CNS HIV Theranostics Based on Intrinsic CEST Contrasts of Antiretroviral Drugs

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Background/Introduction

- Antiretroviral therapy (ART) prolongs the life of human immunodeficiency virus type-1 (HIV-1) infected patients.
- Viral replication in the central nervous system (CNS) persists and elicits neuroimmune activation and is associated with cognitive decline.
- Evidence shows ART can cause adverse clinical outcomes including neuropsychiatric, motor and behavioral events
- Therefore, the ability to follow drug pharmacokinetics (PK) and biodistribution (BD) could serve as a powerful tool to suppress the establishment of viral CNS reservoirs and minimize off-target ART effects within the CNS.

Objective

To develop intrinsic drug chemical exchange saturation transfer (CEST) contrasts to detect ARVs within the central nervous system (CNS) using MRI

Methods

- **CEST contrast of 3TC.** The CEST contrast of 3TC was measured in PBS at 37 °C on a 7 Tesla scanner. Asymmetric magnetism transfer ratio (MTRasym) was calculated from the Z-spectrum.
- **CEST MRI of 3TC-treated mice.** male C57BL/6 mice (14 - 16 weeks old) were treated by oral gavage for five days with 3TC (250 mg/kg) or vehicle. CEST MRI was performed on a 7 Tesla MRI scanner to acquire pixel-by-pixel Z-spectra of brains (Figure 1D and E)
- **Dual-peak Lorentzian fitting.** Dual-peak Lorentzian fitting method was deployed to simultaneously analyze CEST effects of -NH_2 and -OH protons of 3TC (Figure 1A-C). Background CEST signal is represented by a polynomial function for direct saturation (DS) and magnetization transfer contrast (MTC), and a Lorentzian function for the CEST effect at 3 ppm from the amine and amide protons in biomolecules such as glutamate and mobile proteins (Figure 1D). In the second step, the data points in 0.5 – 2.5 ppm are fitted using a dual-peak Lorentzian function to fit –OH and NH_2 simultaneously (Figure 1E).

Results

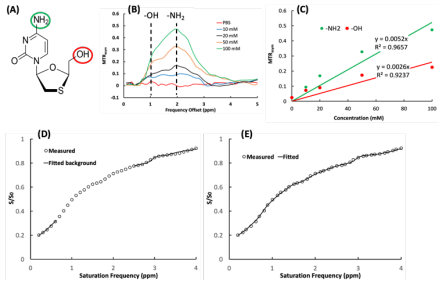
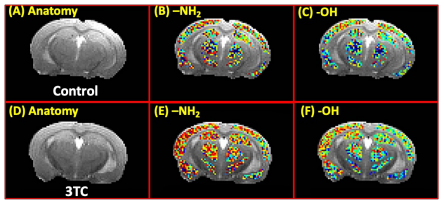


Figure 1. Dual-peak Lorentzian fitting. (A) 3TC chemical structure. (B) *MTRasym* of 3TC. The CEST effects of -NH_2 and -OH protons are at 2 ppm and 1 ppm, respectively. (C) CEST effects of -NH_2 and -OH protons are proportional to 3TC concentration and have a ratio = 2.0. (D) Background fitting using a polynomial function (o: raw data; -: fitted). (E) A dual-peak Lorentzian function fits -NH_2 and -OH.



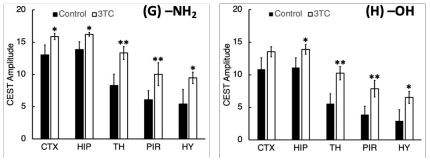


Figure 2. In vivo CEST analysis of 3TC-administarted mice. (A)-(C) Anatomical reference image, -NH_2 CEST amplitude map, and -OH CEST amplitude map of a control mouse. (D)-(F) maps of a 3TC mouse. (G) and (H) Comparisons of CEST effects of -NH_2 and -OH protons. **: $p < 0.05$, *: $p < 0.1$.

Results

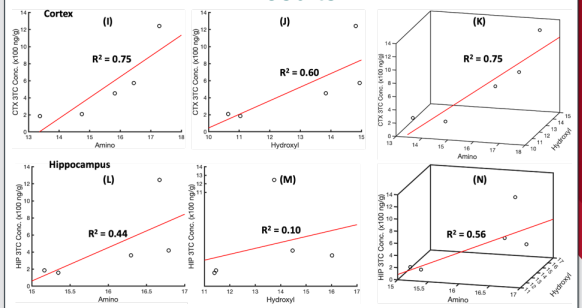


Figure 2. Correlations of -NH_2 (I), -OH (J) and combined CEST effect (K) with drug levels in cortex measured using HPLC-MS. (L)-(N) Correlations of -NH_2 (I), -OH (J) and combined CEST effect (K) with drug levels in hippocampus.

Discussion

- Limitations of traditional drug imaging modalities in which drug molecules are tagged with imaging contrast agents or loaded with imaging contrast agents into nanoparticles: 1) the loading rates of nanoparticles are usually limited, 2) toxicity can be associated with imaging agents and nanoparticles, 3) blood-brain barrier (BBB) penetration of drugs can be compromised.
- The innovation of our algorithm is the simultaneous fitting -NH_2 and -OH protons This extracts the -NH_2 effect of 3TC from other tissue biomolecules such as creatine and glutamine serving to improves the specificity of 3TC detection.
- In summary, we successfully developed a new algorithm that uses the CEST effects of both amino (-NH_2) and hydroxyl (-OH) protons for *in vivo* 3TC detection. The new algorithm shows high specificity for 3TC biodistribution measurements which can be extended to other ARVs.

Acknowledgments

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