Acute liver failure studied by hyperpolarized 1,4-13C2-fumarate in CCl4 injured rat liver

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Introduction: We have developed a diagnostic marker - hyperpolarized 1,4-13C2-fumarate, which takes advantage of the technique dynamic nuclear polarization for magnetic resonance imaging (DNP-MRI)1. This marker allows real-time metabolic studies of a TCA-cycle intermediate, 13C-malate. With this marker we have investigated the metabolism in the liver of CCl4 treated rats. Intra gastric administration of CCl4 into rats is known to induce acute liver failure. The liver cell damage is caused by a radical species2, CCl3-, which damages the electron transport chain and decreases the oxidative capacity of the mitochondria3.

Methods: The in vivo MR experiments were performed on a 2.35T Bruker Biospec Avance II system. A whole body Rat coil, 1H / 13C, diameter 72 mm, was used. Rats were placed prone in the acrylic animal cradle. Physiological parameters; ECG, breathing and temperature was monitored (SA-instruments, USA). 2 ml of the substrate (~50 mM) was injected over 6s.13C-chemical shift image was acquired with the following parameters: FOV 55x55 mm2 x 10 mm, matrix size 16x16, 10 degree RF pulse, TR = 35 ms. Total acquisition time is 11 seconds (due to triggering on breathing). The chemical shift imaging was started 45 seconds after start of the substrate injection. A high resolution proton image was acquired for referencing. The liquid state polarization of 1,4-13C2-fumarate was in all experiments more than 25% at the time of injection into the animal. The rats were imaged before and 24 h after a single dose of 1ml/100g body weight of CCl4 (200mM/L; diluted in olive oil).

Results and discussion: None of the rats revealed detectable signal for 1,4-13C2-malate in the control experiment. 24 hours post CCl4 injure all rats showed a clear malate signal (Fig. 1). In addition the fumarate distribution has changed in the rat liver subsequent to the administration of CCl4. The increase in 1,4-13C2-malate signal as a consequence of CCl4 injure in rat liver is significant. With the sensitivity of the rat body RF coil used, the healthy liver showed no sign of malate (n=5) whereas the CCl4 injured liver showed high an increase in the 1,4-13C2-malate signal of at least a factor of 10 (Fig. 2).

Figure 1. Metabolic images in the rat liver of 1,4-13C2-fumarate and 1,4-13C2-malate after injection of 1,4-13C2-fumarate. Control measurements in the healthy rat liver show no detectable 1,4-13C2-malate signal (top images). The same animals are imaged again, 24 hours after the administration of CCl4. In these experiments a clear 1,4-13C2-malate signal can be detected in all three CCl4 injured livers (bottom images).

Figure 2. Example of Spectra behind chemical shift image of 1,4-13C2-malate and 1,4-13C2-fumarate in a CCl4 treated rat liver (left). The spectral grid is seen superimposed on a slice selective high resolution proton image (right). From these spectra the increase in 1,4-13C2-malate signal is estimated to be at least a factor of 10.

Conclusion: The effect of the hepatotoxin can be assessed by measuring plasma levels of traditional enzymatic markers for liver disease, ALT and AST. The serum levels of the transaminases are likely to reflect necrosis / apoptosis of hepatocytes, caused by CCl4, and do not report directly on changes to the viable hepatocytes, e.g. changes to the metabolic capacity or to the metabolic pathways. These first results show correlation between high malate signal and liver injury. The changes in metabolism of 1,4-13C2-fumarate seen in this model is likely to relate to changes in liver function since elevated levels of fumarate hydratase was measured in the plasma of the CCl4 injured rats. These first results primes further development of hyperpolarized 1,4-13C2-fumarate as a metabolic marker to clarify the link between therapy and pathogenic processes in liver diseases.

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