Preclinical MRI evaluation of human glioblastoma response to an anti-angiogenic treatment

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Introduction:

Despite aggressive surgery, radiotherapy and chemotherapy, malignant gliomas remain uniformly fatal. These tumours stimulate the formation of new blood vessels (angiogenesis). Therapies directed against tumour vasculature or preventing angiogenesis have recently been developed. Monitoring changes in microvasculature should help to evaluate the efficiency of these therapies. Recently, MRI has shown its ability to map different microvascular parameters (blood volume (BV), vessel size index (VSI) and blood brain barrier permeability (BBB perm.)) (1-2). The MRI measurements can be performed repeatedly and safely on the same rat. Moreover, they have the sensitivity to reveal differences between normal and tumoral tissues as well as differences between tumour types (3). The aim of this work was to apply MRI to evaluate the efficiency of a particular anti-angiogenic therapy (a multikinases inhibitor, Sorafenib®, Bayer) on a human glioma model (U87-MG).

Material and methods:

U87-MG human glioma-cells were implanted orthotopically in nude rat striatum (n=40). Animals were divided in two groups (n=20 per group): a 'Control' group with the animals having undergone no treatment, and a 'Treated' group with the animals having received Sorafenib® (100mg/kg daily, per os, between D14 and D28 after tumour implantation). BV, VSI, apparent diffusion coefficient (ADC) and BBB perm. to P846 (Gd-based, 3.5KDa, generous gift of Dr P. Robert, Guerbet, France) were mapped at 2.35T one day before treatment (D13) and 1, 4, 14 days after treatment onset (respectively D15, D18 and D28). Animals were anaesthetized with 2% isoflurane in oxygen/air. ADC, BV and VSI were mapped using diffusion-weighted and multiple gradient-echo/spin-echo MR sequences applied before and after injection of ferumoxtran-10 (Sinerem®/Combidex®, 200µmol Fe/Kg, generous gift of Dr P. Robert, Guerbet/AMAG Pharmaceuticals). BBB perm. was assessed on T_1-weighted images acquired before and after injection of P846 (50µmolGd/kg). Voxel size was 234x234x1000µm^3. In both groups, the same four rats were imaged at each time point. Four additional rats were also imaged per time point and euthanized at the end of the imaging session to enable ex-vivo studies. Tumour volume was computed on T_2w images. Student t-tests (after assessment of homogeneity variance) were used for statistics (*:p<0.05, **:p<0.01, ***:p<0.001).

Results

In the contralateral striatum, ADC, VSI and BV did not vary with time and did not differ between groups (Control: 718±9µm²/s, 5.3±0.2µm and 3.1±0.2%; Treated: 731±40µm²/s, 5.9±0.5µm and 3.4±0.3%) (Fig. 1a-c dotted line; for sake of clarity, only the contralateral data of the Control group are displayed). At any time after implantation and in both groups, ADC and VSI were higher in the tumour than in contralateral striatum. ADC did not differ between control and treated groups, excepted at D28. At this time, ADC in the Treated group was significantly higher than in the Control group (997.1±26.7 vs. 793.1±64.2µm²/s) (Fig. 1a). At D28 (14 days after treatment onset), tumour growth in the Treated group was strongly inhibited compared to in the Control group (28.7±11.1 vs. 117.1±22.9mm^3) (Fig. 1d). VSI did not differ between groups at D13, but at all other time points (D15 to D28) VSI was higher in the Treated group than in the Control group (5.8 ±1.8 to 8.7±1.7µm for Control and 7.2±1.8 to12.4±1.7 for Treated group) (Fig 1b). Before treatment, tumoral BV was higher than contralateral BV in both groups (control tumour: 4.7±0.6; treated tumour: 4.6±0.5; controlateral: 3.3±0.4%). During treatment (D14 to D28), there was no significant change of BV in the Control group (from 4.5±0.5 to 4.1±0.5%), while it decreased in the Treated group decreased strongly (from 4.6±0.5 to 1.86±0.2%) (Fig. 1c). During follow-up, glioma BBB in Control group was permeable to P846. In contrast, treated tumour BBB became impermeable to P846 as early as 4 days after treatment onset (Fig. 2, BBB perm).

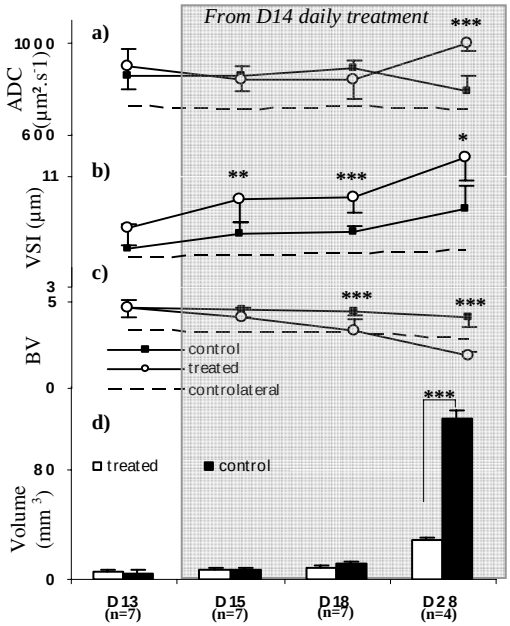

Fig. 1: Evolution of (**a**) ADC, (**b**) VSI, (**c**) BV and (**d**) tumoral volume of U87-MG glioma in control (black squares) and treated (open circles) animals. Mean±SD. *: p<0.05, **: p<0.01, ***: p<0.001 between Control and Treated groups.

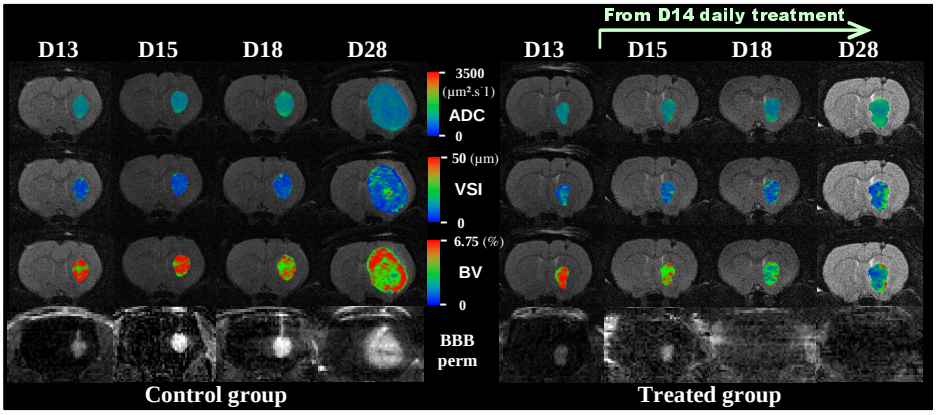

Fig. 2: Evolution of ADC, VSI, BV and BBB permeability to P846 of a U87-MG tumour in control or treated conditions. Treatment (Sorafenib®) was given daily from D14 until D28. Tumoral ADC, VSI and BV color maps have been overlaid on their corresponding T_2w-image. BBB perm. images correspond to the increase in T_1w signal due to the extravasion of P846.

Conclusion:

This study demonstrates that Sorafenib®, an anti-angiogenic treatment, has a significant effect both on tumour growth and on tumour microvasculature. It also shows that MRI imaging of blood volume, vessel size index and blood brain barrier permeability detects significant modifications in tumour vasculature as early as 4 days after treatment onset. Changes in microvasculature (VSI, BV and BBB perm.) occur much earlier than changes in tumour growth or in ADC. These observations are consistent with the mechanism of action of the anti-angiogenic treatment. On each euthanized rat, histological and proteomic studies are in progress to better understand treatment action on tumour microvasculature. Our results suggest that early MRI follow-up of modifications of microvascular parameters (BV, VSI and BBB perm.) permits monitoring the effects of anti-angiogenic treatment on gliomas. This study also indicates that Sorafenib® used alone does not cure U87-MG glioblastoma. It is therefore important, in future works, to combine anti-angiogenic treatment with other treatments such as chemotherapy and/or radiotherapy.

References:

[1] Magn. Res. Med. (2001)45:397-408. [2] Magn. Res Med. (2004)51:533-541 [3] poster #2920, ISMRM (2007).