

## Evaluation of parametric response map (PRM) as an imaging biomarker in the study of a cell therapy in stroke

Rui He<sup>1,2</sup>, Benjamin Lemasson<sup>1,2</sup>, Anaïck Moisan<sup>1,2</sup>, Olivier Detante<sup>2,3</sup>, Chantal Rémy<sup>1,2</sup>, Alexandre Krainik<sup>2,3</sup>, and Emmanuel Luc Barbier<sup>1,2</sup>

<sup>1</sup>U836, Inserm, Grenoble, France, <sup>2</sup>Grenoble Institut des Neurosciences, University Grenoble 1, Grenoble, France, <sup>3</sup>Grenoble University Hospital, Grenoble, France

### Introduction

Stroke is the leading cause of disability in adults. Beyond the narrow time window for thrombolysis, human mesenchymal stem cells (hMSCs) have strong clinical potential<sup>1,2</sup>. To assess the mechanisms underlying the cell-therapy benefit after stroke, imaging is necessary. However, image analysis based on mean values obtained from regions of interest (ROIs) dampers intralesional heterogeneity. An alternative is the parametric response map (PRM), a voxel-based analysis technique, is a promising tool to better investigate parametric changes over time at the voxel level<sup>3</sup>. The purpose of this preclinical study was to evaluate PRM analysis on hMSC therapy after stroke.

### Materials and Methods

Thirty-two Sprague Dawley male rats (250-300g) were used. Twenty rats underwent a transient (90 min) focal cerebral ischemia by occlusion of the right middle cerebral artery (MCAo) and twelve rats underwent the surgery without occlusion at Day 0 (D0) (**sham group**). Rats were anaesthetized by isoflurane, ventilated and equipped with a catheter in the tail vein. MR experiments were performed at 7T (Bruker Avance III) using a volume/surface cross coil configuration. Apparent coefficient diffusion (ADC) was measured during 16 days (D3, 7, 9, 16). At D8, MCAo rats received an intravenous injection of either 1ml phosphate-buffered saline (PBS)-glutamine (**MCAo-PBS group**) or of  $3 \times 10^6$  hMSCs (**MCAo-hMSC group**). MRI protocol: T<sub>2</sub>-weighted sequence (TR/TE=2500/60 ms; voxel size, 234×234×1000 $\mu\text{m}^3$ ), ADC (spin-echo EPI; TR/TE=3000/29 ms; b=0 and 900 s/mm<sup>2</sup>; same voxel size) (Fig.1 (a)-(d)). All images were co-registered to T<sub>2</sub>-weighted images of D3 using a fully automated, affine, mutual information-based, simplex optimization algorithm. Following co-registration, the stroke lesion ROI was manually contoured. For each rat, each time point and ROI, PRM was used to analyze, voxel-wise, changes in ADC. Briefly, PRM was performed by calculating the difference in the ADC values of each voxel in the ROI of D3 with the values of the other time points. A threshold (1210  $\mu\text{m}^2/\text{s}$ , referred to the intact mirrored homologues) was then applied to the absolute difference of each map in a voxel and all like voxels were summed to obtain lesion fractions that showed significantly increased (PRM<sub>ADC+</sub>: red), significantly decreased (PRM<sub>ADC-</sub>: blue) and unchanged (PRM<sub>ADC0</sub>: green) ADC values (Fig.1 (e)-(f)). Between-group comparison was performed using unpaired t-test after checking the variance homogeneity (Levene's test). In case of inhomogeneity of variance, a Mann-Whitney test was used. To evaluate the PRM change, we performed a three groups by three time points mixed design ANOVA, using a Bonferroni correction for multiple comparison. Significance was set at  $p < 0.05$ . Results were expressed as mean $\pm$ SD.

### Results

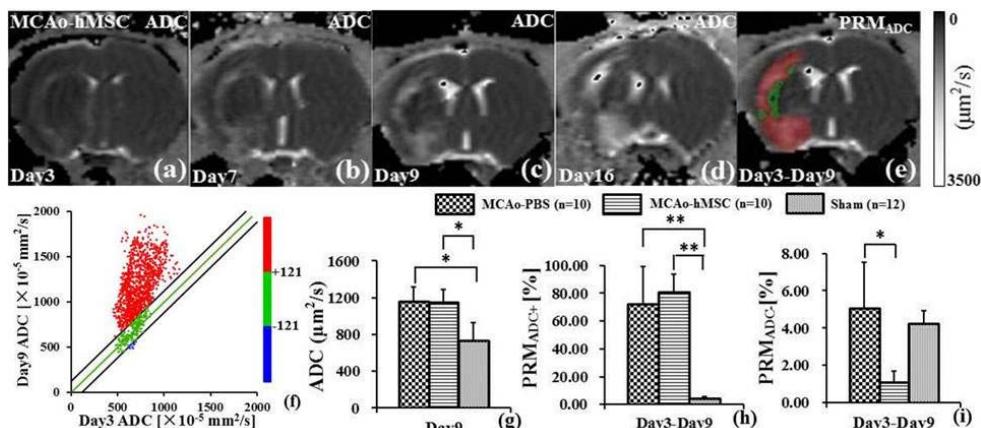
No significant ADC changes were observed between the MCAo and the sham group at Day3 and Day7. At Days 9 (Fig.1 (g)) and 16, the mean ADC in the lesion was significantly increased in both MCAo groups versus sham but was not different between MCAo groups (D9: MCAo-PBS:  $1151 \pm 166 \mu\text{m}^2/\text{s}$ , MCAo-hMSC:  $1142 \pm 144 \mu\text{m}^2/\text{s}$ , sham:  $736 \pm 189 \mu\text{m}^2/\text{s}$ ; D16: MCAo-PBS:  $1582 \pm 415 \mu\text{m}^2/\text{s}$ , MCAo-hMSC:  $1753 \pm 145 \mu\text{m}^2/\text{s}$ , sham:  $789 \pm 32 \mu\text{m}^2/\text{s}$ ). Over time, a trend towards an increased ADC was observed in both MCAo groups. The PRM showed that the fraction of voxels with an increased (PRM<sub>ADC+</sub>) and with a decreased (PRM<sub>ADC-</sub>) ADC over time in both MCAo groups (PBS and hMSC) differed from that of the sham group. The PRM<sub>ADC+</sub> fraction was significantly larger in MCAo groups than in sham group at Day7, Day9 (MCAo-PBS vs sham:  $72.0 \pm 26.9\%$  vs  $4.3 \pm 1.1\%$ ,  $p < 0.05$ ; MCAo-hMSC vs sham:  $80.7 \pm 12.9\%$  vs  $4.3 \pm 1.1\%$ ,  $p < 0.05$ ) (Fig.1 (h)) and Day16 (MCAo-PBS vs sham:  $80.4 \pm 19.0\%$  vs  $5.5 \pm 1.5\%$ ,  $p < 0.05$ ; MCAo-hMSC vs sham:  $92.8 \pm 3.1\%$  vs  $5.5 \pm 1.5\%$ ,  $p < 0.05$ ). Moreover, the PRM<sub>ADC-</sub> fraction was significantly higher in MCAo-PBS than in MCAo-hMSC at Day9 (MCAo-PBS vs MCAo-hMSC:  $5.1 \pm 2.5\%$  vs  $1.1 \pm 0.6\%$ ,  $p < 0.05$ ) (Fig.1 (i)) and was higher in sham than in MCAo-hMSC at Day16 (sham vs MCAo-hMSC:  $5.0 \pm 1.9\%$  vs  $0.6 \pm 0.2\%$ ,  $p < 0.05$ ). The ANOVA showed no effect of either group or time point, and no significant interaction between group and time point.

### Discussion

This study presented a longitudinal study performed on stroke models and analyzed with PRM, a voxel-wise technique, with the aim to highlight ADC changes over time to better assess the response to an hMSC treatment applied at a subacute stage of stroke. These results suggest that PRM could be a reliable method to analyze quantitative ADC maps. Indeed, while mean ADC values were comparable between MCAo groups, PRM showed a differential evolution between animals treated with PBS and hMSC. This change in ADC suggests that hMSC could decrease the post-ischemic cellular edema. In further investigations, the use of multiple b values could improve the accuracy on the determination of the ADC threshold. Moreover, PRM can be used to further analyze multimodal data, including perfusion parameters.

### References

1. Moisan A *et al.* NMR Biomed. 2012; 25: 1340.
2. Eckert MA *et al.* J Cereb Blood Flow Metab. 2013; 33: 1322.
3. Tsien C *et al.* J Clin Oncol. 2010; 28: 2293.



**Figure 1:** (a)-(d): ADC maps of one rat in MCAo-hMSC group for 4 time points, (e): PRM<sub>ADC</sub> color-code overlay of D3 and D9, (f): Corresponding quantitative scatter plot analysis showing the distribution of ADC at baseline compared with D9 (one day after intravenous injection of hMSCs) is shown respectively, (g): Bar plots showing the quantitative analysis of ADC value of D9, (h): Bar plots showing the increased percent ADC value between D3 and D9, (i): Bar plots showing the decreased percent ADC value between D3 and D9. Data is presented as a group mean $\pm$ SD, significant difference between groups was assessed using: \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ .