Detecting CA-lys-TFA – a synthetized novel trifluorinated bile acid in murine gallbladder using *in vivo* ^{19}F MRI

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Introduction

In addition to their traditional role as detergents that facilitate fat absorption, bile acids are potent signaling molecules that affect multiple organs; they modulate gut motility and hormone production, and alter vascular tone, glucose metabolism, lipid metabolism and energy utilization. Changes in fecal bile acids promote colon pathology including cholerrheic diarrhea and colon cancer, the second leading cause of cancer death in the USA. In order to investigate the complex actions of bile acids, we have demonstrated that *in vivo* ^{19}F MRI can be used as an important tool to permit simultaneous monitoring of bile acid levels in the gastrointestinal tract[1]. In our recent publication[2], we reported synthesis of a novel trifluorinated bile acid that can be used for ^{19}F MRI of bile acid enterohepatic circulation, designated CA-lys-TFA (Fig.1) and tested its affinity for the apical sodium dependent bile acid transporter and the Na+/taurocholate cotransporting polypeptide. In the present study, we determined whether CA-lys-TFA was detectable in the murine gallbladder using *in vivo* ^{19}F MRI, and compared measurements of CA-lys-TFA concentration by MRI to those using LC/MS/MS.

Materials and Methods

Ten male C57BL/6 mice (20 to 25 g) were obtained from Jackson Labs, Bar Harbor, Maine. Seven mice were fasted overnight and orally gavaged with 150 mg/kg CA-lys-TFA in 1:1 polyethylene glycol (PEG) 400: Dulbecco's phosphate buffered saline vehicle. After gavage, mice were imaged at either 2 h (mice 1-3) or 7 h (mice 4-7) after dosing. To test the potential clinical utility of CA-lys-TFA as a diagnostic test of bile acid absorption, mice 8-10 were orally gavaged with 50 mg/kg CA-lys-TFA in vehicle once daily for 7 days and imaged on the 7th day either 2 (mouse 8) or 7 h (mice 9-10) after dosing. Prior to imaging, mice were anesthetized with ketamine/xylazine infusion using an indwelling intraperitoneal catheter with maintenance doses injected approximately every half hour during imaging. All *in vivo* ^{1}H and ^{19}F MRI experiments were performed on a Bruker BioSpec 70/30USR Avance III 7T horizontal bore MR scanner (Bruker Biospin MRI GmbH, Germany), equipped with a BGA12S gradient system and interfaced to a Bruker Paravision 5.1 console. A Bruker 40-mm ^{19}F/^{1}H dual-tuned linear volume coil was used to transmit and receive RF signals at 300.28 MHz for ^{1}H and 282.55 MHz for ^{19}F nuclei. Multi slice ^{1}H MR images were acquired using RARE (Rapid Acquisition with Relaxation Enhancement) sequence in the cross view of the sample or the body of the animal with repetition time 2200 ms, echo time 8.9 ms, RARE factor 8, field of view 4 x 4 cm^2, slice thickness 1.0 mm, matrix size 266 x 266, in-plane resolution 150 x 150 μm^2, and number of averages 6. Total acquisition time was 7 minutes 15 seconds. ^{19}F images were acquired using a FLASH (Fast Low Angle Shot) sequence in the same region of the ^{1}H MRI with repetition time 220 ms, echo time 3.078 ms, flip angle 30°, matrix size 32 x 32, in-plane resolution 1.25 x 1.25 mm^2, and number of averages 768. The rest of the parameters were the same as ^{1}H MRI. Acquisition time was 1 hour and 30 minutes. After imaging, mice were euthanized and the gallbladder were removed and ground using a tissue homogenizer in 75:25::acetonitrile:water solvent. Supernatants were analyzed by liquid chromatography (LC) and mass spectrometry (MS)[1]. The experimental protocol was approved by the Committee for the Welfare of Laboratory Animals at the University of Maryland School of Medicine.

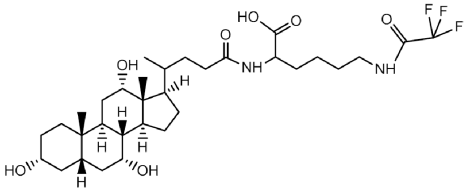

Fig 1. CA-lys-TFA chemical structure. The compound is a trifluorinated derivative of cholic acid, formed by conjugation of cholic acid to trifluoroacetyl L-lysine.

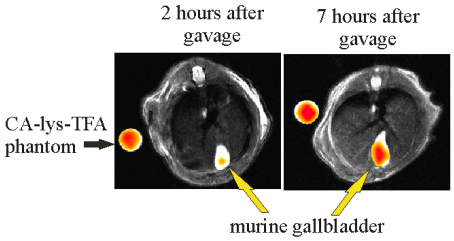

Fig 2. *In vivo* ^{19}F MRI. Two C57BL/6WT mice were gavaged with 150 mg/kg ^{19} F-labeled CA-lys-TFA and imaged using ketamine/xylazine anesthesia at the times shown. The images shown represent overlay of the ^{1}H and ^{19}F MRI images (^{19}F signal is orange). The external CA-lys-TFA phantom was used as the image standard.

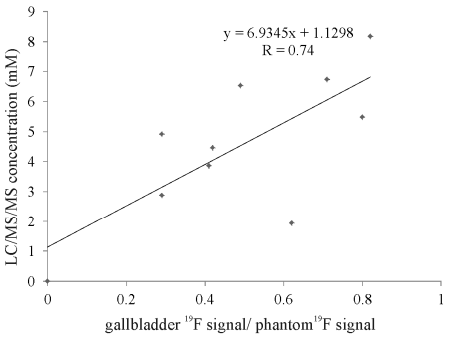

Fig 3. Comparison of CA-lys-TFA levels from MRI imaging from LC/MS/MS. Each data point is from one mouse (n=10 mice total). Linear regression resulted in R = 0.74 and P = 0.015, indicating association between imaged measurement and its subsequent LC/MS/MS analysis.

Results

Representative ^{1}H and ^{19}F MRI images of two mouse gallbladders after oral gavage with 150 mg/kg CA-lys-TFA at 2 hours (Fig.2A) and 7 hours (Fig.2B) demonstrated robust ^{19}F signals in the gallbladders of CA-lys-TFA-treated mice. Compared to the ^{19}F signal from the CA-lys-TFA phantom (30 mM in deuterated methanol), CA-lys-TFA showed a stronger signal at 7 compared to 2 hours after treatment. Fig. 3 shows the correlation between *in vivo* ^{19}F MRI and LC/MS/MS analysis of gallbladder contents from the 10 mice. Pearson product moment correlation analysis resulted in a correlation coefficient (R) = 0.74 and P = 0.015, indicating association between measurements by imaging and subsequent LC/MS/MS analysis.

Discussion

In this study, we demonstrate that CA-lys-TFA can be detected by ^{19}F MRI in the mouse gallbladder with linear dependence measured by LC/MS. *In vivo* ^{19}F MRI showed rapid accumulation of CA-lys-TFA in the gallbladder. These findings indicate that CA-lys-TFA, a multi-fluorinated non-radioactive bile acid analogue, has potential for use in MRI to measure *in vivo* bile acid transport and to diagnose primary and secondary bile acid malabsorption, and other conditions associated with impaired bile acid transport.

Acknowledgement

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References

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