Gradient Echo fMRI Study of Oxygenation Changes in the Livers of Chronic Ethanol-Treated Rats

M. Brauer[1], and L. M. Foley[2]

[1]Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada, [2]Pittsburgh NMR Ctr for Biomedical Research, Carnegie Mellon University, Pittsburgh, PA, United States

Introduction: The metabolism of alcohol by the liver causes increased O_2 demand and potentially decreased O_2 supply due to hepatic lipid accumulation and decreased blood flow through the narrow sinusoidal beds. This study addresses two questions: (1) can fMRI noninvasively assess oxygenation changes in the livers of living rats and (2) does chronic ethanol (CE) treatment alter oxygenation changes in livers of alcoholic rats.

Methods and Materials: The high-fat Lieber-DeCarli all-liquid diet (36% of calories as ethanol or dextrin-maltose) was administered to male Wistar rats for 6 - 8 weeks. For fMRI examination, rats were anesthetized with 2% isoflurane and mechanically ventilated. A T_2-weighted spin-echo fMRI protocol (TE 40 ms, TR 2.14s) provides relatively selective information about sinusoidal O_2 changes. A T_2^*-weighted FLASH gradient-echo fMRI protocol (TE 15 ms, TR 154 ms, 20^o RF pulse) provides more rapid (1.5 min) acquisition of images, although not selective for sinusoidal O_2 changes. Echo time (TE)-dependence studies of fMRI results were done on CE and pair-fed (PF) control rats during normoxia and then during carbogen challenge. A spin-echo (SE) multi-pulse sequence was used to determine T_2 dependence with echo times of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 msec. Each image was obtained as an 80 x 80 matrix, FOV of 8 x 8 cm, a slice thickness of 2.5 mm and with 4 transients per phase encoding step. For T_2* dependence, a single slice FLASH pulse sequence was used with echo times of 6, 8, 10, 14 and 18 ms. Two baseline images were acquired for all groups during normoxic ventilation and two images during hypoxic, hyperoxic and carbogen challenge, respectively. Blood oxygen content was determined after MRI examination but while the animals were still under inhalation challenge. 0.1 - 0.2 ml of blood was taken from the hepatic artery, portal vein and hepatic vein and analysed with a hemoximeter (OSM3 Hemoximeter, Copenhagen) within 5 minutes of removal. Morphometric analysis was done after 5% H_2CO fixation and H&E staining.

Results and Discussion: BOLD contrast in fMRI is based on deoxyhemoglobin being paramagnetic and decreasing T_2- and T_2^*-weighted MRI intensities. Previously we showed that in spin-echo fMRI studies that PF control rats exhibited much greater changes in signal intensity than alcoholic rats during hypoxic (10% O_2), hyperoxia (98% O_2) or hypercapnia (5% CO_2, a vasodilator) challenge. MRI intensity changes correlated well with pulse oximetry measurements ($r^2 = 0.95$). Biochemical lactate / pyruvate (cytoplasmic NADH / NAD^+) also changed less with alcoholics vs. control rats: hyperoxia: 9% vs. 31% decrease; carbogen: 7.7% vs. 38.8% decrease; hypoxia: 10.5% vs. 185% increase. Morphometric measurements showed that sinusoidal area decreased dramatically for alcoholic rats compared to controls, with sinusoidal area of 13.3% ± 2.6% for PF (mean ± SE, n = 7) compared to 4.1% ± 0.9% for CE rats (mean ± SE, n=7, statistically significant $p<0.002$). Blood oxygen sampled from the hepatic artery and portal & hepatic vein show that CE rats have less oxygen in all three vessels than PF rats under all conditions.

With gradient echo fMRI, chronic ethanol-treated rat livers also showed significantly slower rates (> 3min) of response and lower magnitude of response to hypoxia, hyperoxia and carbogen challenges relative to pair-fed controls (mean ± SE, n=5, $p<0.05$). FMRI signal changes for control and alcoholic rats were: hypoxia: 44% ± 3% vs. 14% ± 2% decrease; hyperoxia: 48% ± 4% vs. 27% ± 3% increase; carbogen: 103% ± 30% vs. 9% ± 3% increase. Echo time dependent studies of both spin echo and gradient echo fMRI (delta S / So vs. TE) extrapolated roughly to zero for zero TE time. This indicates that these experiments are true Blood Oxygen Level Dependent (BOLD) effects dependent upon deoxyhemoglobin changes.

These results show that both SE and GE fMRI measurements reflect decreased oxygenation responsiveness in the livers of alcoholic rats to hypoxic, hyperoxic and carbogen challenge, compared to controls. This insensitivity in CE rats is due to decreased sinusoidal blood volume, reflecting accumulation of hepatic fat and denatured protein, and increased tissue pressure in alcoholic rats. The CE rats showed lower blood oxygen levels as well as less responsiveness to challenge. The 5-10 fold greater fMRI intensity changes in liver compared to the brain are due to higher hepatic blood volume (15-20% vs. 2-3%), higher microvascular blood content (60% of total liver blood vs. 33% in brain) and the decreased overall oxygenation of hepatic vs. brain blood.

Conclusion: Carbogen doubles MRI signal intensity in control livers with almost no effect on alcoholic livers - a robust response with clinical diagnostic potential. (Financial support: NIH AA 12077).