Quantitative Assessment of an Antihistamine in Human Liver and Heart In Vivo by 19F MRS at 4T

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Introduction: In the early phases of drug development, it can be of great interest to know a compound's biodistribution. Drug and drug metabolite concentrations in the target tissue, in liver or heart can be of concern for better understanding therapeutic effects, breakdown and elimination metabolism or secondary effects. ^{19}F MRS provides a safe alternative to biopsy and radiotracer techniques to assess *in vivo* concentrations of compounds that have one or several ^{19}F atoms in their structure. In this study, we have developed and validated an external reference ^{19}F MRS method for estimating the concentration of a monofluorinated antihistamine, tecastemizole, and/or its metabolites in human liver and heart *in vivo*. The method was applied in a pilot multiple dose study of 18 healthy volunteers.

Subjects: Written informed consent was obtained for the 14 male and 4 female healthy volunteers who participated in this study approved by the McLean Hospital IRB (Belmont, MA) and Mid*Lands IRB (Leawood, KS). The subjects' average (±sdv) age was 35.3 ±13.0 years and ranged from 19 to 55.

Study design: This was an open label study. After baseline ^{19}F MRS (D0), tecastemizole was administered @ 270 mg/day *per os* for 8 days (D8). ^{19}F MRS was performed twice on D8 for reproducibility assessment *in vivo*. If drug ^{19}F MRS signal was observed on D8, subjects returned 28 days later for post-withdrawal ^{19}F MRS on D36. The T_1 relaxation time of the drug signal was assessed for one subject on D8.

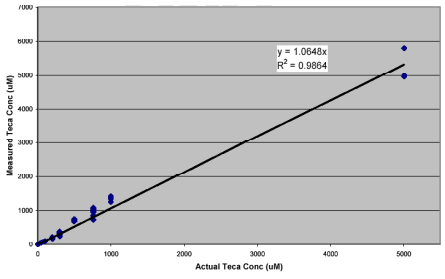
MR methods: MR was performed on a 4T whole body MR system (Varian Inc., Palo Alto, CA), using a flexible dual channel ^{1}H/^{19}F RF surface coil (Clinical MR Solutions, Brookfield, WI) positioned over either liver or heart. An external reference of 20 or 100mM KF w/ Magnevist® solution attached to the coil was present for all MR exams. Proton 2D spin-echo and 3D gradient-echo MRIs were performed to calculate liver or heart volumes by image segmentation. Heart MRIs were cardiac gated from the subject's EKG. ^{19}F MRS was a single 90º square RF pulse, TR=2s, SW=5000Hz, 4096 points, with 912 transients recorded individually. For heart MRS, the pulse was cardiac gated with a minimum TR of 2s. T_1 *in vivo* was assessed by using TRs of 0.5s, 2s and 4s, and *in vitro* by inversion-recovery. For preprocessing, transients were phased individually (to correct for respiratory phase modulation) before averaging. Semi-automatic macro driven processing was performed using Felix 2002 (Accelrys Inc., San Diego, CA) in the frequency domain by numerical integration over predetermined frequency ranges encompassing expected drug and external reference peaks after filtering, phasing and Fourier transform. T_2^* was estimated from linewidths obtained from fitted spectra. The transmit-receive sensitivity profile of the surface coil (determined from 3D RF sensitivity profiles obtained on a calibration standard) was used in conjunction with segmented images of the target organ under study to calculate the effective volumes of the target organ and the reference standard on a per-subject basis. Absolute quantitation was performed by comparison of the ^{19}F MRS signal integrals from the subject and the external reference standard, after correction for T_1 and T_2^* relaxation effects and organ volume.

Validation: The methods for ^{19}F MRS acquisition, processing and quantitation in the validation were identical to those used in the human study. The linearity, limit of detection (LOD), lower and upper limits of quantification (LLOQ & ULOQ), accuracy, precision, and reproducibility of ^{19}F MRS measurement were determined from c.a. 130 measurement sessions using 10 cylindrical 8-liter human-body conductivity-matched tecastemizole w/Gd-DPTA calibration standards with nominal concentrations from 0.5µM to 300µM, performed over a 6 week period. The higher concentration range up to 5000µM was covered by 4 high-conductivity standards. Linearity was assessed longitudinally by weekly ^{19}F MRS of 3 additional (low, medium & high concentration) QC phantoms. Reproducibility was assessed by ^{19}F MRS of the 50µM standard performed at least 3 times per week. System performance stability was verified on each measurement day. Analytical concentrations of all phantoms and external references were assessed by HPLC.

Results: The validation established LOD=0.34±0.19µM (±precision) with an $SNR_{average}$=3.7, LLOQ=2.57±0.49µM with a coefficient of variation (CV) of 15.6% and $SNR_{average}$=22, and ULOQ=5004±4µM with CV=7.9% and $SNR_{average}$=13349. The calibration curve of ^{19}F MRS *versus* HPLC concentrations was linear with slope=1.064, R^2=0.9864 over the LOD to ULOQ concentration range (Fig. 1). The reproducibility concentration measurements on the 50µM standard had a CV of 6.4%. Of the 18 subjects who completed the study, 4 presented a peak on D8 in the drug spectral region (Fig. 2) one of which was from heart. None of these 4 presented ^{19}F MRS signal on the D36 washout MR assessment. T_1 of the drug peak *in vivo* was less than 100ms (0.5s/5) since no saturation effect was found at TR=0.5s. T_1 of the external reference was 100ms. The average T_2* *in vivo* was 1.6±0.6ms (±sdv) and ranged from 0.9 to 2.3ms. Average liver ^{19}F-drug/metabolite concentration *in vivo* was 95±65µM (n=4, 6 measurements) and ranged from 34µM to 179µM. Heart concentration was 41µM (n=1, 1 measurement).

Conclusions & Discussion: A ^{19}F MRS external reference method, based on previous work (1-3), for absolute quantitation of a fluorinated drug in heart and liver *in vivo* was developed and validated using strict quality control criteria. The method was shown to have a lower quantitation limit of about 2.6 µM and a lower detection limit of about 0.3µM. The method applied to a pilot human study measured liver and heart drug concentrations ranging from 34 to 179 µM. Drug was not detected after a 28 day washout period. The method was shown to be very useful in the early development phase of fluorinated drugs for better understanding biodistribution and tissue pharmacokinetics in liver and heart.

References: **1.**JD Christensen et al. MRM 39: 149-154 (1998) **2.** JD Christensen et al. Brain Research 834: 1-5 (1999). **3.** NR Bolo et al. Neuropsychopharmacology 23(4): 428-438 (2000).



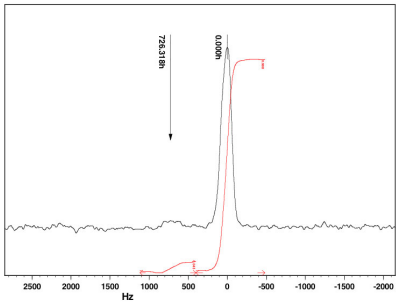


Fig. 1 (left): Calibration curve from validation study. Tecastemizole concentration (µM) measured using the described ^{19}F MRS external reference method *versus* analytic tecastemizole concentration (µM) measured using HPLC.

Fig. 2 (right): ^{19}F MRS spectrum acquired with coil positioned at a subject's liver after 8 days of tecastemizole administration @ 270 mg/day. Arrows indicate peaks of the external reference (KF) at 0 Hz and drug at +726 Hz.