# Vascular permeability to Gd-DOTA and USPIO in C6 and RG2 rat glioma models

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**Introduction:** Dynamic Contrast Enhanced-MRI (DCE-MRI) provides insight into blood brain barrier (BBB) permeability of tumors. Typically, a low molecular weight contrast agent (CA) based on $Gd^{3+}$ is used and data are analyzed using classical pharmacokinetic models to obtain plasmatic volume fraction, influx and efflux constants [1]. To further characterize microvasculature, intravascular contrast agents, such as USPIO, can be used to determine blood volume (BV) and vessel size index (VSI) under stationary conditions [2]. In view of eventually combining these two approaches into a single imaging protocol, we evaluated whether the iron-based CA remains intravascular during a DCE-MRI protocol. DCE-MRI experiments using Dotarem® and Sinerem® were therefore performed on two brain tumor models. The presence of macrophages, potential transporters of iron, was equally investigated.

| Glioma model | C6 | RG2 |
|---|---|---|
| DCE-MRI with Gd-based CA (30min) | 14 | 6 |
| DCE-MRI with iron-based CA (120min) | 2 | 2 |

**Table 1**: Number of animals used for each experiment, per model and per CA.

**Material and methods:** C6 and the RG2 glioma models were used as orthotopic brain tumor models (intrastriatal implantation of $10^5$ cells on Wistar rats and of $5.10^3$ cells on Fischer rats, respectively). 24 rats (16 for the C6 model and 8 for the RG2 model, Table 1) were imaged, 14 days after cell injection.

MR imaging was performed at 2.35T. A baseline $T_1$ map was acquired before the CA injection with an IR-spiral sequence (TR/TE = 4400/1.6ms, 5 interleaves, flip angle = 90°, 11 inversion times, slice thickness = 2mm, FOV = 30*30mm², cartesian matrix after gridding and zero-filling = 128x128). DCE-MRI was performed with spiral imaging. A time span of 30min was covered for the experiment with the Gd-based CA and of 120min for the experiment with the iron-based CA (TR/TE = 600/1.6ms, 5 interleaves, flip angle = 90°, slice thickness = 2mm). Nominal spatial resolution was 470µm. Images were sampled every 10s on the initial 5min and every 20s subsequently. After the acquisition of 5 baseline images, the CA was administered into the tail vein (0.2mmol (of Gd or Fe) /kg, 12mL/h).

Per animal, $\Delta R_1(t)$ maps were derived from the baseline $T_1$ map and the series of DCE-MRI images. Three regions of interest (ROI) were manually drawn (tumor, contralateral and temporal muscle). $\Delta R_1(t)$ was averaged within each ROI.

At the end of the MRI experiments, brains were withdrawn and frozen for histological analysis. Frozen tissue was cut with a cryostat (thickness = 10µm). Macrophages and nuclei were labelled using CD68 antibody and DAPI, respectively, and revealed under epifluorescence (5 rats: 2 for the C6 model and 3 for the RG2 model).

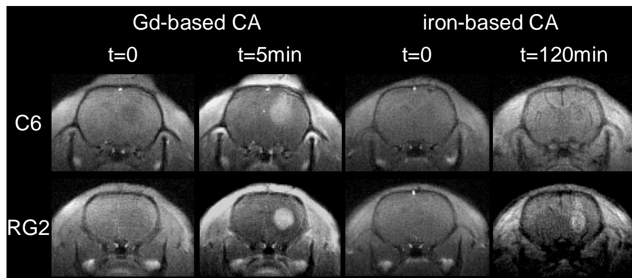


**Figure 1**: $T_1$-weighted images acquired before (t=0) and after Gd-based CA injection (t=5min) and before (t=0) and after iron-based CA injection (t=120min) on one rat for each tumor model and for each CA.

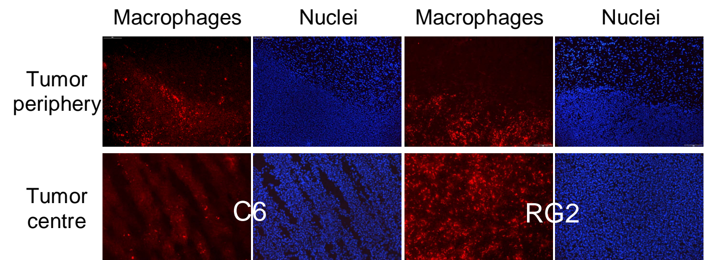


**Figure 3**: Histological images of the periphery and the centre of the tumor for each glioma model. Macrophages and nuclei are labelled in red and blue, respectively.

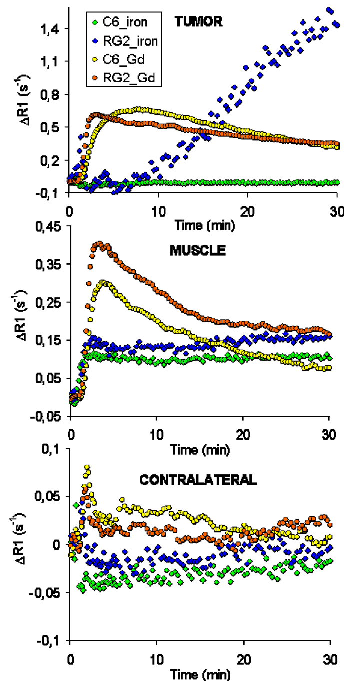


**Figure 2**: Temporal evolution of $\Delta R_1$ within 3 ROI. One rat for each tumor model and for each CA is represented.

**Results:** Fig. 1 shows that both tumor models present signal enhancement in the tumor region using a Gd-based CA, 5min after CA injection. Using an iron-based CA and 120min after CA injection, the whole RG2 tumor is clearly enhanced. For the C6, mild signal enhancement is visible at the periphery. In all cases, muscle signal is increased following CA injection and contralateral brain tissue signal remains unchanged. Fig. 2 shows the temporal evolutions of mean $\Delta R_1(t)$ within each ROI. On the contralateral curves, the influence of the bolus of CA on the signal is clearly visible; signals return to baseline after this first passage. The muscle curves indicate that the Gd-based CA extravasates but not the iron-based CA. However, $R_1$ behaves as a step function due to the $R_1$ increase of blood loaded with the iron-based CA. After injection of the Gd-based CA, the temporal evolutions of $\Delta R_1$ in both tumors correspond to classic extravasation profiles. With the iron-based CA, extravasation is visible in the RG2 tumor. Extravasation of the iron-based CA starts 10min after CA injection and progresses to the end of image acquisition (e.g. 120min after CA injection, result not shown). Fig. 3 presents histological images from a rat bearing a C6 glioma and a rat bearing a RG2 glioma. Nucleus density clearly differentiates the tumor region from the healthy tissue. CD68 labelling indicates the presence of macrophages at the tumor periphery but not in the healthy tissue, in both rats. In the centre of the tumor, the presence of macrophages is important for the RG2 model only.

**Discussion and conclusion:** These results show that the two glioma models studied here present BBB alterations (Gd-based CA extravasation). The extravasation profiles obtained in the muscle region clearly indicate that Dotarem® crosses endothelium without BBB but not Sinerem®. In the tumor region, extravasation of the Gd-based CA is certainly due to the CA concentration gradient between intra and extravascular compartments. The extravasation of the iron-based CA in the RG2 tumor could by similarly explained taking into account the long blood half-life of Sinerem® (about 4.5h) [2]. However, under this assumption, the fact that Sinerem® does not extravasate in the C6 tumor during the first two hours after injection (whereas its extravasation has been observed 24 hours after injection [3]) and the fact that the increase of $\Delta R_1$ in the RG2 tumor begins after a delay of 10min, remain unexplained. Since Sinerem® can be endocytosed by macrophages [3] and given our histological results, an other hypothesis could be proposed. The iron particles might also cross the vascular wall using cellular transporters, which could be the macrophages, even a few minutes after CA injection, or maybe the endothelial cells, themselves. Further histological studies, coupling macrophage and iron labelling are needed to further investigate this hypothesis. Whatever the explanation of this phenomenon, our results indicate that it is possible to join into a single imaging protocol BV and VSI measurements using USPIO, and permeability measurements using a Gd-based CA, provided that the USPIO extravasation is taken into account.

**References:** [1].Tofts, P.S., *et al.* J Magn Reson Imaging, 1999. 10(3): 223-32. [2].Toprès, I., *et al.* Magn Reson Med, 2001. 45(3): 397-408. [3].Corot, C., *et al.* Invest Radiol, 2004. 39(10): 619-25.