Relation between changes in microvasculature detected by MRI and evolutions of the expression of the main angiogenic factors on two different rat brain tumors

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Introduction

Monitoring the evolution of angiogenesis would help not only to predict the aggressiveness of tumors but also the efficiency of anti-angiogenic therapies (1). Blood Volume (BV) and Vessel Size Index (VSI) MR imaging are powerful tools for assessing small changes of the microvasculature (2, 3) and we have recently demonstrated that this methodology can be used repeatedly on the same rats (4). The aim of this study was to perform longitudinal BV and VSI imaging during tumor development of two glioma-models to deal with the known heterogeneity of human gliomas. The accuracy of MRI data was compared to histological data. Finally, we analysed the expression of the main angiogenic factors in relation with the evolution of the microvasculature.

Material and Methods

C6 and RG2 glioma-cells were implanted in rat striata (n=12 per time for each model; 8 rats were followed each time and 4 more rats were imaged once and euthanized for histology). BV and VSI were measured at different time points (Days 11-15-20-25 for the C6-model and Days 6-10-14-18 for the RG2-model) on anesthetized rats (isoflurane 2% in oxygen/air) using T_1w, diffusion and multiple gradient echo-spin echo MR sequences, the latter being acquired before and after intravenous injection of Sinerem® (200µmol Fe/Kg, Guerbet SA). For each animal, the $\Delta\chi$ was measured on blood sample using a CPMG sequence (TE=8 ms, Nb of echoes=400). All experiments were performed at 2.35T (in plane resolution= 234x454µm, thickness=1mm, total acq. time=1.5 hours per animal). Four brains were analyzed by histology after a Collagen IV immunostaining and vessel density, vascular surface (VS) and VSI_{histo} (5)) were measured with the ImageJ software. We analysed the expression of VEGF and Ang-2 by western-blotting and the activity of MMP-2 and -9 by zymography. Two-way ANOVA followed by Bonferronni post-hoc tests and Pearson correlation coefficient were used for statistics.

Results

In the contralateral striatum, neither BV nor VSI varied with time (C6-model: 2.8±0.5% and 5.8±1.6µm, RG2-model: 2.5±0.6% and 4.6±0.8µm) (Fig. 1, blue curves). In the C6-model, the ADC had already increased by D11 in the tumor w.r.t. the periphery or the contralateral side (Fig 2, top). In the RG-2 model, the ADC was reduced in the tumor and strongly increased in the periphery (Fig 2, bottom). In the C6-model, BV remained stable over time in the tumor (Fig 1, red curves) and in the periphery (green curves). In the RG2-model, BV increased in the tumor and in the periphery between the first and the last day to reach 5.1±1.6 and 3.3±0.7%. In the C6-model, VSI was significantly higher in the tumor than in the contralateral striatum at D11 and increased until D25 (19.5±4.8µm). At the periphery, VSI increased later (D20; Fig. 1, top). In the RG2-model, VSI increased in the tumor slower than in the C6-model (11.0±2.0µm at D18). In the periphery, VSI increased until D14 and remained stable afterwards (Fig. 1, bottom).

Histological analysis showed a strong reduction of the vessel density within the C6 tumor w.r.t. the contralateral striatum. This reduction is less pronounced for the RG2 model (Fig 2). No modification of the VS was observed but an increase of the VSI_histo was noted for the C6 model. In contrast, the VS was increased for the RG2 model associated with an increase of the VSI_histo but with a lesser extent than for the C6 model. BV and VSI changes were correlated with VS and VSI histo for both models (r^2>0.70; p<0.01).

The expression of VEGF and MMP-2 was correlated to the tumor volume (obtained from T_{1w} images) for both models (Fig 3). In contrast, an early appearance of the expression of Ang-2 and the activity of MMP-9 was observed for the C6 model (Fig 3, left) while the expression of Ang-2 was correlated to the tumor volume and no activity of MMP-9 was detected for the RG2 model (Fig 3, left).

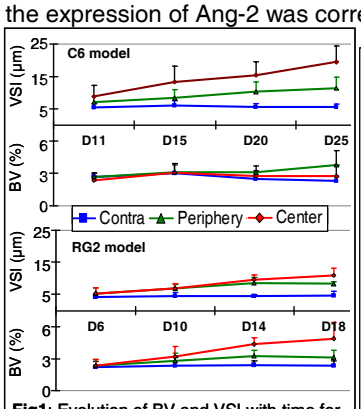

Fig1: Evolution of BV and VSI with time for the C6 and the RG2 models on 3 different ROI's.Mean+/-SD, n=12 per time.

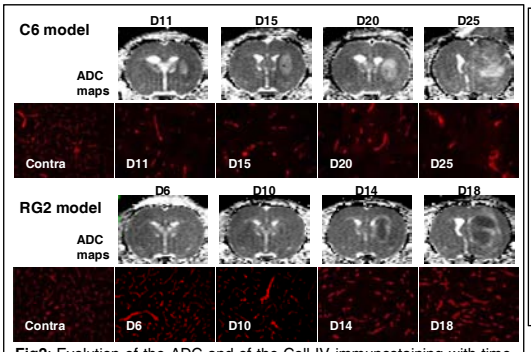

Fig2: Evolution of the ADC and of the Coll IV immunostaining with time for the C6 (top) and the RG2 (bottom) models.

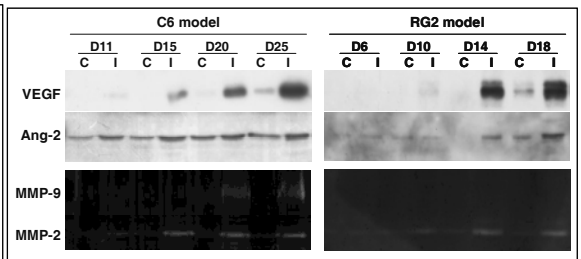

Fig3: Evolution of the expression of the angiogenic factors as function of time for the C6 (left) and the RG2 (right) models on the contralateral (C) and ipsilateral (I) hemispheres. (n=4 per time for each model).

Conclusion

This study demonstrates that the microvasculature evolves very differently with time between both models. We also show a strong correlation between MR and histological data. The early expression of Ang-2 and the activity of MMP-9 observed for the C6 model could explain the reduction of the vessel number and the destabilized aspect of the vessels for this tumor and could explain the different evolution of the microvasculature between both models. Thus, BV and VSI imaging has strong potential for monitoring tumoral microvasculature and assessing the efficiency of antiangiogenic/antivascular therapies. This study also emphasizes the importance of working with different glioma models to conclude about the effect of a therapy.

References :

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