In vivo imaging of vessel diameter, size and density: a comparative study between MRI and histology

Benjamin Lemasson[1,2], Samuel Valable[1,3], Régine Farion[1,2], Alexandre Krainik[1,2], Chantal Rémy[1,2], and Emmanuel L Barbier[1,2]
[1]U836, Inserm, Grenoble, France, [2]Grenoble Institut des Neurosciences, Université Joseph Fourier, Grenoble, France, [3]UMR 6232, CNRS, Caen, France

INTRODUCTION The assessment of angiogenesis contributes to determining tumor grade and prognosis (1). To characterize angiogenesis *in vivo* with MRI, one usually maps the blood volume fraction (BVf) using a bolus tracking approach (2). On the one hand, BVf increases with the number of microvessels within an imaging voxel. On the other hand, BVf may remain constant in case of microvessel diameter increase and vessel density decrease (3), and thus becomes blind to angiogenic processes. Using MRI, one may also image angiogenesis beyond BVf by measuring the changes in transverse relaxivities induced by the injection of an intravascular contrast agent: changes in R_2 (noted ΔR_2 and measured using spin-echoes) and changes in R_2* (noted ΔR_2* and measured using gradient-echoes). Three processing approaches have then been proposed. (**i**) Dennie et al. introduced $\Delta R_2^*/\Delta R_2$ as a non-quantitative estimate of the mean vessel diameter in the voxel (mVD_{MRI}) (4), (**ii**) Based on $(\Delta R_2)^3/(\Delta R_2^*)^2$, Jensen et al. derived an estimate of the microvessel density ($Density_{MRI}$) (5), and (**iii**) Based on $(\Delta R2^*/\Delta R2)^{3/2}$, Tropres et al. defined a quantitative index of the distribution of microvessel diameters within the voxel, the vessel size index (VSI_{MRI}) (6). In this study, we compare these three estimates obtained using MRI and histology from rats bearing either a C6 or a RG2 glioma.

MATERIELS AND METHODS ***Animals:*** Male Wistar (n=15) and Fischer 344 (n=12) rats were used for the C6 and RG2 high grade glioma models, respectively. Each animal was imaged once by MRI and euthanized immediately after for histology. The C6 model was imaged 11 (n=4), 15 (n=4), 20 (n=4) and 25 days (n=3) after tumor implantation and the RG2 model 6 (n=3), 10 (n=4), 14 (n=2) and 18 days (n=3) after tumor implantation. ***In vivo MRI:*** Experiments were performed on a horizontal 2.35 T. Maps of Apparent Diffusion Coefficient (ADC) of water were computed from 3 diffusion-weighted spin-echo images (in X, Y, and Z directions) with b=900 $s.mm^{-2}$ and a reference image (b≈0 $s.mm^{-2}$) (voxel size=234x454x1000 μm^3). A multi gradient-echo and spin-echo MRI sequence (TR=6s, 7 evenly spaced gradient-echoes = [6-42] ms, 1 spin-echo=102 ms, voxel size=234x454x1000 μm^3) was acquired just prior to and 4 min after administration of Sinerem® via the tail vein in about 20 sec (200 µmoles of iron/kg body weight) (Sinerem®, Guerbet SA, France; Combidex®, Advanced Magnetics, Inc, USA). ***Histology:*** At the end of the MRI experiment, the rat was euthanized and its brain was quickly removed, snap-frozen in liquid nitrogen and stored at -80°C. Brains were sliced (20 µm thick sections). Vascular structures in brains were detected by immunohistochemistry (collagen IV). Adjacent sections were stained with hematoxylin-eosin (HE) in order to delineate the tumor. ***Data analysis*****:** Three ROIs (contralateral striatum, contralateral neocortex, and tumor) were delineated on the slice (ADC map for MRI and HE section for histology) containing the largest tumor area. The ROI "tumor" corresponded to the region of modified ADC and was reported on the other MRI maps. Based on the mean ΔR_2 and ΔR_2*, the mVD_{MRI}, $Density_{MRI}$, and VSI_{MRI} were computed and described in (4-6) using Matlab. For histology, the ROIs drawn on the HE sections were reported on the section labelled with Collagen IV. Up to three microscopic field of views were analyzed using ImageJ (i.e. digitization, binarization, and morphological description of each object) per ROI to compute mVD_{histo}, $Density_{histo}$ and VSI_{histo}, as described in (7).

RESULTS Microvascular estimates obtained in normal brain (averaged across neocortex and striatum and across all animals) are in good agreement with previously reported values. Dennie *et al.* reported a mVD_{MRI} of 4.8±0.3 a.u. in the rat brain (4), while we observed 3.7±0.6 a.u. Wu *et al*. reported a $Density_{MRI}$ of 282±43 $vessel.mm^{-2}$ in the mouse brain (7), while we observed 271±83 $vessel.mm^{-2}$. Tropres *et al*. reported a VSI_{MRI} of 4.5±0.8 µm in the rat brain (17), while we observed 4.2±0.9 µm. Figure 1 shows the correlation between the three MRI estimates and their corresponding histological estimates for all ROIs and all animals (data from both tumor models were pooled). A positive correlation is obtained for the three microvascular characteristics studied *in vivo* and *ex vivo*: mVD (Fig. 1a), Density (Fig. 1b), and VSI (Fig. 1c). The lowest correlation between MRI and histology is obtained for Density (R^2=0.50, $p<0.001$). The correlation between mVD_{MRI} and mVD_{histo} is better (R^2=0.62, $p<0.001$) and the best correlation is found between VSI_{MRI} and VSI_{histo} (R^2=0.73, $p<0.001$). One also found a very good correlation between mVD and VSI (R^2=0.79 between MRI estimates; R^2=0.76 between histological estimates; data not shown; $p<0.001$ in both cases).

DISCUSSION In conclusion, this study indicates that MRI and histological estimates of mVD, Density, and VSI obtained on the same animals are well correlated and may be used to characterize angiogenesis *in vivo* beyond BVf. VSI is the closest MRI estimate to histology. As all three estimates may be derived from the same ΔR_2 and ΔR_2* measurements and as they give complementary information, it is worth computing them all. While the results obtained in this study were obtained using a USPIO and a steady-state approach, it has recently been shown that a dynamic approach using Gd-chelate yielded similar results to those obtained with USPIO at steady-state (8). A combination of blood volume fraction, Density, and VSI or mVD (the latter two are well correlated) appears thus desirable to improve the characterization of angiogenesis in patients.

REFERENCES [1]Daumas-Duport *et al.* J Neurooncol 1997. [2]Wintermark et al. Stroke 2005. [3]Valable *et al.* NMR in Biomed 2008. [4]Dennie et al. MRM 1998. [5]Jensen *et al.* MRM 2000. [6]Tropres *et al.* MRM 2001. [7]Wu *et al*. NMR in Biomed 2004. [8]Pannetier *et al.* NMR in Biomed, early view.