Evaluation of Tumor Response to Carmustine and Sorafenib with Magnetic Resonance Imaging in an Orthotopic Human Glioblastoma Model Xenografted in Nude Rats

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INTRODUCTION

Despite aggressive surgery, radiotherapy and chemotherapy, malignant gliomas remain uniformly fatal. These tumors stimulate the formation of new blood vessels (neo-angiogenesis). Many therapies directed against tumor vasculature or preventing angiogenesis have recently been developed. Monitoring changes in gliomas microvasculature should help to evaluate the efficacy of new anti-tumor therapy. Recently, MRI has shown its ability to map different microvascular parameters (blood volume (BV), vessel size index (VSI), blood brain barrier permeability (BBB perm.))[1-2]. MRI measurements can be performed repeatedly and safely on the same rat. Moreover, they are sensitive enough to reveal differences between normal and tumor tissues as well as differences between tumor types[3].

STUDY AIM

To assess the sensitivity of MRI biomarkers to the anti-tumor activity of Carmustine (BCNU, alkylating agent) and Sorafenib(SORA, multikinases inhibitor) in an orthotopic human glioblastoma model (U87-MG) xenografted in Nude rats.

MATERIAL AND METHODS (fig.1)

In a first imaging experiment, 55 Nude rats were orthotopically injected at D0 with 10^5 U87-MG glioma cells. At D11, anatomical images were acquired for tumor size measurement. Rats were randomized at D12 in 3 groups of 16 animals according to tumor volume ($4\pm2mm^3$, figs.1&4a). Treatment started at D14. The first group (BCNU group) received two injections of 10 mg/kg BCNU i.v. (D14 and D27). The second group (SORA group) received daily oral administration of 100mg/kg of Sorafenib from D14 to D28. The third group (Control group) received no treatment. Rat survival was monitored daily. In a second survival experiment, 20 Nude rats were orthotopically injected at D0 with 10^5 U87-MG cells. Rats were randomized at D12 according to body weight in 3 groups of 6 animals. Tumor volume was measured by MRI just before treatment ($4\pm2mm^3$). Treatment started at D12 with the same schedule as described above. Survival was monitored daily, and animals were ethically sacrificed when clinical signs appeared.

BV, VSI, apparent diffusion coefficient (ADC) and BBB perm. to a contrast agent P846 (Gd-based, 3.5kDa, obtained from Dr P. Robert, Guerbet, France) were mapped in tumor and contralateral hemisphere by MRI, at 2.35T, one day before treatment and 1, 4 and 14 days after treatment start (respectively D13, D15, D18 and D28 after tumor cell injection). 12 rats (4 per group) were imaged at each time point and 12 additional rats (4 per group) were also imaged at each time point and sacrificed at the end of the imaging session for ex-vivo studies. At sacrifice, brains were collected and cryo-preserved in OCT for collagen IV immunostaining.

For each imaging session, animals were anesthetized with 2% isoflurane in oxygen/air. Tumor volume was computed from T_{2w} images. ADC, BV and VSI were mapped using diffusion-weighted and multiple gradient-echo/spin-echo MR sequences applied before and after intravenous injection of ferumoxtran-10 (Sinerem®/Combidex®, 200μmol Fe/kg, obtained from Dr P. Robert, Guerbet/AMAG Pharmaceuticals). BBB perm. was assessed on T_1-weighted images acquired before and after injection of P846 (50μmol Gd/kg). Voxel size was $234\times234\times1000μm^3$. Data, averaged across rats, are presented for each region of interest and each group as a function of time. At D13, D15, D18, data from one rat per group were rejected for technical reasons.

Student t-tests (after assessment of variance homogeneity) were used to assess differences (*:p<0.05, **:p<0.01, ***:p<0.001).
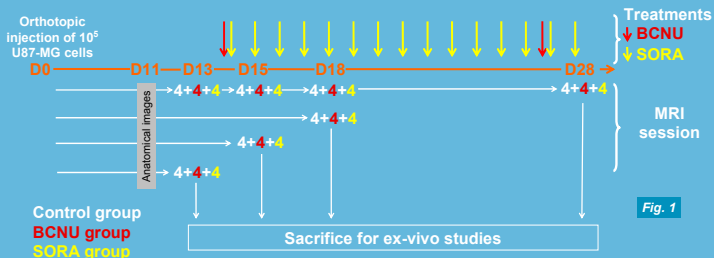
TREATMENT AND IMAGING PROTOCOL

Fig. 1

RESULTS

➢ BCNU and Sorafenib treatments induced a moderate effect on the survival of U87-MG glioma bearing rats. (+16% and +23% respectively) (fig.2).
➢ Both treatments show a significant inhibition of tumor growth compared to the Control group at D28 as measured by MRI (5.7 ± 4.9, 28.7 ± 11.1 and 117.1 ± 22.9 mm3 for BCNU, SORA and Control group respectively) (fig.4a).
➢ Intratumoral ADC in SORA and BCNU groups are higher than in Control group at D28 (+23 and +32% respectively) (fig.4b).
➢ VSI is not significantly different between BCNU and Control groups at any time point. However, VSI is significantly higher in the SORA group than in the Control group at D18 (9.8 ± 1.6 vs. $8.8\pm1.8μm$ respectively) and at D28 (12.7 ± 1.5 vs. $9.3\pm0.9μm$ respectively) (fig.4c).
➢ Intratumoral BV is not modified by BCNU treatment but is strongly decreased by SORA treatment (5.0 ± 0.8 at D13 to $2.6\pm1.0\%$ at D28) (fig.4d).
➢ While BBB remained permeable to P846 in BCNU and Control groups, SORA-treated tumors became non permeable to P846 as early as 4 days after treatment start (fig.3 BBB perm.).
➢ Unlike Control and BCNU groups, SORA treatment induced a strong decrease in intratumoral vessel number between D15 and D28 (fig.3 collagen IV staining).
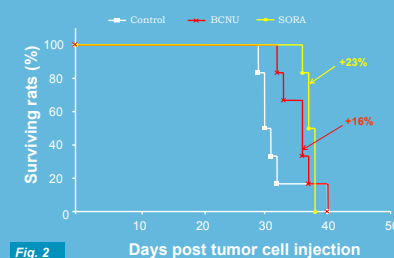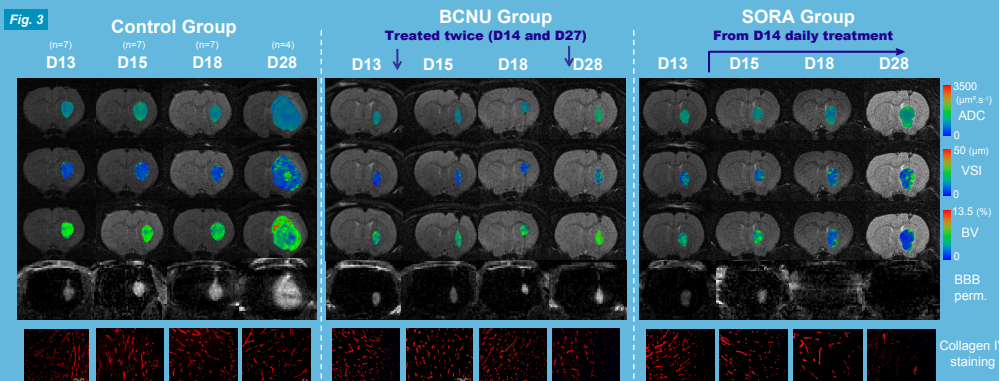

Fig. 2

Fig. 3

Control Group / BCNU Group (Treated twice (D14 and D27)) / SORA Group (From D14 daily treatment)

Collagen IV staining

Fig. 4 Days post tumor cell injection

*: p<0.05, **: p<0.01, ***: p<0.001 between control and SORA groups.
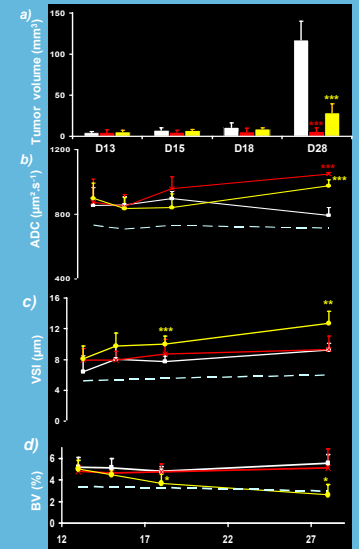***: p<0.001 between control and BCNU groups.

CONCLUSIONS

MRI highlights a tumor growth inhibition induced by Sorafenib and BCNU treatments, despite their moderate effect on the survival of U87-MG-bearing rats. ADC appears sensitive to both treatments only at D28. In SORA group, MRI of BV, VSI and BBB perm. to P846 reveals significant modifications in tumour vasculature as early as 4 days after treatment start (D18) (confirmed by immunohistological studies). This is consistent with the anti-angiogenic activity of Sorafenib. Our results suggest that early MRI follow-up of modifications of microvascular parameters (BV, VSI and BBB perm.) is useful to monitor the effects of anti-angiogenic treatment on glioma models. This study also indicates that Sorafenib alone does not cure U87-MG glioblastoma. It is therefore important, in future works, to combine anti-angiogenic treatment with other treatments (chemotherapy and/or radiotherapy) and microvascular MRI parameters could be helpful to design therapeutic combination.

[1] Troprès et al., Magn. Res. Med. (2001)45:397-408. [2] Troprès et al., Magn. Res Med. (2004)51:533-541 [3] Valable et al., NMR in Biomed, 2008 (in press).