Cerebral blood volume mapping of macro- and microvasculature in mouse brain with 3D gradient echo MRI

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Introduction The cause of Alzheimer's disease (AD) is still unknown and there is no cure for the disease yet. There are indications that impaired cerebral macrovascular and microvascular perfusion play an important role in the development of neurodegenerative diseases like AD (1). To investigate the separate roles of these two vascular compartments on neurodegeneration, we evaluated and optimized the methods for high-resolution mapping of the relative cerebral blood volume (rCBV) to distinguish between macro- and microvasculature with contrast enhanced MR imaging in a transgenic mouse model for AD.

Background Contrast enhanced MRI is used to compute rCBV, measuring the changes in plasma relaxation rate ΔR_2 or ΔR_2* induced by i.v. injection of a contrast agent (CA), using respectively Spin Echo (SE) and Gradient Echo (GE) sequences. To date, steady-state methods with super paramagnetic ultra small iron oxide particles (USPIO) are preferred for rCBV calculation because of their higher signal-to-noise ratio and higher spatial resolution above bolus tracking techniques with Gadolinium-related CA. In particular, the change in the transverse relaxation rates ΔR_2 and ΔR_2^* after injection of USPIO provides an index proportional to the blood volume of the microvasculature and total vasculature, respectively (2). Here we propose a new post processing method for the extraction of quantitative information from both macro- and microvasculature with GE imaging. The two most frequently applied methods to calculate ΔR_2 or ΔR_2* are: 1] the "two-point technique" using pixel-by-pixel logarithmic ratios between signal intensities before (S_{bef}) and after (S_{aft}) CA injection, divided by the echo time (TE). $\Delta R = (1/TE) \log (S_{bef}/S_{aft})$ and 2] the "fitting technique" computing the decay of pixel-by-pixel signal intensities by calculating this with a mono-exponential function with different TEs and extracting the relaxation time T_2 (or T_2*). Relaxation rate maps ΔR_2 (or ΔR_2*) are calculated subtracting $1/T_2$ (or $1/T_2$*) before and after CA. In this paper we present a comparison between the "two-point" and the "fitting" techniques to determine the optimal method for discrimination between rCBV mapping of macro- and microvasculature and for estimation of the ΔR_2*. Discrimination between pixels that belong to macro- and microvasculature is essential when quantification of rCBV in different vascular compartments is required.

Methods MRI measurements have been performed on a 7T/300mm ClinScan (Bruker Biospin, Ettlingen, Germany). A 3D Gradient Echo Flash sequence using six TEs (range 3-13ms) was performed in 7 transgenic APPswe/PS1dE9 mice and in 9 littermate C57BL6/J wild type mice before and after i.v. injection of USPIO (AMI-277, Sinerem®, Guerbet Laboratories, France, 130µg Fe/mouse). For this study a fast GE 3D sequence was chosen to obtain the same resolution in three dimensions, which is preferred when considering small regions of interest (ROIs) as used in mouse brain. Imaging parameters were: flip angle 10°, total resolution 0.16×0.16×0.16 mm, TR=50ms, Bandwidth=540 Hz, FOV=30×30×30 mm with a total scanning time of 20 min per mouse. Relaxation rates ΔR_2* maps of the entire brain were obtained for all mice with the "two point" and "fitting" techniques and results were compared. Image sets from the last three TEs (7-9-13ms) were used for the "two point" technique calculation and thereafter the resulting three ΔR_2* maps were averaged; these TEs were chosen as a compromise between signal to noise ratio and the T_1–weighting CA effects that can occur using short TE and TR (3). The distinction between macro- and microvasculature was performed using histogram analysis of the ΔR_2* maps: pixels included in the main part of frequency-intensity curve are supposed to belong mostly to microvasculature, while pixels with higher intensities are supposed to belong to macrovasculature only (Figure 1). According to this distinction, ΔR_2* maps of macro- and microvasculature were then selected (Figure 2) and diameter range determined in corresponding sections immunohistochemically stained for bloodvessels. Quantitative analysis of rCBV was then performed for each vascular compartment in all mice at 8 and 12 months of age. To assess the blood volume, ROIs that include the entire hippocampus, the prefrontal cortex and the entire brain were drawn on the images.

Results Considering all pixels in the entire brain, a statistical significant difference in ΔR_2* between the "two point" and "fitting" technique was found (respectively 36.7 ± 6 s^{-1} and 29.1± 7 s^{-1}, p= 0.001). However after pixel assignment with histogram analysis, pixels linked to microvasculature show a comparable value of ΔR_2* (respectively 22.9 ± 7 s^{-1} and 23.1± 5 s^{-1}, p= 0.370) while pixels linked to macrovasculature still show a strong statistical significant difference (respectively 95.4 ± 12 s^{-1} and 74.2 ± 18 s^{-1}, p= 0.001). Pixel-by-pixel map comparison showed high amounts of errors (ΔR_2*<0) in the "fitting" technique within the macrovasculature. Errors are proven to be caused by the underestimation of the slope of the exponential fitting curve when CA concentration is high. Significant difference between APPswe/PS1dE9 and the control group was found in the microvasculature compartment of the hippocampus at 12 months (respectively 0.88 ± 0.03 and 1.002 ± 0.06, p=0.004**), as expected from other studies on this mouse model (4). Decrease in rCBV was also found in all mice in hippocampus microvasculature as consequence of aging (1.09 ± 0.07 at 8 months and 0.962 ± 0.08 at 12 months, p= 0.005**). No differences were found in all ROIs without separation of vascular compartments for macro- and microvasculature perfusion. **Data are expressed as $\frac{\Delta R2^* \text{ hippocampus microvasculature}}{\Delta R2^* \text{ total brain microvasculature}}$.

Conclusion We demonstrated that GE MR imaging with histogram analysis provides a valid tool to distinguish and quantify rCBV in macro- and microvasculature. Our data indicate that the fitting technique produces underestimation and errors in the ΔR_2* value of the macrovasculature compartment. On the other hand, the "two point" technique with proper setting is more sensitive and able to measure both macro- and microvasculature. Therefore the "two point" technique with histogram analysis is preferred when a detailed characterization of the architecture of blood vessels is required, as in the case of AD mouse models.

References

1) De la Torre JC. *Alzheimer disease as a vascular disorder: nosological evidence*. Stroke. 2002 Apr;33(4):1152-62. Review.
2) Kennan RP, Zhong J, Gore JC. *Intravascular susceptibility contrast mechanisms in tissues.* Magn Reson Med 1994;31:9– 21.
3) Troprès I, Grimault S, Décorps. *Vessel size imaging*. M. Magn Reson Med. 2001 Mar;45(3):397-408.
4) Hooijmans CR, Rutters F, Kiliaan AJ. *Changes in cerebral blood volume and amyloid pathology in aged Alzheimer APP/PS1 mice on a docosahexaenoic acid (DHA) diet or cholesterol enriched Typical Western Diet (TWD).* Neurobiol Dis. 2007 Oct;28(1):16-29.

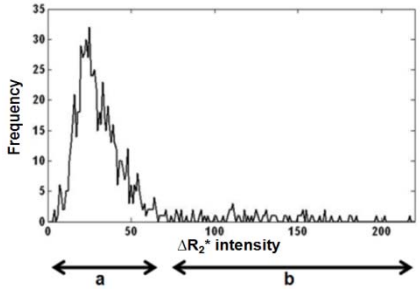

FIG. 1 Frequency-intensity histogram of ΔR_2* of the entire brain (a) ΔR_2* intensity range of microvasculature (b) ΔR_2* intensity range of macrovasculature

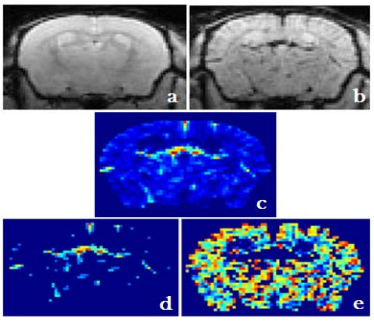

FIG. 2 (a) T2*-weighted image before USPIO injection (b) T2*-weighted image after USPIO injection (c) ΔR_2* map (d) ΔR_2* map of pixels belonging to macrovasculature (e) R_2* map of pixels belonging to microvasculature