Liver Oxygenation Changes in Chronic Ethanol-Treated Rats Studied by fMRI and Morphometric Measurements

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Synopsis: We previously showed that spin-echo fMRI, regarded as relatively selective for microvascular oxygenation changes, showed large 20% decreases in control rat liver image intensity with hypoxia, 20% increase with hypercapnia and a dramatic 40 - 50% increase with hyperoxia [Foley, Picot, Thompson, Yau and Brauer (2003) Magnetic Resonance in Medicine 50: 976-983]. In all cases, alcoholic rats showed much less image intensity change, indicating chronic ethanol-induced microvascular dysfunction. In this study, we used gradient echo fMRI, which is faster but less selective, and showed that chronic ethanol-treated rat livers responded less and more slowly to physiological challenge. Carbogen (95% O_2, 5% CO_2) almost doubled MRI signal intensity within 3 min for control rats with <10% change for alcoholic rats. Morphometric measurements indicate that the sinusoids of alcoholic rats are highly compressed.

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) do fMRI have potential to 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 FLASH gradient-echo fMRI protocol (TE 15 ms, TR 154 ms, 20^o RF pulse) provides rapid (1.5 min) acquisition of images, although not selective for sinusoidal O_2 changes. Morphometric measurements to measure sinusoidal compression of the alcoholic rats' livers were done on formaldehyde fixed tissues with H&E staining. Total and sinusoidal areas were measured for 10 fields per rat using a Nikon 40x objective and imaging softwear.

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, pair-fed 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 changes measured by pulse oximetry ($r^2 = 0.95$). 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, $p<0.05$,): hyperoxia: 44% ± 3% vs. 14% ± 2% decrease; hyperoxia: 48% ± 4% vs. 27% ± 3% increase; carbogen: 79% ± 30% vs. 9% ± 3% increase. Invasive biochemical lactate / pyruvate ratios (cytoplasmic NADH / NAD^+) also changed less with alcoholic vs. controls: hyperoxia: 9% vs. 31% decrease; carbogen: 7.7% vs. 38.8% decrease; hypoxia: 10.5% vs. 185% increase. The 5-10 fold greater fMRI intensity changes in liver compared to the brain are due to the higher 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.

Morphometric measurements showed that sinusoidal area decreased dramatically for the chronic ethanol rats compared to controls. The sinusoidal area for controls was 13.4% ± 2.25%, (mean ± SE, n = 7) compared to 4.2% ± 0.65%, (mean ± SE, n=7, statistically significant $p<0.002$). This shows that the insensitivity of the alchoholic rats is due to a dramatic decrease in sinusoidal blood volume, due to increased hepatocyte size. The liver tissue is increased due to buildup of fat and denatured or oxidized protein in the liver cells, and the liver capsule cannot expand, causing increased tissue pressure. Thus, fMRI is a sensitive, noninvasively and reliable monitor of liver oxygenation changes, with excellent clinical potential. Chronic ethanol treatment makes the liver less responsive to hyperoxic, hypercapnic or hypoxic challenge, likely reflecting microvascular dysfunction.

Long-term alcohol treatment slows and decreases the liver's response to physiological challenge, indicating disruption of microvascular structure and/or regulation. Sinusoids may be blocked by: (1) fatty infiltration / hepatocyte swelling compressing the sinusoids, (2) increased leukocyte adhesion to the epithelial cells due to inflammation and release of TNFalpha or (3) vasoconstriction due to increased endothelin or decreased nitric oxide.