Blood flux imaging in rodent brain using FENSI

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Introduction: Perfusion-MRI is of prime interest to diagnose and monitor non-invasively the vascular changes associated with cerebral diseases such as stroke[1] or tumors[2]. We present here the first preclinical in-vivo application of the Flow ENhanced Signal Intensity (FENSI) perfusion method[3]: we used FENSI to characterize and quantify tumor microvascular flux in a rat glioma model (9L) at high magnetic field (7T). As ASL, FENSI uses magnetically labeled arterial blood water as an endogenous tracer. However, the imaging setup, as well as the tagging strategy, is different in FENSI, as illustrated in Figure 1. In pcASL, a train of short 180° pulses is used to continuously invert the magnetization in one or several arteries. Cerebral perfusion is obtained by acquiring signal in the brain after a delay matching the blood transit time. In FENSI, spins are being repeatedly saturated as they pass through a slice of interest with a train of short 90° RF pulses. The signal intensity difference between "control" and "tag" in the imaging slice is proportional to the microvascular blood flux through the labeling plane.

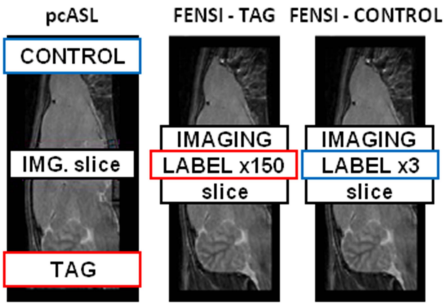

Figure 1. Comparison of a pcASL and a FENSI experiment. Both methods use a train of short RF pulses to respectively invert/saturate the spins magnetization.

Materials and Methods: 10^5 9L glial cells were implanted in the left striatum of 5 SD rats and imaged between D+5 and D+15 on a 7T Bruker PharmaScan system. The sequence used was a FENSI-SE-EPI sensitized to slow velocities in order to access microvasculature (4 segments, TE/TR=13/6000ms, total labeling time 3s, matrix 100x100, in-plane res. 250x280µm^2, labeling/imaging slice thickness=1/6.5mm). The labeling module consisted in a train of short RF pulses (150 pairs of 45° flip angle 0.8 ms sinc pulses). In the "control" preparation module, every other 45° RF pulse was subject to a +π phase shift to refocus the spins and account for MT effects, as in TILT[4]. A triple-pulsed saturation was used in the FENSI «control» image (see Figure 1) to remove the contribution from the static tissue to the signal. T2w images were acquired for tumor localization (RARE, TE/TR=56/3000ms, NX=8, NA=4, res 200x200x1000µm, TA 3min12s). Histology staining was performed on one rat: nuclei (DAPI) and endothelial cells (CD31) concentrations were co-registered with the blood flux map obtained with FENSI. The MT ratio introduced by FENSI was calculated in preliminary tests on phantoms and postmortem rat brain.

Results: In the current implementation, the FENSI method was completely insensitive to MT effects (MTR=0.1±0.2%). Blood flux results on 5 rats in tumor and control regions at different tumor stages are listed in Table 1. Figure 2A presents a typical blood flux map calculated with FENSI one week after injection. The tumor was localized on the T2-weighted image (Figure 2B). We highlight a significant increase (12–48%) in tumor blood flux at an early stage (tumor size < 2mm) compared to contralateral healthy brain blood flux. At late stage (size > 3.5mm), we observe with FENSI a compartmentalization of the tumor (delimited by the red arrows in the blood flux map on Figure 2C). In the flux map the tumor core shows hypointense (55±1 µL/min/cm²), whereas the tumor periphery presents higher perfusion (83±23µL/min/cm²), comparable in several rats to contralateral cerebral blood flux (112±9 µL/min/cm²). Figure 2D presents the results of the histological staining of nuclei (DAPI, blue) and endothelial cells (CD31, green) performed on the tumor shown in Figure 2C. Hyper- and hypo-perfused regions of the tumor delineated with FENSI correlate well with regions of high and low concentration of endothelial cells (i.e. blood vessels) observed with fluorescence microscopy.

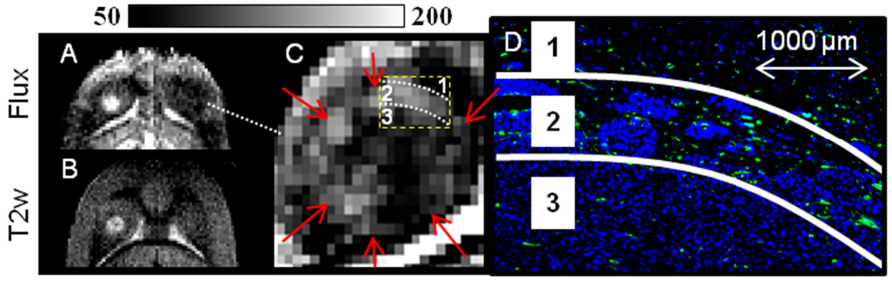

Figure 2. Blood Flux parametric map [µL/min/cm²] calculated with FENSI at (A)D+7 and (B)corresponding T2w image used for tumor localization. (C)Zoom on a Blood Flux map at late tumor stage (D+12). The red arrows indicate the borders of the tumor. (E)CD31/DAPI staining on the same tumor as in C. We co-registered the location of the histological slice with the yellow ROI. The tumor edge [2] presents a higher concentration of endothelial cells than healthy tissue [1] or tumor core [3].

Discussion/Conclusion: We present here for the first time non-invasive, longitudinal quantification of blood flux on a 9L rat tumor model with FENSI.. Based on blood flux parametric maps, the tumor core and periphery display different vascular content. This is in agreement with histology and literature on 9L characterization including the creation of new vasculature in the early stage and the apparition of a solid tumor core in the late stage. FENSI presents an interesting alternative to ASL for micro-perfusion studies, as the blood flux quantification does not require knowledge of transit times and does not suffer from the T1 relaxation of the labeled spins.

TABLE 1. Evolution of tumor blood flux (µL/min/cm²) with tumor size. (*) The animal was sacrificed at D+9, there is no data for this size.

	Tumor size (mm)	**1.3 – 2.0**	**2.1 – 3.5**	**3.6 – 7.0**
Rat 1	Control // Tumor Core // Periphery	136 ± 15 // 130 ± 15	126 ± 20 // 150 ± 24	107 ± 14 // 77 ± 24 56 ± 14 // 83 ± 23
Rat 2	Control // Tumor Core // Periphery	150 ± 20 // 180 ± 30	127 ± 14 // 147 ± 27	104 ± 14 // 66 ± 16 52 ± 10 // 72 ± 14
Rat 3	Control // Tumor Core // Periphery	124 ± 14 // 137 ± 20	96 ± 14 // 70 ± 10	121 ± 14 // 84 ± 19 72 ± 14 // 87 ± 20
Rat 4	Control // Tumor Core // Periphery	131 ± 13 // 144 ± 20	125 ± 12 // 98 ± 33 55 ± 12 // 119 ± 13	(*)
Rat 5	Control // Tumor Core // Periphery	96 ± 12 // 90 ± 8	84 ± 13 // 124 ± 12	107 ± 14 // 55 ± 17 42 ± 9 // 56 ± 16

References: [1]Chalela JA *et al*. Stroke 2000 [2]Wolf RL. *et al.* JMRI 2005 [3]Reynaud O, Ciobanu L. MRM 2010 [4] Golay X *et al.* JMRI 1999
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