In Vivo Proton Magnetic Resonance Spectroscopy of Hepatic Ischemia/Reperfusion Injury in an Experimental Model

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Rationale and Objectives: Hepatic ischemia/reperfusion injury (IRI) occurs during certain hepatobiliary surgeries, hemorrhagic shock, and veno-occlusive disease. Biochemical changes caused by hepatic IRI lead to hepatocellular remodeling, including cellular regeneration or irreversible apoptosis. This study aims to characterize and monitor the metabolic changes in hepatic IRI using proton magnetic resonance spectroscopy (^{1}H MRS).

Materials and Methods: Sprague-Dawley rats (n = 8) were scanned with ^{1}H MRS using 5.0 × 5.0 × 5.0 mm^3 voxel over a homogeneous liver parenchyma at 7 Tesla with a respiratory-gated point-resolved spectroscopy sequence at 1 day before, 6 hours, 1 day, and 1 week after 30 minutes total hepatic IRI. Signal integral ratios of choline-containing compounds (CCC), glycogen and glucose complex (Glyu), methylene proton ($(-CH_2-)_n$), and methene proton (-CH=CH-) to lipid (integral sum of methyl proton ($-CH_3$), $(-CH_2-)_n$ and -CH=CH-) were quantified by areas under peaks longitudinally.

Results: The CCC-to-lipid and Glyu-to-lipid ratios at 6 hours after IRI were significantly higher than those at 1 day before, 1 day, and 1 week after injury. The $(-CH_2-)_n$-to-lipid, and -CH=CH-to-lipid ratios showed no significant differences over different time points. Hepatocellular regeneration was observed at 6 hours after IRI in histology with immunohistochemical technique.

Conclusions: Changes in CCC-to-lipid and Glyu-to-lipid ratios likely reflect the hepatocellular remodeling and impaired glucose utilization upon hepatic IRI, respectively. The experimental findings in the current study demonstrated that ^{1}H MRS is a valuable tool for characterizing either global or regional metabolic changes in liver noninvasively and longitudinally. Such capability has the potential to lead to early diagnosis and detection of impaired liver function.

Key Words: Liver; ischemia/reperfusion injury; MRI; spectroscopy; choline.

Hepatic ischemia/reperfusion injury (IRI) induced by vascular complications contributes to early organ failure and can lead to acute and chronic rejection after liver transplantation (1). Hepatic IRI also occurs during certain hepatobiliary resectional surgeries, hemorrhagic shock, and veno-occlusive disease. IRI is a major cause of acute liver failure, which is associated with high morbidity and mortality (2). Cellular damage can be induced not only during ischemia but also in reperfusion that follows, resulting in both local and systemic organ dysfunction (3,4). Biochemical changes caused by hepatic IRI lead to hepatocellular remodeling, including cellular regeneration or irreversible programmed cell death (5). Early diagnosis and detection of impaired liver function is vital for early and effective therapeutic interventions and thus prevents its progression to liver failure. Through assessing liver function, serology screenings of liver-specific transaminases (aspartate aminotransferase and alanine aminotransferase) have been widely used to monitor hepatic IRI (6–8).

Metabolic changes of liver can provide information and improve the characterization of liver abnormalities (9). Monitoring of liver metabolism through a microdialysis catheter placed in the liver tissue revealed profound metabolic changes during and after IRI, leading to potential evaluation of ischemic preconditioning and clinical application (10). However, microdialysis technique is invasive in nature because implantation of microdialysis probe needs to be accompanied by surgical

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procedures (11). Recently, proton magnetic resonance spectroscopy (^{1}H MRS) has been increasingly employed to investigate liver metabolism in vivo noninvasively in various diseases (12–17). Biochemical changes occurred during hepatic IRI were also investigated in liver extracts (18). However, in vivo and serial study of hepatic IRI model with such MRS technique has been limited.

Continuous measurements of metabolites in liver may provide valuable information to monitor liver metabolism, and contribute to the better characterization of liver abnormalities as compared with liver-specific transaminase measurements. In this study, we hypothesize that hepatic IRI would lead to detectable metabolite changes in liver using ^{1}H MRS. We aim to evaluate the alterations in the liver metabolism longitudinally with ^{1}H MRS using an experimental hepatic IRI model in Sprague-Dawley rats.

MATERIALS AND METHODS

All magnetic resonance imaging (MRI) measurements were acquired on a 7 Tesla MRI scanner with a maximum gradient of 360 mT/m (70/16 PharmaScan, Bruker Biospin GmbH, Germany). A 60-mm quadrature resonator was used for radiofrequency transmission and receiving. All animal experiments were approved by the local institutional animal ethics committee.

Animal preparation

The rodent model of mild total hepatic IRI was performed as described previously in rats (19,20). Sprague-Dawley male rats (260–280 g; n = 8) were anesthetized with isoflurane/air using 1.0–1.5% via a nose cone during surgery. In brief, the abdomen was shaved and a midline incision was made. The common portal vein, hepatic artery, and bile duct in the hepatoduodenal ligament were clamped using a vascular clamp. The liver was inspected for ischemia for 2 minutes. After 30 minutes of hepatic ischemia, the clamp was removed initiating hepatic reperfusion. The liver was again inspected for restoration of blood flow for 5 minutes, then the abdomen was closed and the animal the animal was kept at ambient temperature of 30°C during its recovery from anesthesia.

MR spectroscopy

^{1}H MRS was performed at 1 day before injury, 6 hours, 1 day, and 1 week after hepatic IRI. During imaging, each rat was anesthetized with isoflurane/air using 1.0–1.5% for maintenance via a nose cone and was kept warm under circulating water at 37°C in a heating pad with respiratory monitoring (20,21). Scout images were first acquired in three orthogonal planes with a fast low-angle shot sequence. For ^{1}H MRS, a 5.0 × 5.0 × 5.0 mm^{3} voxel was placed over a homogeneous liver parenchyma with care avoiding large blood vessels. After automatically adjustments of first- and second-order shim terms for localized voxel using the field map based shimming technique (22), a full-width half-maximum line width of water signal of ≤ 40 Hz would be achieved. The water signal was suppressed by variable power radiofrequency pulses with optimized relaxation delays. Outer volume suppression combined with respiratory-gated point-resolved spectroscopy sequence was used for signal acquisition using repetition time ≈ 2000 ms, echo time = 15 ms, spectral bandwidth = 3 kHz, 2048 data points, and 512 averages, similar to previously described (23–25).

Data and statistical analysis

The raw MRS data were zero-filled, apodized with a 2-Hz exponential filter, Fourier transformed, 0th- and 1st-order phase corrected, and baseline corrected (23). Signal integrals of choline-containing compounds (CCC; at 3.2 ppm), glycogen and glucose complex (Glyu; at 3.6–3.9 ppm) and lipid (integral sum of methyl proton (-CH_3), methylene proton ((-CH_2-)$_n$) and methene proton (-CH=CH-) at 0.9, 1.3, and 5.3 ppm, respectively) (12,17,26–28) were quantified by areas under peaks. The CCC-to-lipid and Glyu-to-lipid ratios were measured by dividing peak area of CCC and Glyu by that of lipid. The relative saturated and unsaturated fatty acid fractions were estimated by dividing peak areas of (-CH_2-)$_n$ and -CH=CH- by that of lipid, respectively. Note that fatty acids remained relatively constant during hepatic IRI in earlier studies (18,29), similar signal integrals of lipid were expected over different time points to the mild hepatic IRI in this study. All data were presented as mean ± standard deviation. One-way analysis of variance with Tukey's multiple comparison test was employed to compare differences in ratios of peak areas in different groups, with $P < .05$ considered as statistically significant.

Histology

Three animals were sacrificed at 6 hours, 1 day, and 1 week after injury after ^{1}H MRS, respectively, for immunohistochemical analysis. One normal animal was also sacrificed to serve as a control. To detect proliferating cells, livers were harvested and embedded in paraffin. The livers were then sectioned and incubated with monoclonal antiproliferating cell nuclear antigen (PCNA) antibody (clone PC10, DAKO, Carpinteria, CA) diluted to 1:200 with 0.01 M phosphate buffer solution (pH 7.1) for 60 minutes at room temperature (30).

RESULTS

Figure 1 illustrates the typical voxel placement in the liver and the typical liver ^{1}H MRS spectra at 1 day before injury, 6 hours, 1 day, and 1 week after hepatic IRI from the same animal. Eight resonance peaks were detected and identified: 1) -CH_3 (at 0.9 ppm); 2) (-CH_2-)$_n$ (at 1.3 ppm); 3) allylic proton (-CH=CH-CH_2-; at 2.0 ppm); 4) glutamine and glutamate complex (Glx; at 2.2 ppm); 5) diallylic proton (=CH-CH_2-CH=; at 2.8 ppm); 6) CCC (at 3.2 ppm); 7)