer's Disease (AD), an age-related condition [1, 2]. Additionally, other factors associated with vascular dementia, such as atherosclerosis, cerebral amyloid angiopathy, and cardiovascular disease increase the risk of developing AD. MR Angiography (MRA) is an invaluable, non-invasive technique to study the complexity of the neurovasculature both clinically and pre-clinically. Unlike Time-of-Flight MRA techniques that rely on endogenous properties of the vascular space, contrast enhanced-MRA (CE-MRA) is based on a blood pool agent and is less dependent on hemodynamics and the field of view. For this study, we utilized recently developed Gd-loaded micelles as a long living blood pool agent to achieve highly resolved $(100um)^3$ isotropic angiograms in mice in less than two hours. This approach was used to monitor three-dimensional neurovascular changes in individual C57BL/6 wild type mice over one year of normal aging.

Materials and Methods: Synthesis: Gd-loaded micelles were synthesized using a thin-film method in which Gd-DTPA, PEG, and Rhodamine bound lipids were combined. The chloroform-methanol solvent was gradually removed under heat and vacuum. Micelles were then formed when hydrated with the physiological buffer HEPES. Dynamic light scattering revealed a diameter of 12-15nm per micelle thus preventing leakage from the vasculature. The micelles maintain a plasma half-life of 1.4 hrs and a relaxivity of 11.6 s^{-1} mM^{-1} at 60MHz [3], both significantly higher than the corresponding values of Magnevist. MRI: All *in vivo* studies were performed in C57BL/6 wild type mice using a 7-T Bruker micro-MRI system interfaced to a 200mm horizontal bore magnet (Magnex Scientific, Yarnton, UK) with 750-mT/m and actively shielded. A modified 3DGE sequence acquiring a self-gating signal was used retrospectively for motion artifact-free image reconstruction. The effect of several micelles doses in comparison to Magnevist was monitored over a 2 hour period using a series of 30-minute $(150um)^3$ isotropic resolution scans and, upon determination of the appropriate dose, angiograms were acquired using an 87-minute $(100um)^3$ isotropic resolution scan, both performed using previously described parameters (N=7 at 2-4 months old, N=6 at 14-16 months old) [4]. All mice were anesthetized with Isofluorane and body temperature was maintained using a heating pad. Data Analysis: The resulting brain volumes were aligned through a series of rigid, affine, and non-linear registration steps using software provided by the Mouse Imaging Centre (Toronto, Canada) and using tools developed by the Montreal Neurological Institute (Montreal, Canada). The neurovasculature was assessed qualitatively using Maximum Intensity Projections (MIPs) and changes were quantified by an intensity-based segmentation of the vasculature using a thresholding function in which the vasculature was defined as the mean signal intensity of brain tissue + 2 standard deviations (ImageJ) (CA Berrios-Otero, personal communication). The defined threshold was utilized for an intensity-based segmentation of the neurovasculature for 3D visualization of this quantification.

Results and Discussion: The $(100um)^3$ angiograms were used to visualize neurovascular details and showed a decrease in detectable vasculature after 1 year of aging. This decrease was seen in the vascular volume obtained from the average brain of the 14-16 month old mice when compared to the average brain of the 2-4 month old mice (data not shown). This decrease was further visualized in 5 individual mice that were scanned at both ages. Fig 1. a&b illustrate an example of a mid-sagittal brain MRI slice obtained from the 3D MRA data set of an individual mouse followed over one year and Fig 1. c&d, the corresponding MIPs, show obvious microvascular enhancement differences in various areas of the brain (see red arrows in Fig 1. c). When using the previously defined criterion for vascular segmentation, we obtained a similar enhancement seen in the MIPs (Fig 2. a&b). The number of voxels corresponding to neurovasculature was quantified and demonstrated a significant decrease ($p<0.05$, two-tailed student's t-test) with age (Fig 2. c).

Conclusion: Our protocol enabled effective longitudinal monitoring of neurovascular changes with excellent contrast enhancement and high anatomical detail. Quantification of the vascular volume during normal brain aging in C57BL/6 wild type mice revealed a significant age-dependent decrease in neurovasculature. This study in normal aging mice serves as a baseline for future studies of age-dependent diseases such as Alzheimer's Disease.

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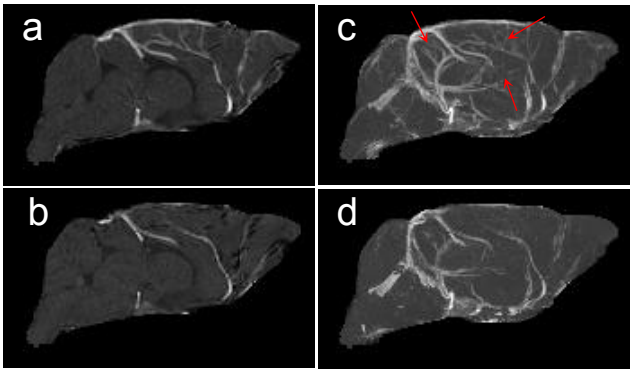

Fig 1: 2D mid-sagittal views from $(100um)^3$ 3D CE-MR angiograms of an individual mouse at 4 months (a) and 16 months old (b) and corresponding MIPs (c and d, respectively) revealing a decrease in detectable vasculature after aging one year.

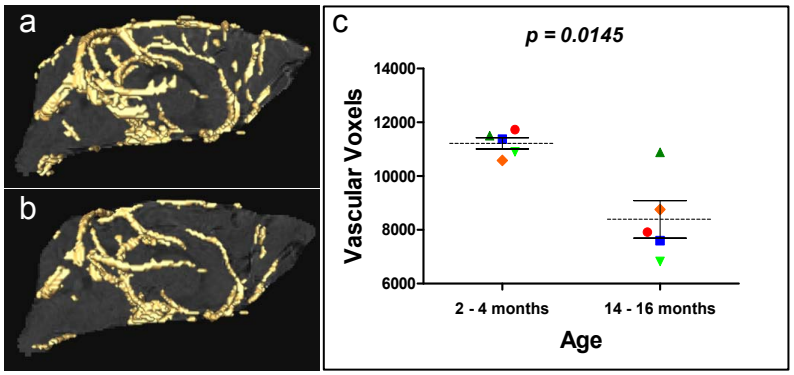

Fig 2: Intensity-based vascular segmentation superimposed onto a 2D mid-sagittal section at 4 months old (a) and 16 months old (b). Quantification of voxels corresponding to neurovasculature demonstrated a significant age-dependent decrease ($p=0.0145$); same color and shape data points represent individual mice, with the dashed lines showing the average number of vascular voxels in each age group (c).