In vivo ^{19}F MRI to detect biliary excretion of ^{19}F-labeled drugs in mice

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Introduction

Current methods to measure bile acid (BA) transport *in vivo* are limited to ^{75}SeHCAT [1,2] and luciferase-FXR imaging [3]. Radioactive ^{75}SeHCAT, available only in Europe, is likely to be discontinued by Amersham. Luciferase-FXR imaging, used only in transgenic mice expressing the reporter gene, provides very limited anatomical detail [3]. To overcome these limitations, we conceived of an innovative, non-radioactive approach; ^{19}F-labeled BA tracers and *in vivo* MRI in rodents. After excretion from the liver, BAs are stored and concentrated in the gallbladder (GB), providing the potential to detect a ^{19}F signal of sufficient strength. Because rats lack a GB, mice better serve this purpose. A fluoridated test agent was required to validate this novel approach. Because fluoridated hydrocarbons, particularly isoflurane are commonly used to anesthetize mice, and hepatic metabolism of this agent is reported [4,5] we chose isoflurane (5 fluorides/molecule) as an ideal test agent to determine whether normal anesthetic concentrations of isoflurane were detectable in the mouse GB using *in vivo* ^{19}F MRI.

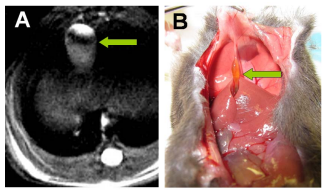

Fig.1. Identification of the mouse gallbladder using *in vivo* proton MRI (A) and post-imaging animal dissection (B).

Materials and Methods

Mice (male C57BL/6, 20 to 25 g) were fasted overnight before imaging. *In vivo* MRI experiments were performed on a Bruker Biospec 7.0-Tesla 30-cm horizontal bore scanner using Paravision 5.0 software (Bruker Biospin MRI GmbH, Germany). A Bruker 30-mm ^{19}F/^{1}H dual-tuned surface coil was used to transmit and receive radiofrequency (RF) signals at 300.28 MHz for ^{1}H and 282.55 MHz for ^{19}F nuclei. Mice were anesthetized in an animal chamber with a gas mixture of O_2 (1 L/min) and 3% isoflurane. Animals were placed supine in a Bruker animal bed and the RF coil was positioned and fixed with surgical tape in the region of interest on the animal body. The animal bed was moved to the center of the magnet and the isoflurane level changed to 1.5% and maintained at this level for the remainder of the experiment. To monitor animal respiration and temperature, an MR-compatible small-animal monitoring and gating system was used. Mouse body temperature was maintained at 36-37^{o}C using a warm water circulator. The experimental protocol was approved by the Committee for the Welfare of Laboratory Animals at the University of Maryland.

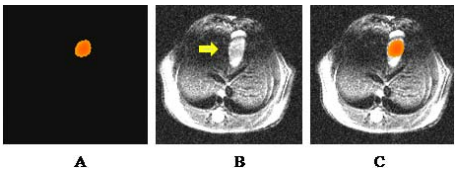

Fig.2. In vivo ^{19}F MRI (A), ^{1}H MRI (B), and merged ^{19}F and ^{1}H images (C) of isoflurane in a mouse gallbladder.

Three-slice (axial, mid-sagittal, and coronal) scout rapid acquisition with fast low-angle shot MR imaging (FLASH) was used to localize the GB. High resolution proton density-weighted anatomic images were acquired by rapid acquisition with relaxation enhancement sequence in the axial view with TR/TE = 1847 or 2631/11 ms, RARE factor = 8, field of view (FOV) = 6 x 6 mm^2, in-plane resolution = 0.15 x 0.15 mm^2, and slice thickness (st) = 1 mm. Total acquisition time was $\leq$ 18 min. Low resolution ^{19}F images were acquired using FLASH sequence in the region of the anatomic ^{1}H MRI with TR/TE =123 or 245/6 ms, excitation pulse angle = 30^{0}, FOV = 6 x 6 mm^2, in-plane resolution = 1.875 x 1.875 mm^2, and st = 4 mm. Total acquisition time was < 2 h 36 min. Mice inhaled isoflurane 50 to 94 min before ^{19}F MRI.

After inhaling isoflurane for 3 h, mice were euthanized and the liver and GB removed and ground using a tissue homogenizer in 75:25:: acetonitrile:water solvent. Homogenized tissue was centrifuged (9600 X g for 1 min; 4°C). Supernatants were analyzed by liquid chromatography-mass spectrometry (LC/MS) using a TSQ Vantage (Thermo Scientific) MS with Accela 1250 pump and PAL HTC-Accela1-TM autosampler. The column was a Phenomenex Gemni C18 (50 X 4.6 mm, 3 u, 110 A). The mobile phase (1.2 mL/min, water and acetonitrile) was a gradient of: 0-0.5 min, 40% ACN; 0.5 − 1.5 min, 40% to 95% ACN; 1.5 − 2.5 min, 95% ACN; 2.5 − 2.6 min, 95% to 40% ACN; and 2.6 − 3.6 min, 40% ACN. MS was performed using an APCI probe, in negative mode; ion transition m/z 183 → 163 was used to quantify isoflurane. Data collection and processing used Xcalibur 2.3.0 with LC Quan 2.6.0 software.

Results

Initial experiments identified the mouse GB by ^{1}H MRI. As shown in Fig. 1A, the GB was identified in the anterior upper abdomen of 6-wk-old male C57BL/6 mice. The identity of the GB was confirmed by immediate post-imaging animal dissection (Fig. 1B). Isoflurane signal (orange) was detected in mouse GB with no background noise (Fig. 2A). Corresponding ^{1}H anatomic images revealed the GB in the same region with sharp imaging contrast (Fig. 2B). ^{19}F signals were further localized by fusing ^{19}F and ^{1}H MRI images (Fig. 2C). ^{19}F signal was not detected in GBs of control mice anesthetized with injected ketamine and xylazine instead of inhaled isoflurane (not shown). Following isoflurane anesthesia, all 3 test mice revealed robust ^{19}F signals in the GB (representative images in Fig. 2). LC-MS confirmed that isoflurane is metabolized in the liver and accumulates in GB bile; in three additional test mice, isoflurane was detected in both the liver and GB bile (range = 3.2 - 4.7 µg isoflurane/GB bile). Isoflurane was not detected in the liver or GB of a control animal not treated with isoflurane, or in the empty GB of an isoflurane-treated mouse.

Discussion

Hepatic metabolism is a minor component of isoflurane excretion. Hence, we focused on imaging isoflurane in the GB, where exobiotics and metabolites are both stored and concentrated after excretion by the liver into the biliary tree. We reasoned that we were likely to detect the strongest ^{19}F signal in the GB. This study provides an important proof-of-concept showing that ^{19}F-labeled drugs can be detected at concentrations as low as 3.2 µg in mouse GB bile using a novel *in vivo* ^{19}F MRI approach. We plan to use this novel approach to quantify biliary excretion and enterohepatic circulation of both existing and novel ^{19}F-labeled drugs; ^{19}F-labeled drug design and a LC-MS protocol is under development.

References

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