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EXCI-CEST: Exploiting pharmaceutical excipients as MRI-CEST contrast agents for tumor imaging

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ABSTRACT

Chemical Exchange Saturation Transfer (CEST) approach is a novel tool within magnetic resonance imaging (MRI) that allows visualization of molecules possessing exchangeable protons with water. Many molecules, employed as excipients for the formulation of finished drug products, are endowed with hydroxyl, amine or amide protons, thus can be exploitable as MRI-CEST contrast agents. Their high safety profiles allow them to be injected at very high doses. Here we investigated the MRI-CEST properties of several excipients (ascorbic acid, sucrose, *N*-acetyl-D-glucosamine, meglumine and 2-pyrrolidone) and tested them as tumor-detecting agents in two different murine tumor models (breast and melanoma cancers). All the investigated molecules showed remarkable CEST contrast upon i.v. administration in the range 1–3 ppm according to the type of mobile proton groups. A marked increase of CEST contrast was observed in tumor regions up to 30 min post injection. The combination of marked tumor contrast enhancement and lack of toxicity make these molecules potential candidates for the diagnosis of tumors within the MRI-CEST approach.

1. Introduction

Medicines could not be made without the use of pharmaceutical excipients that contribute notably to guarantee efficacy and safety of the final pharmaceutical product (Casas et al., 2015). Moreover, excipients perform multiple functions, besides completing the formulation volume, such as improving bioavailability, administration and acceptance of the treatment by the patient (Loftsson, 2015; Narayan, 2011; Wening and Breitkreutz, 2011). Another fundamental characteristic of excipients is their pharmacological and toxicological inactivity that allows them to be used at high doses (Abrantes et al., 2016). Several natural products, simple substances and mixtures are commonly used in formulating medicines, with chemical structures that vary from small molecules to polymers.

Interestingly, most, if not all of these molecules, possess exchangeable protons (hydroxyl, amine, amide groups) that can be potentially detected by chemical exchange saturation transfer (CEST) magnetic resonance imaging (MRI) (van Zijl and Yadav, 2011; Vinogradov et al., 2013). This technique enables the indirect visualization of molecules via magnetization transfer between exchangeable protons and bulk water protons. By applying a selective radiofrequency irradiation to the mobile protons, the induced saturation is transferred to the bulk water protons, thus inducing a reduction of the water signal (Liu et al., 2013). Several natural molecules and polymers (glucose, glycogen, glycosaminoglycan, sialic acid, gelatin) have already been exploited as MRI-CEST contrast agents, since these molecules have precedence of use with human exposure (Chan et al., 2012; Jin et al., 2017; Liang et al.,

Abbreviations: MRI, magnetic resonance imaging; CEST, chemical exchange saturation transfer; i.v., intravenous.

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2015; Ling et al., 2008; Shinar et al., 2014; van Zijl et al., 2007; Walker-Samuel et al., 2013). Also metabolites, drugs and polypeptides/proteins have been investigated to demonstrate their capability to generate contrast within this approach (Bar-Shir et al., 2015, 2014; Cai et al., 2015; Haris et al., 2012; Li et al., 2016; Liu et al., 2016; Longo et al., 2014a; McMahon et al., 2008; Zaiss et al., 2013). Moreover, several diamagnetic CEST agents have been proposed as exogenous probes for tumor imaging (Geraldes and Laurent, 2009). However, diamagnetic molecules require high doses to discriminate their contrast from direct water saturation and from endogenous magnetization transfer effects, due to the small chemical shift difference from water.

These considerations limit the effective use of exogenous molecules as CEST agents to those possessing low *in vivo* toxicity. According to these considerations, researchers firstly turned their attention to already clinically approved contrast agents, such as iodinated contrast media, exploiting their high safety profile and FDA approval (Aime et al., 2005b; Longo et al., 2011). Consequently, radiographic agents have been exploited for assessing tumor microenvironment properties, including perfusion (Anemone et al., 2017; Longo et al., 2016b), acidosis (Chen et al., 2015; Jones et al., 2015; Longo et al., 2016a, 2014b; Sun et al., 2014) and for assessing renal functionality (Longo et al., 2013, 2017).

Pharmaceutical excipients have attracted our interest since them can be used at very high dose due to their well-known safety profiles. In addition, excipients do not have any pharmacological effects, in contrast to active pharmaceutical ingredients. Ideally, a MRI-CEST contrast agent should possess good solubility and high safety profile, it should accumulate enough in the region of interest to produce contrast; afterwards, it should be excreted through the kidneys without long-term accumulation (Aime et al., 2005a; Sherry et al., 2009). The present investigation reports the MRI-CEST properties of several pharmaceutical excipients (sucrose, *N*-acetyl-D-glucosamine, ascorbic acid, meglumine and 2-pyrrolidone), as novel, biocompatible MRI contrast agents for molecular imaging of tumors. We describe the *in vitro* MRI-CEST contrast enhancing properties and the *in vivo* investigation of these molecules in two murine tumor models.

2. Methods

2.1. Materials

All chemicals (Sucrose, *N*-acetyl-D-glucosamine, Meglumine, 2-pyrrolidone, Ascorbic acid) were purchased from Sigma-Aldrich (Sigma Aldrich, Milan, Italy).

2.2. In vitro MRI CEST acquisition

Phantoms containing vials of phosphate buffer solution of Sucrose, *N*-acetyl-D-glucosamine, Meglumine, 2-pyrrolidone or ascorbic acid were prepared at a concentration of 30 mM and titrated over a range 6–7.4 pH units. CEST-MRI experiments were performed on a vertical 7 T MRI scanner Bruker Avance 300 (Bruker, Ettlingen, Germany) using a fast spin-echo sequence with centric encoding after presaturation pulses varying in power (1.5, 2.0, 3.0 and 6.0 μT) for 5 or 7 s at 37 °C. A modified RARE sequence including a magnetization transfer module was used to acquire CEST-weighted images from −10 to +10 ppm with increments of 0.1 ppm around the water resonance.

2.3. Cell lines for xenograft tumor models

TS/A cells, derived from a metastasizing mouse cell line, originated from a mammary adenocarcinoma which arose spontaneously in a BALB/c female, were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100U/mL Penicillin with 100 μg/ml Streptomycin (Pen/Strep) and 2 mM L-Glutamine (Nanni et al., 1983). B16-F10 cells, an established murine melanoma cell line, were cultured in DMEM supplemented with 10% FBS, 100 μg/ml penicillin and 100 μg/ml streptomycin. B16-F10 cells were obtained from American Type Culture Collection (ATCC).

2.4. Animal experiments

6-old-week female BALB/c mice (n = 5 for each molecule) were inoculated subcutaneously with 2.5×10^5 TS/A cells in 100 μL of PBS on both flanks and 6-old-week male C57BL/6 mice (n = 5 for each molecule) were inoculated subcutaneously with 3×10^5 B16-f10 cell in 100 μL of PBS on both flanks. BALB/c and C57BL/6 mice (Charles River Laboratories Italia S.r.l., Calco Italia) were maintained under specific pathogen free conditions in the animal facility of the Molecular Biotechnology Center, University of Turin, and treated in accordance with the EU guidelines (EU2010/63). All in vivo studies were conducted according to approved procedures of the Institutional Animal Care and Use Committee of the University of Torino.

Before imaging, mice were anaesthetized with a mixture of tiletamine/zolazepam (Zoletil 100; Vibac, Milan, Italy) 20 mg/kg and xylazine (Rompun; Bayer, Milan, Italy) 5 mg/kg and during the acquisition their breath rate was monitored throughout in vivo MRI experiments using a respiratory probe. Cannulation of the lateral tail vein with a catheter was exploited for intravenous injection of the investigated molecules.

2.5. In vivo MRI CEST acquisition and analysis

A Bruker 7T Avance 300 MRI scanner (Bruker Biospin, Ettlingen, Germany) equipped with a 30 mm 1H quadrature coil was used to scan mammary adenocarcinoma (TS/A cell line) and melanoma (B16-f10 cell line) tumor bearing mice 15 days post-inoculation. After the scout image acquisition, T_{2w} anatomical images were acquired with a Fast Spin Echo sequence and the same geometry was used for the following CEST experiments. CEST images were acquired with a single shot FSE sequence with centric encoding (TR: 6000 ms, TE: 4.0 ms) after a CW-RF presaturation pulse of $B_1 = 1.5\ \mu T \times 5$ s from a single axial slice with high in-plane resolution of 234 μm (FOV 3 cm, MTX 96, zero filled to 128, slice thickness 1.5 mm) with 55 frequency offsets unevenly spaced in the range ±10 ppm. Each investigated molecule was administrated intravenously at the dose of 1.2 g/kg b.w. with a single bolus of 100 μL followed by continuous infusion at a rate of 500 μL/h and CEST images were acquired before and every 10 min up to 30 min.

CEST images were analyzed using homemade scripts implemented in MATLAB (The Mathworks, Inc, Natick, MA). The Z-spectra were interpolated, on a voxel-by-voxel basis, by smoothing splines, B_0-shift corrected and saturation transfer efficiency (ST%) was measured by punctual analysis at 1.2 ppm (Terreno et al., 2009). For in vivo images, difference contrast maps (ΔST%) were calculated by subtracting the ST contrast after each molecules injection from the ST contrast before the injection on a per voxel basis. Extravasation fraction of each molecule was calculated as the percentage of pixels showing a ΔST% above the threshold (2%) in the manually-defined tumor region of interest.

2.6. Statistical analysis

Calculations were performed using GraphPad Prism (GraphPad Software, La Jolla, CA, USA) software package; data are presented as mean ± SD unless otherwise stated. Statistical significance was established at $P < 0.05$.