

Measurement of tumor vascular integrity changes in an orthotopic glioma model in rats induced by antiangiogenic treatment using DCE-MRI

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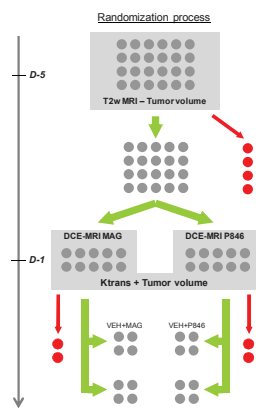
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Introduction

Glioblastoma is the most aggressive subtype of brain tumor. Antiangiogenic compounds are being tested in clinical trials, for this pathology, mostly in combination with other molecules. The availability of an imaging biomarker of efficacy could help optimize the dosing and scheduling of these combinations. The aim of this study was to validate DCE-MRI, using two contrast agents of different molecular weight, as an imaging biomarker of the efficacy of sorafenib (Nexavar®, BAY 43-9006) in an orthotopic glioma model in Nude rats.

Material and Methods

- U-87 MG human glioma cells (10^5) were inoculated by stereotactic injection in the right *caudate nucleus* of 24 Nude rats 24 h after whole-body irradiation (7 Gy, ^{60}Co). All rats were first imaged 10 days after cell injection to measure their baseline tumor volume (TV) using T2w MRI.
- Based on their TV, 20 rats were randomized into 2 groups of 10 rats and imaged 14 days after cell injection using either Gd-DTPA (Magnevist®, 0.94 kDa) or P846. P846 is a medium-molecular weight (3.5 kDa), gadolinium-based contrast agent provided by Dr P. Robert (Guerbet).
- In both groups, rats were randomized into 2 groups of 4 animals based on their tumor volume and on their tumor vascular status following a full DCE-MRI protocol (two parameters: Ktrans from pharmacokinetic modeling of the contrast agent uptake curve, and IAUC60, the initial area under the gadolinium concentration time curve after 60 seconds).
- In both subgroups of 8 rats, each animal received a daily per os administration of either sorafenib (100 mg/kg/adm) or its vehicle for 14 consecutive days. The treatment started 15 days after cell injection, this day is considered as D0.
- All 16 rats were then imaged at 1, 3 and 7 days after treatment start (D1, D3 and D7). At each time point, voxelwise maps of DCE-MRI parameters were derived from imaging data and their distribution analyzed using regions of interest within the tumor and the contralateral lesion-free tissue.



Dynamic contrast enhanced imaging protocol:

- MRI was performed on a 4.7T Pharmascan horizontal bore (Bruker, Germany). The animals were maintained under anaesthesia via a constant flow of isoflurane at 2-3% delivered by a nose cone.
- Morphological description and tumor volume were assessed with a T2w RARE sequence (TE/TR = 36/3500 ms, FOV = 30x30 mm, slice thickness = 1 mm).
- DCE-MRI: follow up of contrast agent uptake in the tumor during 8 minutes after an intravenous bolus injection (Magnevist®, 0.1 mmol/kg or P846 0.025 mmol/kg) using a T1w FLASH sequence (TE/TR/flip angle = 3 ms/50 ms/60°, slice thickness = 1 mm) at a temporal resolution of 12.8 s per image.
- Contrast agent uptake curves were derived from signal enhancement in selected regions of interest (ROI) (i.e. in tumor or contralateral tissue, drawn using morphological images) and characterized by:
 - K^{trans}: the volume transfer constant was determined by fitting the curve using a two-compartment kinetic model (Tofts et al., JMRI, 1999), using an in-house developed plugin of ImageJ.
 - IAUC60: the initial area under the curve was computed by integration between injection time and 60 s after injection time.

All procedures with animals were submitted to the Animal Care and Use Committee of Pharmacy and Medicine University (Dijon, France). Principe d'éthique de l'expérimentation animale. Directive n° 86/609 CEE du 24 Nov. 1986, Décret n° 87/848 du 19 Oct. 1987, Arrêté d'Application du 19 Avril 1988.

Workman et al. Guidelines for the welfare and use of animals in cancer research. Br J Cancer. 2010;102(11):1555-1577.

Results

Fig. 1: (A) Morphological T2w images, (B) Maps of the IAUC in the brain and (C) corresponding contrast agent concentration curves in the tumor of a representative animal of each group: animal imaged with Magnevist and treated with vehicle, animal imaged with P846 and treated with vehicle, animal imaged with Magnevist and treated with sorafenib, animal imaged with P846 and treated with sorafenib.

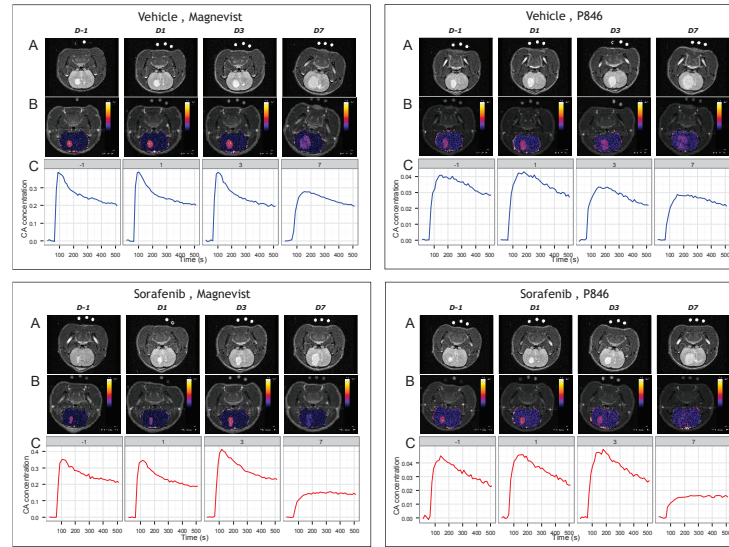
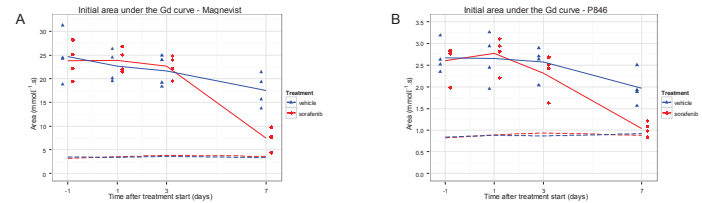
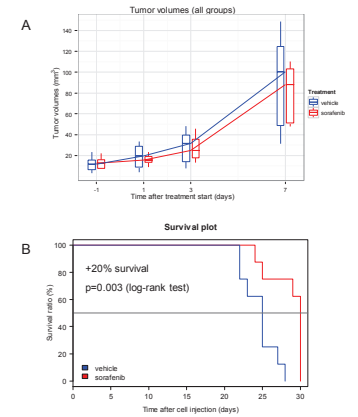


Fig. 2: Evolution of the IAUC after the treatment start in the tumor (points represent each animal and solid lines group means) and in the contralateral tissue (dashed lines) for animals imaged with Magnevist® (A) and with P846 (B) and treated with vehicle (blue) or sorafenib (red).



Results

Fig. 3 : (A) Tumor volume evolution after treatment start measured on the morphological T2w images in the vehicle (blue) and sorafenib (red) groups. (B) Survival curves of the vehicle and sorafenib treated groups.



- The day of treatment start was chosen 14 days after tumor implantation to ensure a suitable tumor volume for DCE-MRI (mean 7.3, SD 3.8 mm³). When started at this stage, sorafenib treatment had no significant effect on tumor growth, but increased the survival of U-87 MG tumor-bearing rats by 20%.

- Both contrast agents induced a contrast enhancement in the tumor but not in the contralateral tissue (only diffusion).

- Using either Magnevist® or P846, 7 days after treatment start, sorafenib significantly decreased DCE-MRI parameters (IAUC and K^{trans}) in the tumor compared to the vehicle-treated groups. No effect of sorafenib was observed on the contralateral tissue.

Conclusions

- The blood-brain barrier in the contralateral tissue remained impermeable to Magnevist® and P846 at all stages of tumor development and after sorafenib treatment.

- In U-87 MG tumors, sorafenib decreased vessel perfusion and permeability, as measured by DCE-MRI.

- In conclusion, we have shown that DCE-MRI, with either Magnevist® or P846 contrast agents, is a suitable non-invasive imaging technique to show changes of tumor vasculature integrity induced by antiangiogenic treatment such as sorafenib in orthotopically-implanted glioma in Nude rats.