Quantitative molecular MR imaging of U87 brain tumor angiogenesis using a novel RGD Gd-based emulsion

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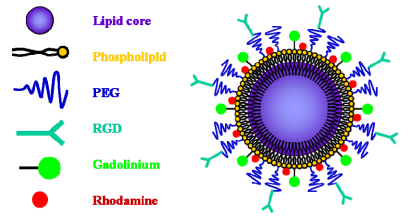

Fig.1. Schematic representation of the RGD-emulsion

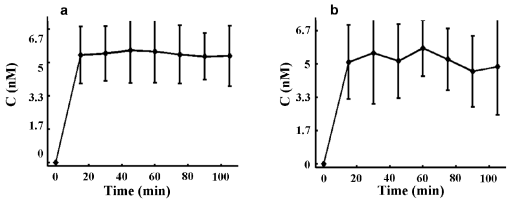

Fig.2. Concentration of the RGD-emulsion (a) and non-RGD emulsion (b) in the retro-orbital sinus during the experiment

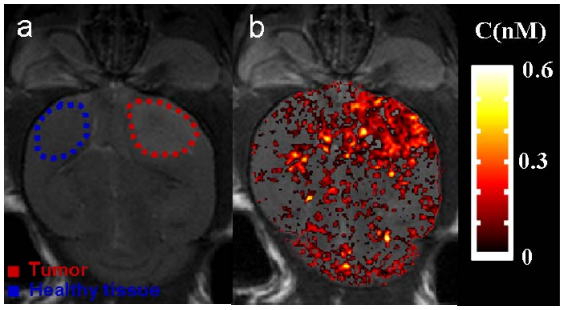

Fig.3. (a) Anatomical T_{2w} image of the mouse brain showing a 3mm U87 tumor in the right parenchyma. (b) Concentration map of the RGD-emulsion 75min after injection overlapped on the T_{2w} anatomical image.

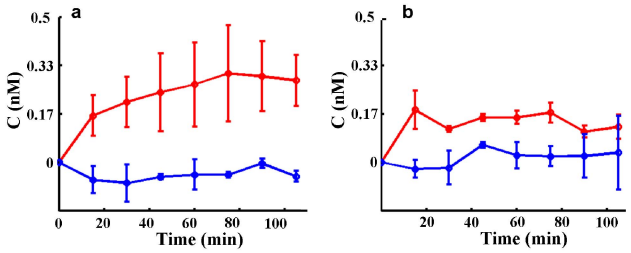

Fig.4. Concentration of RGD-emulsion (a) and non-RGD emulsion (b) during the experiment in the tumor ROI (red) and the control ROI (blue)

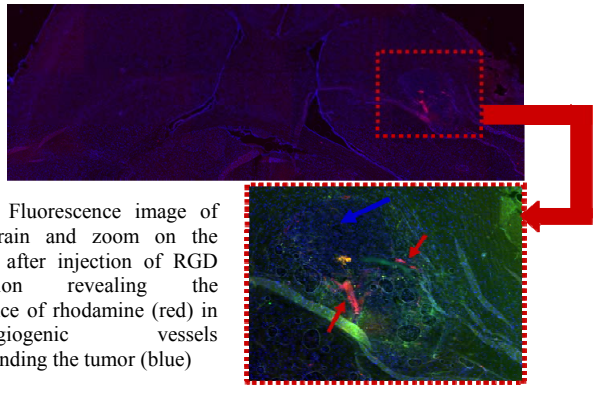

Fig.5. Fluorescence image of the brain and zoom on the tumor after injection of RGD emulsion revealing the presence of rhodamine (red) in neoangiogenic vessels surrounding the tumor (blue)

Background

Molecular magnetic resonance imaging is an increasingly used tool to investigate tumors angiogenic activity. In our work we used a high relaxivity Gadolinium-based (Gd-based) emulsion grafted with RGD peptides to target $\alpha_v\beta_3$ integrin over-expressed during tumor angiogenesis induced in mice brain [1]. This study proposes a methodology allowing *in vivo* quantification of the Gd-based emulsion concentration by acquiring dynamic T_1 maps.

Materials and Methods

Contrast agent. The emulsion consists in a lipid droplet (hydrodynamic diameter ~ 170nm) incorporating around 30000 gadolinium ions and surrounded by approximately 5000 PEG molecules to control its biodistribution. Rhodamine was added in order to use fluorescence imaging after the animal sacrifice. The RGD emulsion was obtained by the additional grafting of approximately 2500 RGD peptides (Fig.1). For control experiments, an equivalent non-RGD emulsion was used.

Animal protocol. U87 human glioblastoma cells (~120000, 2µL) were injected in the right caudate putamen of n = 5 nude mice. Imaging was done between 15 and 20 days after cells injection, when the tumor size was around 3-4mm. A catheter was installed in the caudal vein of the mouse to allow the contrast agent injection inside the magnet. Mice were separated into two groups: the targeted group was injected with the RGD emulsion (3 mice) while the control group was injected with the non-RGD emulsion (2 mice). Injected dose was 1nmol/kg of nanoparticles for both groups. Mice were sacrificed after MRI acquisition and their brain collected after an intra-cardiac perfusion of paraformaldehyd to remove blood.

MRI acquisitions. MRI experiments were performed on a 7T preclinical scanner (Bruker, Germany) using a 2.8-cm-diameter birdcage ^{1}H coil. Images were acquired before and every 13 minutes after injection with an IR-TurboFLASH sequence (TE/TR=2.5/5ms, 60 inversion times from 45 to 4765ms spaced by 80ms). T_1 maps were generated using the approach proposed by Deichmann et al. [2]. The longitudinal relaxivities of the two Gd-based emulsions were measured separately *in vitro* in an agar matrix assuming that they hardly differ from those in the brain tissue ($r_1 \sim 240000 mM^{-1}.s^{-1}$ in term of nanoparticles).

Data processing. Parametric T_1 maps were generated using a pixel by pixel fitting procedure written in Matlab (The MathWorks Inc, USA). Concentration maps after injection were calculated from corresponding T_1 maps using the T_1 map before injection (T_{10}) and the measured r_1: $C = 1/r_1 \times (1/T_1 - 1/T_{10})$. Regions of interest were taken in the tumor, in the healthy tissue (Fig.3a.), and in the retro-orbital sinus to follow the contrast agent concentration during around 2 hours.

Results

The complete methodology combining high relaxivity Gd-based emulsion and the acquisition of quantitative concentration map allows us to achieve *in vitro* a sensitivity threshold below 100 picomolar of nanoparticles. Figure 2 shows the concentration in the retro orbital sinus along the experiment for the RGD and non-RGD emulsions. Both curves reach a plateau just after the injection demonstrating a long term vascular remanence of these contrast agents. Figure 4 reveals that the concentration of both emulsions is more important in the tumor than in the control ROI (Fig. 4). It reflects that the tumor is more vascularized than healthy tissues due to neoangiogenesis. Furthermore the RGD emulsion seems to accumulate in the tumor all along the experiment (see figure 3 and 4) suggesting a binding effect of the targeted emulsion. This accumulation is not observed in the case of non-RGD emulsion. The specific binding was confirmed by histology (Fig.5): after intra-cardiac perfusion of mice injected with RGD emulsion, rhodamine was only observed in the neoangiogenic vessels surrounding the tumor.

Discussion and Conclusion

This study shows that quantitative molecular MR imaging of brain tumor angiogenesis is possible using a high relaxivity and targeted Gd-based contrast agent combined with quantitative T_1 imaging. A proof of concept of its binding was obtained *in vivo* with brain tumors induced in nude mice.

References

[1] Hsu et al, Mol Imaging Biol (2006), 8: 315-323
[2] Deichman et al, MRM (1999), 42: 206-209