In vivo LipoCEST CA accumulation around U87 mice brain tumor demonstrated by in vivo CEST MRI and ex vivo fluorescence microscopy

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Introduction

Recently, Guerbet (WO 2006/032705) and Aime S. et al. [1] have introduced LipoCEST, a promising class of contrast agents for MR-based molecular imaging and MR-monitored drug delivery [2]. LipoCEST CA are lip-bilayer filled with a huge amount of paramagnetic complexes. By applying frequency selective presaturation prior to MR imaging, LipoCEST CA allows to achieve sub-nanomolar sensitivity in vitro. Yet, to our knowledge, paramagnetic liposomes were only detected in vivo following systemic administration as T2 MRI contrast agents. Recently, such PEGylated “stealth” paramagnetic liposomes have been shown to be avidly taken up by macrophages [3-4]. For LipoCEST CA, similar cellular uptake could be extremely detrimental for its detection in vivo leading to doubts over its future applicability. In this study, we propose to demonstrate that LipoCEST can be detected in vivo after i.v injection using CEST-MRI and to confirm such observation using ex vivo fluorescence microscopy.

Subjects and Methods

Animal preparation. Tumor was induced by i.c injection of 1.2x106 glioma U87 human cells in a single immuno-depressed "nude" mouse brain. Experiments were performed 10 days after tumor induction when tumors were between 2-4mm wide.

MRI acquisition. Brain CEST images were acquired using a MSME sequence (TE/TR=54/5000ms, resolution 150x150x660μm, Taq=14min) preceded by a CW saturation pulse (Tsat =400ms, Bsat=7μT, Δsat=-6ppm) on a 7 T small animal MRI scanner (Bruker, Germany) using home-made 2.8cm-diameter quadrature volumic H1 coil. Images were acquired before (pre-injection) and 1-hr (post-injection) after i.v injection in the caudal vein of 10mL/kg b.w. of PEGylated LipoCEST with fluorescent rhodamine on its surface (Guerbet, France).

Z-spectrum acquisition. Z-spectrum was acquired using the same parameters than CEST images with Bsat=7μT, Δsat=[-20;20]ppm (see Fig.1).

Image analysis. %CEST images were obtained by the subtraction of images acquired with saturation applied at 6 and -6ppm normalized by the reference image without saturation. %CEST contrast was analyzed in different regions-of-interest corresponding to the entire "brain", the "tumor" and the area "controlateral" to the tumor.

Fluorescence microscopy. After intracardiac puncture and sacrifice of the mouse, the brain was extracted immediately and immersed in PFA solution for 24h then in sucrose solution for fixation of tissue. Fluorescence microscopy was performed on a slice corresponding to the tumor region. Cells nuclei were stained with DAPI (Fig.3.A) and fluorescent image was acquired at the frequency of emission wavelength of rhodamine.

Results

As illustrated by Fig.2, following i.v injection of LipoCEST, the mean CEST effect in the whole brain was found to be 10% higher 1h after the injection than before whereas it was 43% higher in the tumor (Tab.1). The fluorescence microscopy (Fig.3.B) confirms the presence of rhodamine-LipoCEST CA in vessels at the rim of the tumor (white arrow).

Discussion and Conclusion

To our knowledge, this is the first report of LipoCEST CA detection following systemic administration in vivo. Our observation is validated by fluorescence microscopy ex vivo. Yet, our observations are not conclusive and many questions about the fate of LipoCEST in vivo remain.

First, we need to establish the compartmentation of rhodamine-LipoCEST and free rhodamine by immuno-histology. Secondly, we should look more precisely to the dynamic of the LipoCEST CA eventually by applying the same multimodal approach than Delli Castelli et al. [3]. The next step of our study will focus on using functionalized LipoCEST with RGD peptide to demonstrate the feasibility of αvβ3 receptors mapping in the U87 mice brain tumor model.

References


<table>
<thead>
<tr>
<th>RDI</th>
<th>Pre-injection</th>
<th>Post-injection</th>
<th>Relative variation</th>
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<tbody>
<tr>
<td>Tumor</td>
<td>3.1 ± 2.0</td>
<td>4.3 ± 2.3</td>
<td>+ 43%</td>
</tr>
<tr>
<td>Controlateral</td>
<td>2.9 ± 2.3</td>
<td>3.1 ± 2.2</td>
<td>+ 7%</td>
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<tr>
<td>Brain</td>
<td>2.9 ± 2.2</td>
<td>3.2 ± 2.3</td>
<td>+ 10%</td>
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Tab.1. Mean, standard deviation and relative variation of CEST signal in "all brain", "tumor" and "controlateral" ROIs.