Proton MRS of Changes of Lipid Unsaturation during Liver Regeneration

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Introduction

Partial hepatectomy (PHx) is a curative measure for hepatic malignancies, and has been performed more frequently with advances in diagnosis and surgical techniques [1]. Liver dysfunction is observed in postoperative period, and there is an increasingly important role of liver stem cells in liver regeneration[2]. A real time and specific monitoring method of liver regeneration is required. In a ^{31}P-MRS study, energy depletion was observed and the ratios of adenosine triphosphate (ATP) to inorganic phosphate (Pi) decreased after PHx. The regeneration is at maximum at 24-48 h post-operation, which is supported by findings in DNA synthesis[1]. An increase in unsaturation of liver fatty acid was reported in rat liver after PHx *ex vivo*, and peaked at day 1 and day 5[3]. Moreover, lipid metabolism is linked to a gene that found in regenerating liver[4]. However, data on the corresponding genetic models varied when correlating the gene with triglyceride to assess liver regeneration. This showed that triglyceride could be uncoupled from liver regeneration[5]. The use of ^{1}H-MRS to monitor unsaturation of lipids could provide more information of lipid metabolism as compared with ^{31}P-MRS, and could be a promising and alternative method to assess liver regeneration non-invasively. This could give us insight into the precise role of lipids in liver regeneration.

Methods

Animal Preparation: Male Sprague-Dawley (SD) rats (280-300 g; $N = 3$) were under investigation. A standard 70% hepatectomy was performed in which the left and median lobes of the liver were removed[1], and the weight of each animal was measured at the time point of MRS measurement. MRS was performed on the animals before and after PHx at 12h, 24h and 120h.

In Vivo MRS: ^{1}H MRS experiments were performed on a 7T Bruker MRI scanner with a 60 mm RF Tx/Rx quadrature resonator. Animals were anesthetized with ~1.5 % isoflurane, and body temperature was maintained at about 36.5°C with respiratory monitoring. Scout images were first acquired in three orthogonal planes with a FLASH sequence. A 5×5×5 mm^3 voxel was placed on a homogeneous liver parenchyma, without involving large blood vessels. MAPSHIM protocol was applied for shimming, and each voxel was shimmed by the first- and second-order localized voxel shimming based on field map technique. A FWHM linewidth of water signal of <30 Hz was achieved. The water signal was suppressed by variable power RF pulses with optimized relaxation delays (VAPOUR). Outer volume suppression (OVS) combined with respiratory-triggered point-resolved spectroscopy (PRESS) sequence was used for signal acquisition, with TR = 2 respiratory cycles (~2.0-2.5 s), TE = 15 ms, spectral bandwidth = 3 kHz, 2048 data points, 512 averages, and total scan time of ~15 min.

Data Analysis: MR spectra were processed using the MR spectroscopic analysis package provided by manufacturer. The raw data were zero-filled, apodized with a 2-Hz exponential filter, Fourier transformed, 0th- and 1st-order phase corrected, and baseline corrected. Major peaks with good SNR were evaluated, which including signal integrals of saturated lipid (–CH_3, –CH_2–, Sat), unsaturated lipid (–CH=CH–, Unsat) and choline (CCC) at 0.9-1.5, 5.3 and 3.2 ppm, respectively. The area under peak of lipid signals and the choline signal were manually quantified. The Sat and Unsat were measured by normalizing the peak area to CCC.

Results

In Fig. 1a, the ^{1}H-MRS spectra of the liver before and after PHx at 12 h and 24 h were shown with Fig. 1b indicates the position of voxel. Peaks of Sat and Unsat lipids had changed at different time points. An increase in Sat and Unsat was observed at 12h and 24 h post-PHx, respectively (Fig. 2). Sat had a maximal increase at 12h (Fig. 2a), and Unsat had a maximal increase at 24h (Fig. 2b). Both lipids decreased at 120h post-PHx. The body weight of the rats decreased by 4% and 7% in average at 12h and 24h post-PHx, respectively, and increased by 4% in average at 120h post-PHx.

Discussion

In this study, we monitored the change in the three major components in the liver, which included Unsat, CCC and Sat. No significant change was observed in CCC, and total lipids increased. We compared Unsat:CCC with Sat:CCC, as the degree of unsaturation was reported to increase after PHx[3]. There was over 95% of the functioning hepatocytes enter the cell cycle, and DNA synthesis was found at 12h and reached a maximum at 24h post-PHx, which indicated the maximal liver regeneration[6]. A maximal increase in Sat was at 12 h and that in Unsat was at 24h. This could be ascribed to the initial phase of liver regeneration at 12h. When the liver regeneration reaches maximum at 24h, Unsat was the highest with Sat remained high. According to DNA synthesis studies, most of the liver regeneration takes place at day 3 and the mass of the liver restores at day 7 post-PHx[6]. At 5 day after PHx, level of unsaturation of liver fatty acid was at maximum *ex vivo*. Authors suggested that a high level of unsaturation could inhibit liver regeneration[3]. That is not much regeneration will be expected at day 5. Our study showed that both the Unsat and Sat decreases on day 5. With reference to the time line of liver regeneration, this decrease could be due to the slow down or inhibition of liver regeneration. However, Unsat was not at maximum. This discrepancy in Unsat at day 5 could be due to the difference between the *ex vivo* and *in vivo* settings. Unsat consists of a number of molecules, such as DHA, polyunsaturated fatty acids and triglycerides. We investigated the unsaturated compounds macroscopically *in vivo*, while the *ex vivo* study focused on a pool of unsaturated compounds. The change in the body weight of the rats, i.e. a decrease at the 24h and an increase at day 5 post-PHx, was supplementary information to indicate the restored liver mass at 3-7 day of PHx. Therefore, monitoring of both Sat and Unsat allows us to study the postoperative liver regeneration. Experiments will be carried out on a larger sample size to study this change further.

Conclusion

Proton MRS study of the change of unsaturated lipids in the liver after PHx allows us to monitor the liver regeneration. We found a high unsaturated lipid content at 24h post-PHx, at which liver regeneration is maximum. This could be related to liver regeneration. Hence, ^{1}H-MRS could assess the liver regeneration in a multi-component manner.

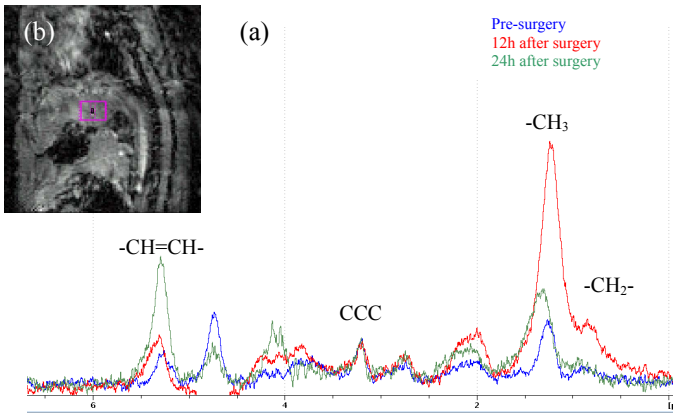

Fig. 1 (a) ^{1}H-MRS spectra of the liver (voxel shown in 1b) pre- and post-PHx at 12h and 24h.

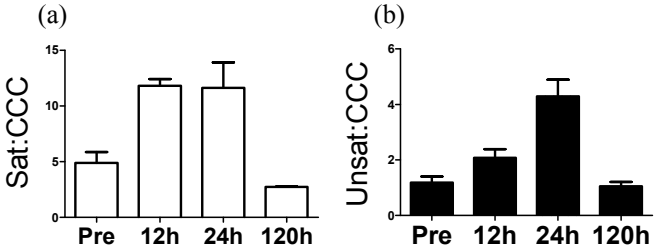

Fig. 2 (a) Saturated lipid:Choline pre- and post-PHx; (b) Unsaturated lipid:Choline pre- and post-PHx.

References [**1**] Corbin IR et al. Hepatology 2002;36:345-353. [**2**] Duncan AW et al. Gastroenterology 2009;137:466-481. [**3**] Kishino T et al. Lipids 2000;35:445-452. [**4**] Fernandez MA et al. 2006;313:1628-1632. [**5**] Newberry EP et al. Hepatology;2008:48:1097-1105. [**6**] Anderson SP et al. 2002;36:544-554.