The Potential Advantage of High Resolution In Vivo 31P-MRS in the Assessment of Nonalcoholic Fatty Liver Disease

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) includes a broad range of liver diseases such as fatty liver, nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis [1]. Despite increasing prevalence of NAFLD [2], biopsy remains the current gold-standard for assessing the disease [3]. To develop a diagnostic means of assessing the disease, 31P-MRS has been explored extensively. However, the severe spectral overlaps and low SNR in 31P-MRS spectra at clinical field strength are clearly limiting factors. To address this issue, studies employing proton decoupling and nuclear Overhauser enhancement (NOE) have also been reported [4-7]. Using these techniques phosphoethanolamine (PE), phosphocholine (PC), glycerophosphorylethanolamine (GPE), glycerophosphorylcholine (GPC) and even nicotinamide adenine dinucleotide phosphate (NADP) can be resolved with improved SNR [4-7]. However, there have been only a few in vivo studies in association with NAFLD with such enhanced spectral dispersion and SNR [6, 7]. To this end, we have investigated in vivo at 9.4T whether or not the separate quantification of PE, PC, GPE, GPC and NADP is advantageous over the conventional phosphomonoester (PME), phosphodiester (PDE) and nucleotide triphosphate (NTP) measurement in assessing NAFLD, in animals with different disease severity. Meanwhile, the data in previous 31P-MRS studies have been presented with different reference peaks for peak normalization, thereby making inter-laboratory comparison difficult. To address this issue, therefore, we have presented our data using those commonly used reference peaks. Then, multivariate analyses were performed to efficiently manage the increased number of variables.

MATERIALS AND METHODS

Animal Preparation: The animal research protocol was approved by the IACUC. A total of 44 male Sprague-Dawley rats entered the study. For the induction of NAFLD, 29 rats received i.p. injections of CCl4 mixed with vegetable oil [8] for 3-16 weeks. There were 15 control rats, among which 5 rats received pure vegetable oil at the same frequency.

31P-MRS: All MR data were collected on a 9.4T animal MR scanner using a 1H/31P double-tunable surface coil (Bruker Biospec 94/20 USR). For spatial localization, an ISIS sequence [9] was used with a typical voxel volume of 2.5 cm^3. The sequence parameters were: TR=6000 ms, 1024 data points, spectral bandwidth=8000 Hz, 512 signal averages.

Histopathology: Following MRS scan, livers were harvested for histopathology. Liver pieces were stained with hematoxalin and eosin (HE), and Masson's trichrome (MT). Livers were scored for steatosis, necrosis, inflammation and fibrosis. Severity scores ranged 0-3 for steatosis, necrosis and inflammation, and 0-4 for fibrosis.

MRS Data Analysis: MRS data were processed by using MRUI [10]. The individual peak areas were estimated by using AMARES, and then divided by total phosphorous signal (P_{total}; to be denoted without the denominator specified). They were also normalized to those commonly used references (PME+PDE and β-NTP (to be referred to as NTP)).

Statistical Analysis: For statistical analysis, rats were divided into control, NASH and cirrhosis groups according to the histopathological findings. Multivariate analyses were performed by using SIMCA (Umetrics Inc.). To examine the correlations between the histopathological parameters and the 31P-MRS measures a partial least-squares regression (PLS) was performed. Then, partial least-squares discriminant analyses (PLS-DA) were performed. Briefly, rats were randomly divided into a training (n=28) and a test (n=16) set for the construction and validation of a statistical model, respectively. Initially, all 31P-MRS measures were included and a PLS-DA model was produced using the training set. Then, a set of MRS measures were defined that contributed most in the differentiation of those three animal groups, based on the variable influence on projection (VIP>1) as a measure of the relative discriminatory potential (RDP). Using this set of MRS measures with VIP>1 an optimized model was constructed and evaluated for its predictability on both the training and the test sets.

	Control (n=15)	NASH (n=17)	Cirrhosis (n=12)
steatosis	0.00 ± 0.00	1.12 ± 1.05*	1.08 ± 0.79*
necrosis	0.00 ± 0.00	2.41 ± 1.00*	3.00 ± 0.00*
inflammation	0.47 ± 0.52	1.35 ± 0.79*	2.83 ± 0.39*&
fibrosis	0.00 ± 0.00	2.35 ± 0.79*	4.00 ± 0.00*&
PE	0.06 ± 0.03	0.08 ± 0.04	0.09 ± 0.04
PC	0.06 ± 0.02	0.03 ± 0.01*	0.04 ± 0.01*
PME	0.12 ± 0.05	0.11 ± 0.04	0.13 ± 0.05
Pi	0.10 ± 0.02	0.11 ± 0.02	0.10 ± 0.02
GPE	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.01&
GPC	0.04 ± 0.01	0.05 ± 0.02	0.03 ± 0.01&
PDE	0.07 ± 0.01	0.09 ± 0.02*	0.05 ± 0.02*&
PME+PDE	0.20 ± 0.05	0.20 ± 0.04	0.18 ± 0.06
NADP	0.11 ± 0.03	0.09 ± 0.03	0.09 ± 0.02*
UDPG	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
NTP	0.15 ± 0.01	0.16 ± 0.03	0.16 ± 0.03

Table 1 *(* p<0.05 vs. control, & p<0.05 vs. NASH) Pi: inorganic phosphate, UDPG: uridine diphosphoglucose*

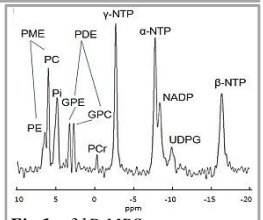

Fig.1 *31P-MRS spectrum*

RESULTS

Histopathology: The two control groups did not differ in any of the MRS measures (p>0.177), and therefore they were grouped together as control (n=15). Of 29 treated rats 17 and 12 rats were assigned to the NASH and the cirrhosis groups, respectively. The detailed histopathological characteristics of the animal groups were listed in Table 1.

31P-MRS Spectra: A representative spectrum is shown in Fig.1. The PME and PDE spectral regions are all well resolved into PE and PC, and into GPE and GPC, respectively. The NADP resonance is observed separately from α-NTP. The high SNR of the spectrum is clearly demonstrated.

Comparisons between Animal Groups: Table 1 summarizes the comparisons among the animal groups with respect to the MRS measures. There was a trend towards increasing PE with increasing disease severity. On the other hand, PC of both NASH and cirrhosis groups were significantly lower than that of control (p<0.002). Consequently, PME did not differ between the animal groups. The cirrhosis group had lower GPE and GPC than the NASH group (p<0.006). There were significant differences in PDE between all animal groups (p<0.044). NADP was significantly lower in the cirrhosis group relative to the control group (p<0.05).

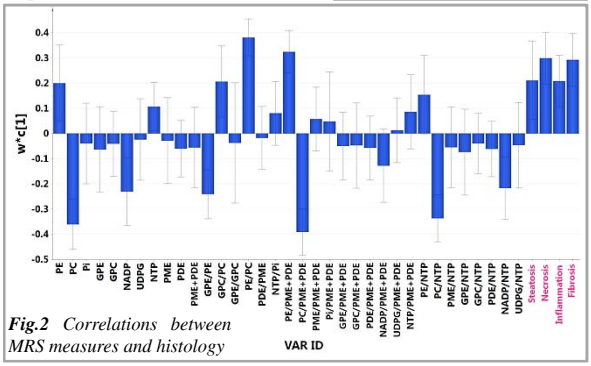

Fig.2 *Correlations between MRS measures and histology*

Correlations between 31P-MRS and Histopathology: Using a PLS analysis the correlations between the histopathological parameters and the MRS measures are shown in Fig.2 where large w*c[1] values (either positive or negative) indicate high correlations with histopathology. For instance, the histopathological parameters have relatively high, positive correlations with PE/PC and PE/(PME+PDE), and negative correlations with PC and PC/(PME+PDE). Note that the majority of those MRS measures with high correlations with the histopathological parameters are those detectable only in highly resolved spectra.

Separation of Animal Groups by a PLS-DA Model: In an initial PLS-DA model, the RDPs of the MRS measures in the differentiation of the three animal groups are shown in Fig.3(a) in terms of VIP. Note that most of the 14 MRS measures with VIP>1 (from PC/(PME+PDE) through PDE/PME in Fig.3(a)) are those detectable only in highly resolved spectra. Using these 14 MRS measures an optimized model was constructed, and the resulting score plot is shown in Fig. 3(b) where the optimized model allows good separations of the animal groups. Using this optimized model, 79% (22/28) of the rats in the training set were correctly classified and 69% (11/16) of the rats in the test set were correctly predicted.

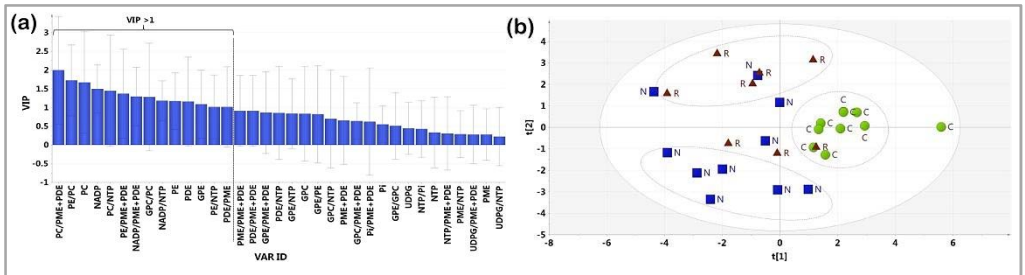

Fig.3 *(a) The variable influence on projection (VIP) of the MRS parameters as a measure of the relative discriminatory potential in the differentiation of the three animal groups. (b) Score plot (C: control, N: NASH, R: cirrhosis)*

DISCUSSION

Given the increasing prevalence of NAFLD and availability of high field clinical MR scanners, the investigation of the role of those additional peaks separately quantifiable in highly resolved spectra in assessing NAFLD is important. The use of different reference peaks for peak normalization in the previous 31P-MRS studies makes inter-laboratory comparison difficult. Our approach of presenting the data normalized to those commonly used reference peaks followed by multivariate analyses minimizes such difficulty. In our study, PDE differed between all animal groups. However, as it was higher in NASH and lower in cirrhosis with respect to control, it may not be used as a single MR measure for assessing NAFLD. The PDE/PME that was lower in cirrhosis with respect to both control and NASH in our study reconfirms its role as a 31P-MRS biomarker of liver cirrhosis. However, in our study it could not separate between control and NASH. The altered NADP metabolism found herein supports its potential role as an additional MR biomarker in NAFLD. In the PLS analysis including all of the MRS measures, relatively higher correlations with histology were found with those separately detectable only in highly resolved spectra. The advantage of separately quantifying these MRS measures was also clearly demonstrated in the construction of the optimized PLS-DA model that allowed the classification and prediction of the animals according to disease severity with ~ 70-80% accuracy. In conclusion, PE, PC, GPE and GPC that can be separately quantifiable in highly resolved spectra may further improve the efficacy of 31P-MRS in the diagnosis of NAFLD. The altered NADP level in diseased liver suggests its potential role as an additional MR biomarker in NAFLD.

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