Transforming the herpes simplex virus type-1 thymidine kinase (HSV1-tk) into an MRI reporter gene

Amnon Bar-Shir[1,2], Guanshu Liu[1,3], Nirbhay N Yadav[1,3], Yoshinori Kato[1], Galit Pelled[1,3], Piotr Walczak[1,2], Michael T McMahon[1,3], Martin G Pomper[1], Marc M Greenberg[4], Peter C van Zijl[1,3], Jeff W Bulte[1,2], and Assaf A Gilad[1,2]

[1]*Department of Radiology, Johns Hopkins University, Baltimore, Maryland, United States,* [2]*Cellular Imaging Section, Johns Hopkins University, Baltimore, Maryland, United States,* [3]*F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, Maryland, United States,* [4]*Department of Chemistry, Johns Hopkins University, Baltimore, Maryland, United States*

Introduction

Chemical exchange saturation transfer (CEST) MRI based reporter genes have great potential for probing protein function changes due to mutations (genetic) or impaired regulation (epigenetic) that are the essence of every abnormality or disease. However, CEST studies of proteins are limited by the small chemical shift difference of their exchangeable protons with water ($\Delta\omega$ <3.6 ppm), which results in interference from endogenous CEST contrast and, depending on the irradiation field, from direct water saturation. To overcome this drawback, we used the enzyme herpes simplex virus type-1 thymidine kinase (HSV1-tk), a widely used reporter gene for positron emission tomography (PET), which relies on entrapment of the imaging probe in HSV1-tk expressing cells following phosphorylation (*1, 2*). The imino NH protons of the HSV1-tk's substrate, thymidine (dT), is a good candidate for CEST MRI applications because of its large chemical shift difference with water ($\Delta\omega$ ~ 5-6 ppm) and high exchange rate (*3, 4*). We show here that a rational chemical modification of dT improves its CEST-MRI properties by increasing the imino proton's pK_a and reducing its exchange rate (k_{ex}) with water. The synthetic dT analog 5-methyl-5,6-dihydrothymidine showed excellent CEST contrast and high sensitivity and specificity to HSV1-tk both *in vitro* and *in vivo*, thus adding HSV1-tk to the growing family of CEST based reporter genes (*5, 6*).

Materials and Methods

In vitro: All compounds were dissolved in 10 mM PBS at 20 mM concentration. CEST MRI experiments were performed at 11.7 T and 37 °C. CEST-weighted images were acquired with a modified RARE pulse sequence (TR/TE=6000/9.4 ms) with a 4000 ms pre-saturation pulse (see Table 1 for saturation field strengths). The mean Z-spectra were calculated from an ROI for each sample after B_0 correction of each voxel using WASSR (*7*). $MTR_{asym} = (S^{-\Delta\omega}-S^{+\Delta\omega})/S^0 \times 100$ was computed at $\Delta\omega$ = 5 ppm for the imino protons of all compounds. pK_a values of the imino protons of dT and its analogs were calculated using the MarvinSketch 5.3.3 software. Exchange rates were quantified using the QUESP (*8*) method with saturation field strengths of 1, 2, 3, 4, 5, 6, 8 and 10 μT. *In vivo*: 9L rat glioma, engineered to express HSV1-tk ($9L^{HSV1\text{-}TK}$) and control, non-expressing cells ($9L^{WT}$) were transplanted into the brains of adult NOD-SCID male mice resulting in a bilateral tumors. One week after cells implantaions 0.2 mL of compound **2** (160 mg/kg) were injected intravenously (i.v) and CEST-MRI data were aquired at 11.7 T MRI scanner (Biospec, Bruker). A series of four S^{-5ppm}/ S^{+5ppm} (5.0 μT/4000 ms) data sets were acquired 1, 2 and 3 hours after i.v injection.

Results and Discussion

Initially, five pyrimidine-based molecules were identified as putative imaging agents (Table 1). Increasing the pK_a value of the imino proton of dT from pK_a = 9.96 to 11.6 was achived by reduction of its 5,6-double bond to obtain dihydropyrimidine-based nucleosides (compounds **1** or **2**). This lead to a significant decrease of the exchange rate (k_{ex}) between this proton and the water protons (Table 1) at physiological pH. These dihydropyrimidine-based nucleosides fulfill the "slow to intermediate regime" condition (i.e., $k_{ex} \leq \Delta\omega$) required for generating high CEST contrast. Conversly, compound **4**, which had the lowest pK_a value, showed negligble CEST contrast at 5-6 ppm.

	 dT	 1	 2	 3	 4
pK_a	9.96	11.60	11.57	11.0	7.97
k_{ex} (sec^{-1})	29×10^2	7.0×10^2	13×10^2	23×10^2	Fast
MTR_{asym} (3μT)	5%	14%	10%	7%	0.3%
MTR_{asym} (5μT)	15%	19%	21%	16%	1%

Table 1: Chemical structures of dT and its analogs and their characteristics.

Interestingly, the phosphorylation by HSV1-tk of compounds **1** and **2** was the same as the native substrate dT, and the phosphorylated compounds showed no difference in the CEST contrast. Additionally, compound **2** is not phosphorylated by endogenous mammalian kinases thus making it an ideal substrate for HSV1-tk as a CEST reporter gene.

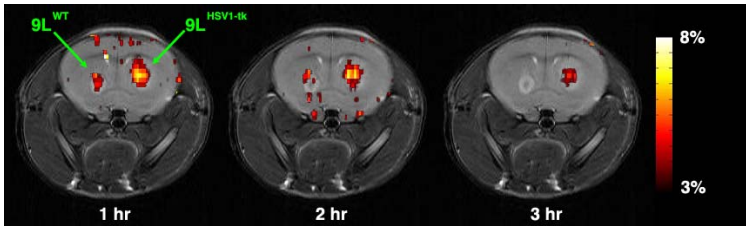

Figure 1. In vivo CEST-MR images (MTR_{asym} maps at 5ppm) of the distribution of compound 2 after i.v injection. Left hemisphere: wild type 9L tumor ($9L^{WT}$); Right hemisphere: 9L tumor expressing HSV1-tk ($9L^{HSV1\text{-}tk}$).

Figure 1 demonstrates that by i.v injection of compound **2**, HSV1-tk expressing cells ($9L^{HSV1\text{-}tk}$) could be distinguished from control non-expressing cells ($9L^{WT}$). The MTR_{asym} map obtained at 5 ppm 1 hour after i.v. injection (Figure 1, left panel) shows an increase in CEST contrast at 5 ppm for both tumors. This is a result of contrast agent leakage from highly permeable blood vessels in the tumor. However, after 2 hours (middle panel) clearance of the agent was observed only in the control tumor ($9L^{WT}$). Three hours after injection, the agent was observed only in the $9L^{HSV\text{-}tk}$ tumor, indicating accumulation of the agent due to phosphorylation by HSV1-tk.

Conclusion

This study shows that compound **2** (5-methyl-5,6-dihydrothymidine) is a sensitive probe designed for using HSV1-tk as a new CEST based MRI reporter gene *in vivo*.

References:

1. S. Soghomonyan *et al.*, *Nat Protoc* **2**, 416 (2007). 2. S. S. Yaghoubi, S. S. Gambhir, *Nat Protoc* **1**, 2137 (2006). 3. K. Snoussi, J. W. Bulte, M. Gueron, P. C. van Zijl, *Magn Reson Med* **49**, 998 (2003). 4. K. M. Ward, A. H. Aletras, R. S. Balaban, *J Magn Reson* **143**, 79 (2000). 5. A. A. Gilad *et al.*, *Nat Biotechnol* **25**, 217 (2007). 6. G. Liu *et al.*, *J Am Chem Soc* **133**, 16326 (2011). 7. M. Kim, J. Gillen, B. A. Landman, J. Zhou, P. C. van Zijl, *Magn Reson Med* **61**, 1441 (2009). 8. M. T. McMahon *et al.*, *Magn Reson Med* **55**, 836 (2006).