Dual-injection of a low- and a macro-molecular-weight-contrast media to monitor the blood-brain barrier status in a glioma model under therapy

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Introduction: Numerous anti-tumor therapies modify the permeability (increase or decrease) of tumoral or healthy vessels. Changes in vessel permeability can be estimated by Dynamic Contrast-Enhanced-MRI (DCE-MRI). Some studies have shown that the sensitivity of DCE-MRI depends on the size of the contrast medium (CM) (1). In this study, we monitored the change in vessel permeability induced by an antiangiogenic treatment (Sorafenib) and by synchrotron-based Microbeam Radiation Therapy (MRT) in a rat gliomsarcoma model (9LGS) and in normal surrounding tissues. Low- and macro-molecular-weight-CM (LMCM and MMCM, respectively) DCE-MRI was performed within the same imaging session on each rat before and three times after the start of the treatment, searching for possible increase or decrease in vessel wall permeability.

Material and methods: Fisher 344 rats (n=80) were orthotopically injected at day 0 (D0) with 10^4 9LGS glioma cells. MRI was performed at 4.7T (Bruker, Avance III console). At D7, T_2-weighted images were acquired to measure tumor size. Rats were then randomized in 4 groups (n=20 per group) with similar tumor volume (4.6±2.5 mm^3). Treatment started at D10 ($D10_{(T0)}$). Untreated group received no treatment. SORA 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 $D18_{(T8)}$). MRT group was treated by (MRT) at $D10_{(T0)}$. MRT+SORA group was treated by MRT at $D10_{(T0)}$, animals also received a daily oral administration of Sorafenib (100 $mg.kg^{-1}$) between $D11_{(T1)}$ and $D18_{(T8)}$. MRI was performed one day before and 1, 5 and 8 days after the start of the treatment (respectively $D9_{(T-1)}$, $D11_{(T1)}$, $D15_{(T5)}$ and $D18_{(T8)}$). To evaluate the impact of repeated anaesthesia, each group was divided into two subgroups: 4 rats were imaged at every time point ("longitudinal" subgroup); 16 rats were imaged once ("single-time" subgroup, 4 animals per time point). Thus, a total of 8 rats per group were imaged at each time point. At all time points, vascular integrity to a MMCM and to a LMCM (P846 (2) and Gd-DOTA, both Gd-based contrast agents, 3.5 and 0.5 kDa (r_1: 15 and 3.3 $s^{-1}.mM^{-1}$ for P846 and Gd-DOTA, respectively)respectively; Guerbet, France) was assessed using a DCE-MRI approach. Briefly, multiple T_{1w} images (n=60, 15.6 sec per image) were acquired using a RARE sequence (TR/TE = 800/4.2 ms, 7 slices, FOV=30 mm, voxel size = 234x234x1000 μm^3). After the acquisition of 4 images, P846 was administered as a bolus via the tail vein in about 1 sec (25 $\mu moles.kg^{-1}$ body weight). After the acquisition of 50 images, Gd-DOTA was then administered in the same way (200 $\mu moles.kg^{-1}$ body weight). To assess the integrity of vessels, we calculated the area under the signal intensity curve during the 2 min and 36 sec (corresponding to 10 images) that followed CM injection and after baseline substraction (AUC); a coarse indicator of vessel permeability (3). For each CM, AUC was calculated (AUC_{P846} and $AUC_{Gd\text{-}DOTA}$) (Fig 2a). Unpaired Student t tests were used for statistical analysis (*: $p<0.01$)

Results: We observed no difference in DCE-MRI between the "single-time" and the "longitudinal" subgroups, at any time point. Consequently, for the 4 groups, values from the "single-time" and "longitudinal" subgroups were pooled.

Impact of the treatments on tumor AUC. Before the start of the treatment ($D9_{(T-1)}$), tumor vessels from each group were similarly permeable to P846 (Fig 1a). At all time point after treatment onset ($D11_{(T1)}$, $D15_{(T5)}$ and $D18_{(T8)}$), the tumor AUC_{P846} in MRT group was comparable to that measured in the untreated group (Fig 1a). *A contrario*, the tumor AUC_{P846} in SORA and MRT+SORA groups became significantly lower than that of the untreated group from the first day after the start of treatment. This difference remained stable at $D15_{(T5)}$, $D18_{(T8)}$ (Fig 1a). At all time points, the tumor $AUC_{Gd\text{-}DOTA}$ in treated groups were comparable to the one found in the untreated group, except at $D18_{(T8)}$ ($AUC_{Gd\text{-}DOTA}$ in the MRT+SORA group became lower than in the untreated group) (Fig 1b). At $D15_{(T5)}$, for each group, the two injections of CM led to clear signal variations in the tumor (Fig 2a-d). After the 1st CM injection (P846), tumor signal intensity increased rapidly and reached a plateau about 3 min after injection. These plateaus slightly decreased in untreated and MRT groups and conversely slowly increased in SORA and MRT+SORA groups (Fig 2a-d). In the tumor, at $D15_{(T5)}$, the increase in signal intensity after P846 injection was visually less important in groups treated by antiangiogenic drugs (SORA and MRT+SORA; Fig 2c-d) than in untreated and MRT groups (Fig 2a-b). The increase in signal intensity after Gd-DOTA injection is clearly visible and is not different between all groups (Fig 2a-d).

Impact of the treatments on healthy ipsilateral striatum AUC**.** For each group and at each time point, ipsilateral striatum vessels were not permeable to P846 (Fig 1c). Before the start of the treatment ($D9_{(T-1)}$), ipsilateral striatum vessels of all groups were not permeable to Gd-DOTA (Fig 1d). In absence of treatment or under Sorafenib treatment, ipsilateral striatum vessels in SORA group remained not permeable to Gd-DOTA (Fig 1d). *A contrario*, at $D15_{(T5)}$ and $D18_{(T8)}$, ipsilateral striatum $AUC_{Gd\text{-}DOTA}$ in MRT and MRT+SORA groups became significantly larger than in the untreated group (Fig 1d). No modification of the signal intensity was observed in the healthy ispsilateral striatum at $D15_{(T5)}$ in untreated group despite the injections of 2 CM (Fig. 2e). At the same time point, in MRT group, signal intensity in healthy ipsilateral striatum was very slightly enhanced by P846; and after injection of Gd-DOTA the signal intensity clearly increased (Fig 2f).

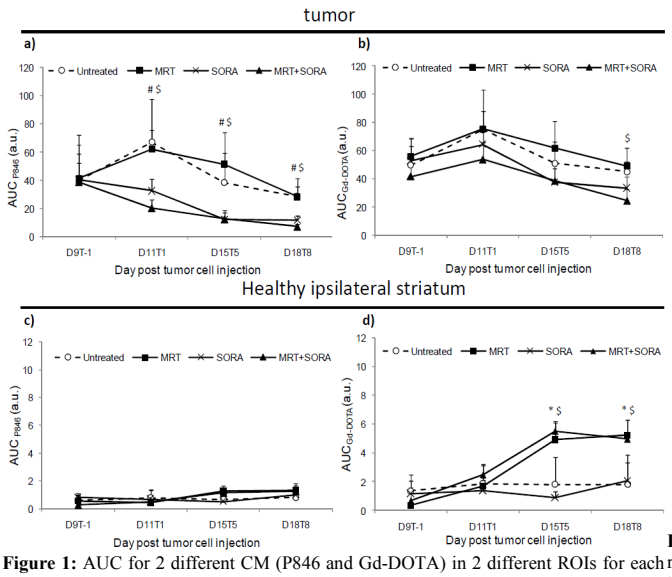

Figure 1: AUC for 2 different CM (P846 and Gd-DOTA) in 2 different ROIs for each group and as function of time. Average value of (a-c) AUC_{P846} and (b-d) $AUC_{Gd\text{-}DOTA}$ from (a-b) tumor ROIs and (c-d) ipsilateral striatum ROIs. Mean ± SD. *: MRT *vs.* untreated group; #: SORA *vs.* untreated group; $: MRT+SORA *vs.* untreated group.

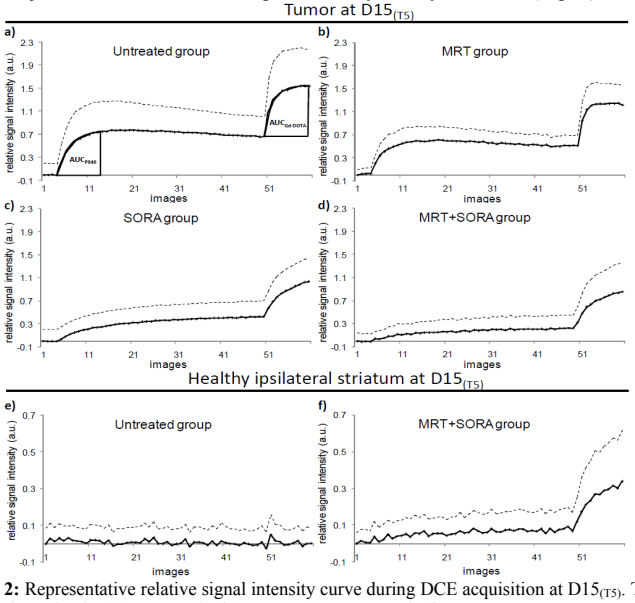

Figure 2: Representative relative signal intensity curve during DCE acquisition at $D15_{(T5)}$. Tumor relative intensity in (a) untreated, (b) MRT, (c) SORA and (d) MRT+SORA groups. Healthy ipsilateral striatum in (e) untreated and (f) MRT groups. For sake of clarity, the signal intensity of the first point in time was subtracted from the data. Images 4 and 51 corresponded to the injections of P846 and Gd-DOTA, respectively. Mean (dark line) ± SD (dashed line) for one ROI. On graphic a) AUCs calculated for both CM were illustrated

Conclusions: Antiangiogenic and radiation therapy have different effects on the CM extravasation in tumoral and healthy tissues, suggesting different modifications of the vessel permeability. This study suggests that the choice of CM for a DCE-MRI depends on the physiological question to be addressed. It also suggests that the use of two CM within the same MRI session is feasible.

References: (1) Turetschek et al. ***JMRI*** 2004; (2) Heilmann et al. ***Contrast Media Mol Imaging*** 2009; (3) Cheng ***JMRI*** 2009.