

Proton MR Spectroscopy for *in vivo* Quantification of Small Animal Brain Alcohol Kinetics

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Introduction: The *in vivo* kinetics of acute alcohol brain levels can be monitored in real time with proton magnetic resonance spectroscopy (MRS). We measured the uptake and clearance of brain ethanol in rats after intraperitoneal (IP) alcohol injection over a 1-1.5 hour duration with temporal resolution ranging from 4 minutes to 4s. The observed time course of alcohol brain concentration followed a consistent pattern characterized by a rapid absorption, an intermediate distribution, and a linear clearance. In a sample of 8 healthy Wistar rats, the intercept of the linear clearance term, extrapolated to time of injection, correlated with the administered dose per unit of lean body mass.

Methods: Ten adult Charles River Wistar rats (504g-671g, 150-250 days old) were scanned in a 2-hour session before and after ethanol infusion IP (9 animals) or intragastrically (IG, one animal). Data were acquired on a clinical 3T GE Signa human MRI scanner with standard gradients (40 mT/m, 150 T/m/s) and a custom rat brain head coil. Anesthesia was provided by 1-3% isoflurane plus oxygen (~2 liters/min). After sedation, a flexible catheter was inserted in the IP cavity (9 animals) or in the stomach via the esophagus for IG injection. Prior to MRS acquisition, T1-weighted multislice whole-body images were acquired before and after injection of 1cc, 1% Gd-DTPA to ensure proper catheter placement. A 3-plane localizer scan (TE/TR=2.1/54ms, FOV=80mm, 256x128, 5mm thick, 10 slices/plane) was used for prescription of coronal fast spin-echo (FSE) images (TE1/TE2/TR=18/90/2200ms, FOV=40mm, 256 x 256, 2.5mm thick, 12 slices). A 0.25 cc brain voxel (10x5x5 mm) was prescribed and a series of single-voxel, 2D J-resolved spectra (2, 3) were acquired before and after bolus injection of 1 g/kg ethanol in approximately 5cc volume during. MRS duration was 1-1.5 hours, and time resolution was 4:40 min (3 animals, TE1/TR=35/2000ms, Δ TE=10ms, 16 t1 steps, 8 averages per t1 step) or 2:20 min (5 animals, 2 averages). For two studies, PRESS spectra at TE=144ms were acquired with two-averages and a 2s TR to yield a 4s sampling. All spectra were phased using the residual water for each TE as a reference. The data were apodized with a 6 Hz Gaussian filter, zero filled, and Fourier transformed. For each 2DJ acquisition the TE-average spectrum was used for signal estimation of ethanol at 1.3 ppm, normalized by the unsuppressed water signal at TE=35ms. The temporal sequence of the ethanol peak was fitted with two exponentials and a single linear term. Lean body mass (LBM) was assumed to be approximately the same for all animals at 80% of weight for the smallest rat.

Results and Discussion: Fig 1 shows a representative time course of the ethanol signal amplitude for IP injection with a 2:20-minute resolution. The curve (red) fits the data (blue) demonstrating a rapid uptake, followed by an intermediate exponential decay and a slower linear clearance. Fig 2 shows a correlation between the intercept and alcohol dose per unit of lean body weight. Fig 3 shows an example of a 4s temporal resolution sampling IG (Fig 3a) and IP (Fig 3b), demonstrating the increased time resolution with the expected increase in variance for individual sampling points and marked flattening of the rapid uptake component with IG relative to IP infusion, consistent with previous reports based on blood samples (5).

The expected brain concentration of alcohol after IP injection is governed primarily by absorption in the first 5-10 min, followed by a combination of absorption, metabolism and elimination, and after 30 min, primarily elimination. The intercept of the linear component of the alcohol elimination curve at time zero represents the theoretical alcohol dose (5). Retro-orbital blood was drawn at the end of the MRS session and alcohol concentration determined for 4 animals. Using the slope, intercept, blood-draw time, LBM, and alcohol concentration at blood draw time, the estimated theoretical concentration at time zero was 139 mg/100 ml and the MRS estimated dose/lean body mass was 132 mg/100 gm.

Conclusion: The data demonstrate the feasibility of quantifying the stages of *in vivo* brain alcohol kinetics in small animals. When applied to animal models of alcohol preferring and non-preferring Wistar rats, this technique offers the opportunity to study uptake and clearance of alcohol under controlled conditions in behaviorally different groups. The high correlation of the linear intercept with alcohol dose/LBM and the MRS estimates of initial dose base on blood samples indicate that this MRS procedure provides a valid *in vivo* method for quantifying alcohol uptake and elimination kinetics and relative brain concentration.

Support: NIH AA13521 (INIA), AA05965

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