Assessment of vascular reactivity in two rat brain gliomas (C6 and RG2) by Blood Volume fraction MRI during CO_2 challenge and correlation to mature vessels

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Introduction:

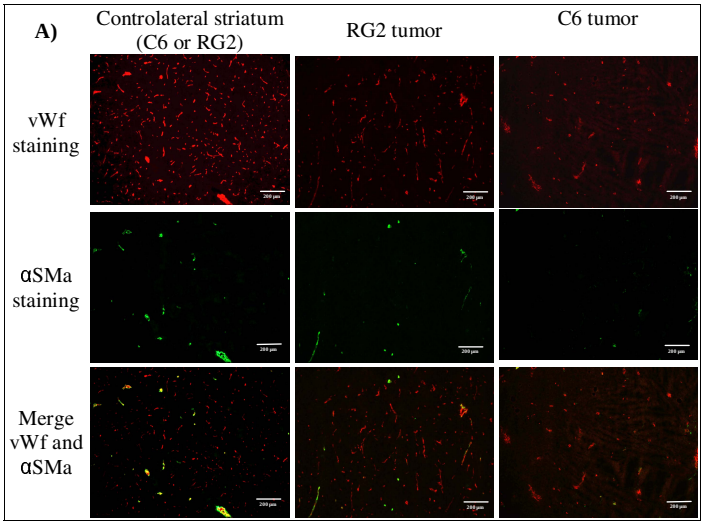
Microvascular maturation may vary across tumor types (1). Using histology, structural microvascular maturation is assessed by measuring the pericyte coverage index. Using MRI, functional microvascular maturation might be assessed by measuring vasoreactivity and blood volume fraction (BVf) under hypercapnic challenge. C6 and RG2 glioma, both permeable to Gd chelates, exhibit different molecular profiles (namely expressions of VEGF and Ang-2) (2). These two models might thus present differences in microvascular maturity. In this study, we compare both structural and functional microvascular maturation – assessed by histology and MRI – of these two orthotopic rat models.

Material and methods:

C6 and RG2 glioma-cells were implanted in rat striatum (n=5 per model, Wistar rats for the C6-model and Fisher rats for the RG2-model). BVf were mapped at day 10 (D10) (for RG2 model) and D12 (for C6 model) after tumor cell implantation. For MR imaging, animals were anesthetized with 2% isoflurane in gas mixture. MRI was performed at 7T with a Bruker Avance 3 console using a volume/surface cross coil configuration. Anatomical T2weighted images were first acquired. Then a multiple gradient echo MR sequence (MGE)(TR=2000ms, GE=[3-55]ms, FOV 30x30mm², matrix 64x64, 1mm-thick, 1.36min per image) was acquired 17 times: 4 times under air condition, 5 times under hypercapnia (air+10% CO_2), 8 times under air condition. The same MR sequence was repeated with the same breathing conditions after injection of iron oxide particles (Sinerem®/Combidex® Guerbet, France/Amag Pharmaceutical, 200µmol Fe/Kg, tail vein injection). Inspired CO_2 fraction was continuously monitored with a capnometer (CWI, Inc.). BVf maps were reconstructed from paired MGE experiments before and after injection of iron oxide particles. At the end of the MRI experiment, animals were euthanazied. Their brains were quickly removed, frozen in -40°C isopentane and stored at -80°C. Brain was sliced at -20°C with a cryostat (10 µm thick sections). For each rat, three slices were used to detect by immunohistology endothelial cells (antibody against Von Willebrand Factor (vWf)) and smooth muscle actin (αSma). Data, averaged across rats, are presented for 2 regions of interest (whole tumor and contralateral striatum (contra)) and for each model.

Results:

As there was no difference between the contralateral striatum BVf values of both groups (Wistar rats for the C6-model and Fisher rats for the RG2-model), these data were pooled. In controlateral striatum, during the 4 first acquisitions (air condition) basal BVf was stable (3.6±0.1%, Fig. 1B). Under hypercapnic condition, BVf increased and reached a plateau (4.4±0.3%, Fig. 1B). When returning to air condition, BVf returned to basal level (3.7±0.4%, Fig. 1B). In RG2 model, tumoral BVf was stable during air condition (5.8±0.1%, Fig. 1C). Under hypercapnia, RG2 tumoral BVf increased slowly between each measurement (from 6.3±1.0 to 7.7±0.7%, Fig. 1C). When returning to air condition, BVf decreased slowly but not to the baseline (6.3±0.4%, Fig. 1C). In the C6 model, tumoral BVf remained stable across all conditions (4.2±0.1%, Fig. 1C). Immunohistology showed the presence of mature vessels in the contralateral striatum of both models (Fig. 1A). In RG2 tumors, mature vessels could be detected while in the C6 tumors, only very few mature vessels could be observed (Fig. 1A).



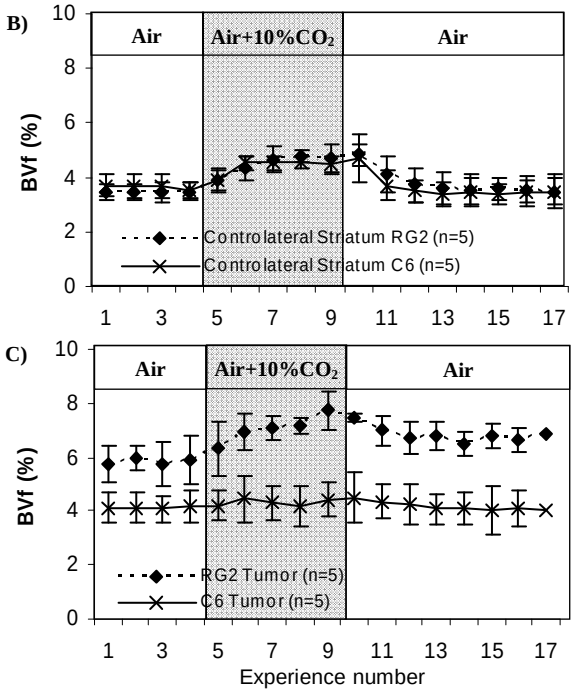


Figure 1: A) Contralateral striatum and tumor stainings (representative of 5 rats per group) of endothelial cell (vWf; red), smooth muscle actin (αSma; green) and merge of both stainings. (B) Blood Volume fraction over time in both contralateral striata (C6 and RG2). (C) Blood Volume fraction over time in the two tumor types. Error bars represent one standard deviation.

Conclusions:

Using MRI of vasoreactivity, BVf measurement during CO_2 challenge showed that 2 glioma models, RG2 and C6, harbored different functional microvascular maturation. These observations are in good agreement with structural microvascular maturation detected by immunohistology (quantification of pericyte coverage index under progress). A previous study (2) had shown that angiopoietin-2 was overexpressed during early development in the C6 model but was not in the RG2 model. This difference in Angiopoiein-2 expression between the two tumors might explain the absence of vascular reactivity in the C6 model. This study indicates that MRI vasoreactivity measurement might be an important biomarker in neurooncology to better understand microvascular physiopathology and improve medical treatment.

References:

[1]Packard et al. Neoplasia 2003 [2] Valable et al. NMR Biomed. 2008.