Evaluation of the relationship between ISO2 MR measurement and hypoxia: impact of an antiangiogenic treatment on a gliosarcoma model

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Introduction: Glioblastomas (GBM), the most angiogenic brain tumors, exhibit either hyper-vascularised and necrotic areas. Despite this highly vascular phenotype, most tumor cells are in hypoxia (1). Indeed, the new vessels are structurally abnormal. They are dilated and tortuous, leading to a chaotic blood flow. The existence of vessels does not guarantee a good oxygenation of tumor tissue. This hypoxia can select a sub-class of cancer cells which possess the ability to survive without oxidative metabolism and continue to proliferate despite these conditions. Hypoxia is associated with an aggressive phenotype that promotes tumor growth and metastasis (2). Furthermore, hypoxia renders tumor chemo-and radio-resistant. Recently, different studies showed that local blood oxygen saturation (ISO2) could be estimated by MRI (3). Antiangiogenic therapies have demonstrated their ability to change tumor vasculature, should also alter tumoral oxygenation. In this study, we evaluate (i) the relationship between ISO2 estimated by MRI and tissue hypoxia estimated by immunohistology and (ii) the impact of an antiangiogenic (Sorafenib) treatment on the vasculature (blood volume fraction; BVF) and the oxygenation (ISO2) of a gliosarcoma model (9L).

Material and methods: Fisher 344 rats were orthotopically injected at day 0 (D0) with 10^6 9L glioma cells (n=48). At D7, T2-weighted images were acquired to measure tumor size (4.7T, Bruker Avance III console). Rats were then randomized in 2 groups (n=24 per group) with similar tumor volume (4±2.5 mm³, data not shown). Treatment started at D10 (D10(T0)). Untreated group received no treatment. Treated group received a daily oral administration of Sorafenib (100 mg.kg⁻¹ Nexavar®, Bayer Corporation) between the 1st and the 8th day after the start of the treatment (D10(T0) to D18(T8)). BVf and ISO2 were mapped 1 day before and 1, 3, 5 and 8 days after starting the treatment (D9(T-1), D11(T1), D13(T3), D15(T5) and D18(T8), respectively). BVf was mapped using a steady-state approach (a multiple gradient-echo/spin-echo MR sequence was acquired before and after intravenous injection of ferumoxtran-10 (Sinerem®, 200 µmol Fe.kg⁻¹, obtained from Guerbet)). ISO2 was mapped using a recently proposed method and based on the quantitative BOLD approach(3). For each group, 4 rats were imaged at every time point. At each time, 4 additional animals per group were imaged and then sacrificed for ex-vivo studies. We therefore obtained, for each group and each time, MRI data from 8 animals and ex-vivo data from only 4 animals. One hour before sacrifice, rats were injected with pimonidazole (100 mg.kg⁻¹; Hypoxyprobe-1, Chemicon). After sacrifice, immunostaining of pimonidazole was performed (10 µm thick slice). The percentage of necrotic/ hypoxic area within the tumor was calculated from the sum of pimonidazole stained areas and necrotic areas. The fraction of pixels within the tumor ROI and with ISO2 < 40% was estimated on MRI data as a blood SO2 <40 % is known to induce tissue hypoxia (4). Then, the correlation between the percent of low ISO2 values (< 40%) estimated by MRI and the percent of hypoxic/necrotic area estimated by immunohistology was computed.

Results: Visually, in untreated tumors, we observed an increase in the number of pixels with low ISO2 values over time. Meanwhile, in the same group, an increase in pimonidazole stained areas was observed (Fig1a). In untreated tumor, a good correlation was found (R² = 0.812) between the hypoxic-necrotic areas measured from pimonidazole staining and the fraction of tumor pixels with ISO2 < 40% (Fig1b). This correlation was not found in treated tumors. ISO2 in contralateral striatum did not change over time (68 ± 2 %; mean across all time points; Fig1c). Values of ISO2 in untreated tumors were not different from that in contralateral striatum (70 ± 2 %; mean across all time points). In contrast, there was a decrease between D9(T-1) and D15(T5) in ISO2 of tumors treated with Sorafenib (from 71 ± 5 to 54 ± 5 %, respectively; Fig1c). At the last imaging time, ISO2 in treated tumors was similar to that estimated in untreated tumors and contralateral striatum (61 ± 4, 69 ± 3 and 66 ± 8 % at D18(T8), respectively; Fig1c). Before treatment, BVf measured in tumors of both groups was higher than in contralateral striatum (5.9 ± 0.5, 5.5 ± 0.5 % versus 3.0 ± 0.5 % for untreated, treated tumor and contralateral striatum respectively; Fig1d). In untreated tumor and in contralateral striata, BVf did not vary over time (Fig1d). In untreated tumor, however, there was a decrease in BVf between D9(T-1) and D15(T5) (from 5.5 ± 0.5 to 3.3 ± 0.7, respectively; Fig1d) and a trend to increase at the last imaging time (Fig1d). The fraction of ISO2 <40% was similar in both groups before treatment (< 3 %). During tumor growth, this fraction slowly increased in untreated tumors (from 3 ± 3 to 22 ± 10 %, between D9(T-1) and D18(T8)). This increase was more pronounced in treated tumors (from 3 ± 3 to 39 ± 10 %, between D9(T-1) and D15(T5)) before a decrease at the last imaging time (33 ± 9 %; at D18(T8)).

Conclusions: SO2 is the fraction of oxyhemoglobin in the total blood hemoglobin and is therefore different from tissue PO2. However, it has been reported that, for local SO2 values lower than 40%, tissue cells may be found in hypoxic conditions (3). In tumor tissue, our results suggest that ISO2 could be used as a reporter of tumor hypoxia. Indeed, in this study on 9L gliosarcoma, ISO2 estimated by MRI appears qualitatively and quantitatively related to the hypoxic/necrotic area in the untreated tumor. In treated tumor, the lack of correlation could be ascribed to a reduction in vessel wall permeability to pimonidazole consecutive to the antiangiogenic therapy. We also observed that ISO2 measured in the contralateral striatum was stable over time, with values (< 70 %) comparable to normal physiological values. Intratumoral ISO2, in the untreated group is identical to that of the contralateral tissue and remains stable over time. However one can observe a very large heterogeneity of ISO2 values in the tumor especially in the later phases. In the treated tumor, ISO2 and BVF estimates decreased over the time. While the decrease in tumoral BVF values yield “normal” BVF values (Fig 1d; D13(T3)), the concomitant decrease in ISO2 values suggest an abnormal tumor perfusion. This becomes even clearer in Fig. 1e where the fraction of tumor pixel with ISO2 < 40 % becomes much larger than in untreated tumors. In conclusion, this study suggests that ISO2 could be a sensitive reporter of the hypoxic effects of antiangiogenic therapies. However, complementary studies, using other SO2 measurement techniques, need to be performing to valid these preliminary results.