EXCI-CEST: exploiting pharmaceutical excipients as safe MRI-CEST contrast agents

Dario Livio Longo[1,2], Fatima Zzahra Moustaghfir[3], Lorena Consolino[1,2], Giuseppe Digilio[4], and Silvio Aime[1,2]

[1]*University of Turin, Torino, Italy,* [2]*Molecular Imaging Center, University of Turin, Torino, Italy,* [3]*University of Vrije Brussels, Brussels, Belgium,* [4]*Environmental and Life Science, University of Eastern Piedmont "Amedeo Avogadro", Alessandria, Italy*

Target audience:

Those interested to explore new and ready-to-use molecules as MRI-CEST contrast agents possessing safety profile.

Purpose:

Molecules possessing exchangeable protons may be exploited as Chemical exchange saturation transfer (CEST) contrast agents. They include sugars, metabolites, peptides and X-ray iodinated contrast media [1-4]. Those diamagnetic molecules possess suitable exchangeable protons resonating at a close distance from bulk water signal, thus requiring high doses to discriminate their contrast from direct bulk water signal and from endogenous magnetization transfer effect. This consideration limits the effective use of molecules as in vivo CEST agents to those possessing low in vivo toxicity. Many molecules, employed as excipients for the formulation of finished drug products, can be used at very high amount due to their safety profiles. In contrast to active pharmaceutical ingredients, they don't have pharmacological effects and their LD50 may vary between 1 to 5 g / kg body weight. We investigated the CEST properties of several excipients (sucrose, mannitol, ascorbic acid, N-acetyl-D-glucosamine, 2-pyrrolidone, meglumine) both in vitro as well as in mice tumor models. Being all the examined molecules already approved for drug formulation at high doses, one may foresee for these chemicals a possible translation for human investigations.

Methods:

High-resolution NMR spectra on a Bruker Avance 600 spectrometer operating at 14 T were acquired to identify the chemical shift of the exchangeable proton pools and the pH dependence of their exchange rate constants at several temperature and pH values. Saturation transfer efficiency (ST%) was measured in phantoms of a 30 mM solutions in phosphate buffer in the pH range between 5.5 and 7.9. CEST experiments were carried out on a 7 T MRI scanner Bruker Avance 300 using a fast spin-echo sequence preceded by saturation pulses varying in power (1.5-9 μT) and length (1 to 9s) at 37°C.
In vivo detection on TSA breast tumor bearing mice (n=3 for each molecule) and on B16 melanoma tumor bearing mice (n=3 for each molecule) was assessed by i.v. injection at doses of 0.5-1.0 g/kg b.w. by MRI-CEST applying a RF pulse of 1.5 and 3 μT x 5s and ST% calculated.

Results/Discussion:

The chemical shifts of the exchangeable proton pools of the considered excipients lie in the range of 0.5 to 1.5 ppm for molecules possessing hydroxilic groups (sucrose, mannitol, ascorbic acid, N-acetyl-D-glucosamine, meglumine) and in the range 2.5-3.0 ppm for amide groups (pyrrolidone and N-acetyl-D-glucosamine) and at 3.2 ppm for amine group (meglumine) in respect to bulk water signal, respectively. Sucrose and meglumine are the molecules showing the higher contrast in the range 0.5-1.0 ppm (ca. 30% for a 30 mM solution), whereas ascorbic acid and 2-pyrrolidone show the lower ST contrast (ca. 5-10%); N-acetyl-D-glucosamine and meglumine clearly show high ST contrast at both the exchangeable proton pools (ca. 15-25%) as showed in Figure 1.
We observed a pronounced increase in the CEST contrast in TSA breast tumor model for the following molecules: ascorbic acid, N-acetyl-D-glucosamine and sucrose, with an average increase of 5 to 8% in comparison to the pre-contrast ST curve (Figure 2). The observed differences in the CEST contrast in the two tumor models (B16 and TSA), may be likely associated to different accumulation effects as well as to changes in tumor vascularization levels influencing the amount of detectable ST contrast.

Conclusions:

The investigated excipient molecules show remarkable MRI-CEST properties allowing their in vivo visualization as MRI-CEST contrast agents in two different tumor models. Moreover, their safety profile allows possible translation to clinical application.

Acknowledgement / References:

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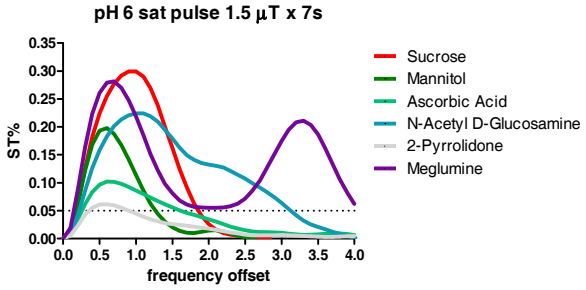

Fig. 1 CEST contrast (ST%) for excipient molecules (30 mM in PBS at pH=6, 37°C, at 7T saturation pulse B_1 = 1.5 μT x 7s).

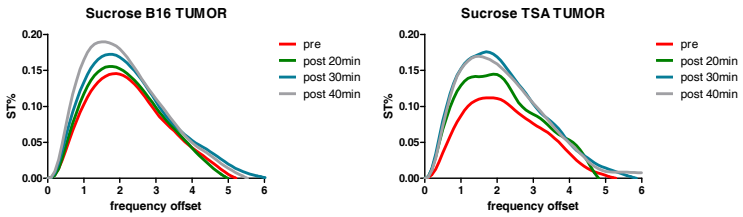

Fig. 2 in vivo contrast showing ST curves before and after the injection of sucrose at a dose of 0.5 g /k g bw in TSA breast tumor (left) and in B16 melanoma bearing mouse (saturation pulse B_1 = 3 μT x 5s).