Design of Thymidine Analogs as CEST Reporters for Imaging of HSV1-TK Expression

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Introduction

The gene encoding the enzyme Herpes Simplex Virus type 1 Thymidine Kinase (HSV1-TK) is in widespread used for gene therapy as well as for positron emission tomography (PET) imaging and has been used clinically (*1*). Once expressed by cells, HSV1-TK phosphorylates not only thymidine but also a range of nucleoside analogs. These analogs (the reporter probes) can freely cross the cell membrane prior to phosphorylation but accumulate in HSV1-TK expressing cells upon phosphorylation (*2, 3*). Unlike PET, an imaging probe for MRI will eliminate the need of radio-labeled probes and allow combining high-resolution anatomical information with sub-cellular genetic information. The imino NH protons of nucleosides are good candidates for chemical exchange saturation transfer (CEST) based MRI applications, because their chemical shift difference with water (Δω~5-6ppm) minimizes contributions from direct water saturation and endogenous CEST contrast (*4, 5*). We demonstrate here that chemical modification of deoxynucleosides improves their CEST-MRI characteristics, thus making them potential reporter probes for MRI of HSV1-TK expression.

Materials and Methods

All compounds were dissolved in 10mM PBS at 40mM concentration. CEST MRI experiments were performed on an 11.7T Bruker Avance system. A modified RARE (TR/TE=6000/9.4 ms, RARE factor =16, 2mm slice thickness, FOV=14x14 mm, matrix size=128x64, resolution= 0.11x0.22mm, and NA=2) including a magnetization transfer (MT) module (B1 = 4.7 μT/4000ms) was used to acquire CEST weighted images from -8ppm to 8ppm (step=0.2ppm) around the water resonance (0ppm). The absolute water resonant frequency shift was measured using a modified Water Saturation Shift Reference (WASSR) method (*6*), using the same parameters as in CEST imaging except TR=1.5 sec, saturation pulse of 250 ms, B1 =0.5 μT and a sweep range from -3ppm to 3 ppm (step= 0.1ppm). Data processing was performed using custom-written scripts in Matlab. Mean Z-spectra were used from a ROI for each sample, after B0 correction for each voxel. MTR asymmetry $=(S_{-\Delta\omega} - S_{+\Delta\omega})/ S_0$ was computed at different offsets Δω for the imino protons of the different thymidine analogues studies. pKa values of the NH exchangeable protons of thymidine and its analogs were calculated using MarvinSketch version 5.3.3 program.

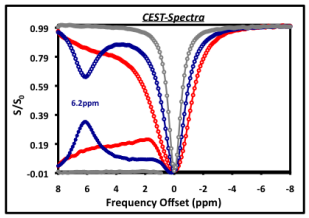

Figure 1: CEST-spectra of Thymidine (red), TTP (blue) and PBS (gray)

Results and Discussion

Initially, we examined the effect of phosphorylation of thymidine to thymidine triphosphate (TTP) on the CEST-MRI profile (Figure 1). Interestingly, TTP provided much higher CEST contrast as compared to thymidine with a narrower, better-defined peak at 6.2ppm. Since the acidity (pKa value) of the NH proton does not change due to phosphorylation, the difference in the CEST contrast and the sharper peak may be related to electrostatic or steric effects of the three negatively charged triphosphate groups. This important result implies that phosphorylation of Thymidine, and thus, the HSV1-TK activity, can be detected by CEST-MRI.

In order to further improve the probe, we focused on the imino protons of uridine-like pyrimidines. These are good candidates for CEST-MRI (*4*) even though they generate broad peaks and have high exchange rates with water protons. In order to slow the exchange rates of these NH protons, their pKa values should be increased. We found that modifying thymidine to 5,6-Dihydrothymidine (DHT) increases the pKa value of the NH proton from 9.96 to 11.60, thus decreasing its exchange rate with the surrounding water protons at physiological pH and making it a good candidate for CEST-MRI. Figure 2 shows the Z-spectra, MTR asymmetry spectra of thymidine (red), DHT (blue) and PBS (gray) and the MTR asymmetry maps. It is clear that DHT has higher MTR asymmetry values (35% drop of water signal in the CEST-MRI) and a sharper, narrower and better-defined NH peak at 4.9ppm compared to the broad peak of the respective NH proton of thymidine. These results demonstrate that the CEST-MRI characteristics of deoxynucleoside analogs can be controlled by chemical modifications, which change the pKa values and therefore the exchange rates of their NH protons with the water protons. DHT is a synthetic analog of thymidine, which should not be phosphorylated by mammalian thymidine kinase, and therefore has the potential to be used as a CEST-based reporter probe for imaging HSV1-TK expression by MRI. Moreover, further genetic alterations of the enzyme can improve the turnover rate and specificity of the HSV1-TK for this CEST-based substrate.

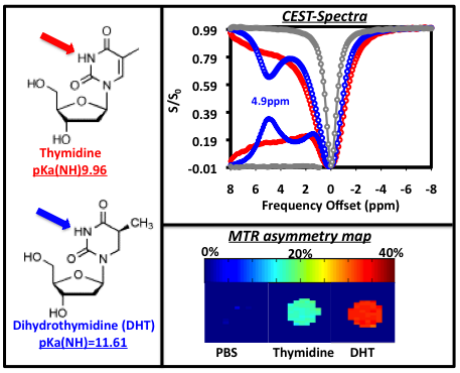

Figure 2: The CEST-spectra and MTR asymmetry map show the CEST characteristics of Thymidine (red), DHT (blue), and PBS (gray). Arrows indicate the NH (imino) exchangeable protons.

Conclusion

Chemical modification of the native HSV1-TK substrate (thymidine) to a synthetic analog (e.g., DHT), can considerably improve its CEST-MRI contrast thus making it suitable probe for MR imaging of the reporter gene HSV1-TK.

References

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