Semi-quantification of lipoCEST CA *in vivo*. Application to molecular imaging of $\alpha_v\beta_3$ integrins expressed during angiogenesis using targeted lipoCEST CA in a tumor mouse brain model

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Introduction

Recently, Guerbet (WO2006/032705) and Aime S. [1] have introduced lipoCEST, a promising contrast agent for MR-monitored drug delivery and molecular imaging [2] which allows achieving sub-nanomolar sensitivity *in vitro*. They can be functionalized by grafting peptide in order to target specific biomarker such as the integrin $\alpha_v\beta_3$ expressed in angiogenesis. Several studies have already shown that their detection *in vivo* was feasible [3,4,5] in spite of relative modest chemical shift (2-28 ppm) and endogenous MT contrast. However, the quantification of such CA remains challenging *in vivo* [6] but is a crucial aspect for molecular imaging. Therefore we developed a semi-quantitative analysis tool based on a 4-pools model of water exchange processes [7] in order to estimate lipoCEST CA concentration from the exogenous MTRasym effect and correct for errors induced by B_0 and B_1 field inhomogeneities. In this study, we first validated our semi-quantitative analysis tool *in vitro* using a calibrated lipoCEST phantom. Secondly, we applied our tool to establish semi-quantitative maps of lipoCEST CA in a tumor mouse brain model.

Subjects and Methods

Animal model. The study was performed on 24 "nude" mice 15 days after brain tumor induction by i.c. injection of U87 human cells (tumor size ~ 2-5mm).

LipoCEST CA. Two groups of Tm(III)-lipoCEST were used ($C_{lipoCEST}$=48nM, Guerbet): RGD-functionalized lipoCEST (affinity in the nanomolar range) and bare lipoCEST. A phantom containing 6 lipoCEST concentrations (0/0.1/0.5/1/10/25nM) was used for quantification validation.

MRI acquisitions. CEST images were acquired at 7T using a MSME sequence preceded by a CW saturation pulse (T_{sat}=400ms, B_{1sat}=7µT, δ_{sat}=±8ppm) before (t=0') and after i.v injection (t=18'/30'/42'/60'/72'/84'/96'/108') in the caudal vein of 200µL of RGD or control-lipoCEST. MTRasym images were expressed as 100*($Image_{OFF}$-$Image_{ON}$)/$Image_{Ref}$. CEST contrast was analyzed in the "tumor" and the "controlateral" regions.

Semi-quantitative analysis tool. As illustrated by Fig.1, semi-quantitative lipoCEST maps were obtained by minimizing the cost function $|MTRasym(r)_{exp} - MTRasym(C_{lipoCEST},r,B_0,B_1)_{sim}|$ which is the difference between experimental MTRasym and simulated MTRasym using a 4-pools model based of modified Bloch equations (H_A: bulk protons; H_B: macromolecular protons; H_C: amide protons; H_D: protons shifted by lipoCEST). Model parameters were determined by fitting of *in vivo* Z-spectra acquired in rodent brain (for pools A, B and C) and by fitting of *in vitro* Z-spectrum acquired on lipoCEST phantom (for pool D, see Fig.2).

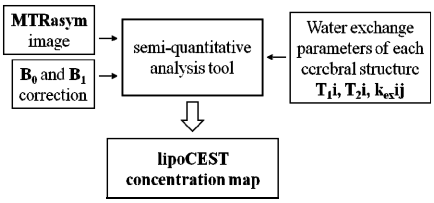

Fig.1 Flow chart of our semi-quantitative analysis tool

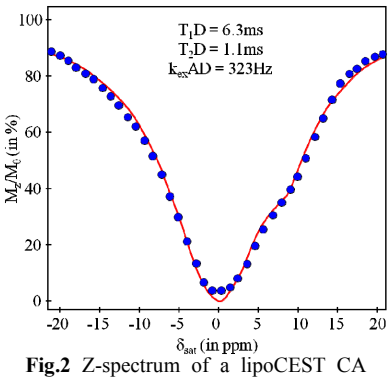

Fig.2 Z-spectrum of a lipoCEST CA (blue dots) and its adjustment using 4-pools model (red curve)

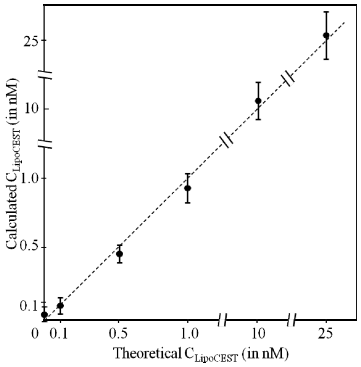

Fig.3 In vitro validation of semi-quantification tool on 6 different lipoCEST concentrations

Results and discussion

As shown by Fig.3, the lipoCEST CA concentrations calculated using our semi-quantitative analysis tool were consistent with theoretical concentrations of calibrated lipoCEST phantom. The precision of our analysis tool was estimated to be 0.2nM *in vitro*. This tool was applied *in vivo* and concentration maps of RGD-lipoCEST (Fig.4a) and Ctrl-lipoCEST (Fig.4b) were established 1h post-injection showing higher accumulation of RGD-LipoCEST in the tumor region. In order to better appreciate the distinct fate of lipoCEST CA, lipoCEST concentrations were averaged in "tumor" and "controlateral" ROIs and through the animal cohort (Fig.5). Interestingly, lipoCEST concentration was almost comparable in "tumor" and "controlateral" ROIs for the bare lipoCEST (Fig.5, green and yellow curves) and in "controlateral" ROI for the RGD-lipoCEST (Fig.5, red curve). However, a significant and *a priori* specific enhancement of the tumor region was observed following the injection of RGD-functionalized lipoCEST after 1h post-injection ($p<0.01$, Fig.5, blue curve). If a lipoCEST concentration decrease was observed for all control conditions, a plateau is rapidly reached at a concentration of 1.6±0.3nM (mean±SD, n=12) with RGD-lipoCEST in "tumor" ROI arguing in favor of an accumulation of intact RGD-lipoCEST following specific binding to the $\alpha_v\beta_3$ target. Typical time-constants T_{in} for accumulation and T_{out} for wash-out were estimated on average concentration curves using simple bi-exponential function $A.(1-\exp(-t/T_{in})).\exp(-t/T_{out})$. T_{in} were comparable between the four conditions (11 to 16min) whereas T_{out} with RGD-lipoCEST in "tumor" ROI (609min) was longer that in control conditions (~110min). This shorter T_{out} was compatible with half-life of flowing liposomes (~1h30).

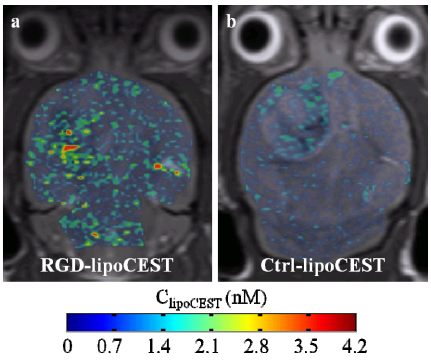

Fig.4 LipoCEST concentration maps acquired 1h after i.v. injection of either RGD-lipoCEST (**a**) or Ctrl-lipoCEST (**b**)

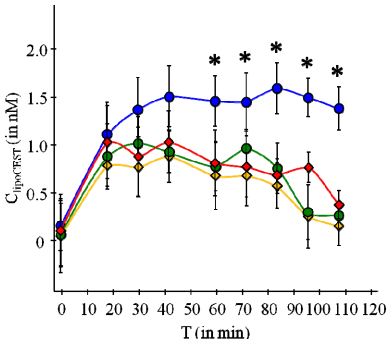

Fig.5 Mean lipoCEST concentration in "tumor" (blue (RGD) and green (Ctrl) curves) and "controlateral" (red (RGD) and yellow (Ctrl) curves) ROIs after group averaging (n = 12)

Conclusion

In this study, we showed that semi-quantification of lipoCEST CA was feasible thanks to a semi-quantitative analysis tool based on a 4-pools model taking into actual relaxation parameters for each brain structure and B_0 and B_1 fields values. This allows us to go beyond simple detection of lipoCEST CA and opens the way for compartment modeling of our targeted lipoCEST CA kinetics *in vivo*.

References

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