Hepatic phospholipids in alcoholic liver disease assessed by proton-decoupled $^{31}$P magnetic resonance spectroscopy

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Background/Aims: Alteration of the phospholipid composition of hepatic biomembranes may be one mechanism of alcoholic liver disease (ALD). We applied proton-decoupled $^{31}$P magnetic resonance spectroscopic imaging ($^{1}$H–$^{31}$P MRSI) to 40 patients with ALD and to 13 healthy controls to confirm that metabolic alterations in hepatic phospholipid intermediates could be detected non-invasively.

Methods: All patients underwent liver biopsy. Specimens were scored in non-cirrhosis (fatty liver ($n = 3$), alcoholic hepatitis ($n = 2$), fibrosis ($n = 4$), alcoholic hepatitis plus fibrosis ($n = 16$)), and cirrhosis ($n = 15$). $^{1}$H–$^{31}$P spectra were collected on a clinical 1.5-Tesla MR system and were evaluated by calculating signal intensity ratios of hepatic phosphomonoester (PME), phosphodiester (PDE), phosphoethanolamine (PE), phosphocholine (PC), glycerophosphorylethanolamine (GPE), and glycerophosphorylcholine (GPC) resonances.

Results: The signal intensity ratio GPE/GPC was significantly elevated in cirrhotic ($1.19 \pm 0.22$; $P = 0.002$) and non-cirrhotic ALD patients ($1.01 \pm 0.13$; $P = 0.006$) compared to healthy controls ($0.68 \pm 0.04$), while PE/PC and PME/PDE were significantly elevated in cirrhotic ALD patients compared to controls ($1.68 \pm 0.60$ vs. $0.97 \pm 0.31$; $P = 0.02$, and $0.38 \pm 0.02$ vs. $0.25 \pm 0.01$; $P = 0.002$, respectively) and non-cirrhotic patients.

Conclusions: The data support that $^{1}$H–$^{31}$P MRSI appears to distinguish cirrhotic from non-cirrhotic ALD patients and confirms changes in hepatic phospholipid metabolism observed in an animal model.

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1. Introduction

Chronic alcohol consumption may lead to severe morphological and functional alterations of the hepatocyte including changes in the chemical composition and structure of biomembranes [1,2]. This membrane injury is predominantly caused by ethanol-induced changes in phospholipid metabolism [2,3]. It has been shown in rats and baboons, that chronic alcohol ingestion resulted in a decrease of hepatic polyenylphosphatidylcholine (PPC), a major constituent of biological membranes [4–6]. Various mechanisms contribute to the observed reduction of hepatic PPC, in particular, a lower production rate of phosphatidylcholine from phosphatidylethanolamine due to an acetaldehyde-mediated inhibition of phosphatidylethanolamine-N-methyltransferase (PEMT) [7,8]. Furthermore, chronic alcohol ingestion results in a reduced availability of the methyl groups that are necessary for phosphatidylcholine generation [9]. This disturbed hepatic methyl transfer is a result of various effects of alcohol...
including folate, vitamin B₁₂, and vitamin B₆ deficiencies as well as decreased formation of S-adenosylmethionine (SAM), the active methylating compound [7,9–13].

Phosphorus-31 magnetic resonance spectroscopic imaging (³¹P MRSI) has been applied in liver disease of various etiologies [14–23] as well as in alcoholics with [24,25] and without liver injury [26] to determine non-invasively relative concentrations of hepatic phosphorus-containing compounds. A ³¹P MR spectrum of the human liver in vivo shows intense resonances of (a) phosphomonooesters (PME), containing information on the membrane-phospholipid precursors phosphocholine (PC) and phosphoethanolamine (PE), (b) phosphodiesters (PDE), containing information on the cell-membrane degradation products glycerophosphorylcholine (GPC) and glycerophosphorylethanolamine (GPE), (c) inorganic phosphate (Pᵢ), and (d) adenosine 5'-triphosphate (ATP). In previous ³¹P MR spectroscopy (MRS) liver studies, only the abnormalities of relative PME and PDE concentrations in patients with alcoholic liver disease (ALD) were measured [22,24–26] because with conventional ³¹P MRS it is impossible to resolve the constituents of the PME and PDE resonances. The PME resonance is comprised of signals from PE and PC, and the PDE resonance is comprised of signals from GPE and GPC. Resolved resonances of PE, PC, GPE and GPC can be obtained by means of proton-decoupled ³¹P MRSI (¹H–³¹P MRSI) [27]. Proton decoupling is a double resonance technique that causes a collapse of scalar-coupled multiplets into singlets so that resonances become detectable. Thus, in the present study this method was used to determine whether ethanol-mediated changes in the hepatic membrane phospholipid composition observed in rodents and baboons can also be detected in humans, and, if so, whether differences between various types of ALD can be seen.

2. Methods and materials

2.1. Patients

The study included 40 chronic alcoholics (29 males, 11 females; mean age: 49 years) with a daily alcohol intake of more than 100 g who were admitted to the Dept. of Medicine, Salem Medical Center Heidelberg for alcohol detoxification therapy or for therapy of complications of ALD, and 13 healthy volunteers (7 males, 6 females; mean age: 35 years) with an alcohol intake of less than 100 g per week. All patients underwent standardized serum analysis, ultrasound imaging, and liver biopsy as part of their initial clinical assessment. Patients with hepatitis B and C and autoimmune hepatitis were excluded from the study. Liver biopsy was performed under sonographic control from lateral (segment VII) using a standard biopsy needle with a diameter of 0.9 mm. The length of the biopsy was approximately between 1.5 and 2.0 cm. Biopsy area and the area of spectroscopic analysis were most frequently identical and in some cases rather close to each other. Biopsy specimens were scored in non-cirrhosis [fatty liver (n = 3), alcoholic hepatitis (n = 2), fibrosis (n = 4), fibrosis plus hepatitis (n = 16)] and cirrhosis (n = 15). In addition, the percentage of hepatic fat was evaluated histologically. Patient and laboratory data are listed in Table 1. For statistical reasons we grouped the patients with steatosis, hepatitis, and fibrosis and referred to them as non-cirrhotics (NC) (n = 25). Accordingly, we compared healthy controls (HC), NC, and cirrhotics (C).

The study was approved by the Ethical Committee of the University of Heidelberg and each person gave written consent.

2.2. Proton-decoupled ³¹P MR spectroscopic imaging (¹H–³¹P MRSI)

Patients were examined 4–20 days after their last alcohol consumption. (¹H–³¹P MRSI) was performed using a clinical 1.5-Tesla MRI system (Magnemot Vision; Siemens, Erlangen, Germany), which was equipped with two radiofrequency systems enabling proton-decoupling during ³¹P signal detection. A double-tuned (¹H, ³¹P) planar surface coil with two concentric loops (14 cm diameter of ³¹P-loop) was applied for spin excitation and signal detection. The surface coil was embedded in the table of the tomograph and patients were placed in right lateral position. Scout view images were obtained in three orthogonal directions for checking correct patient positioning. The ¹H-loop was used for MR imaging, adjusting the magnetic field homogeneity (shim), and decoupling. The ³¹P-loop was used to acquire spectroscopic data.

Interactive shim was performed by monitoring the proton resonance of tissue water within the sensitive volume of the ¹H-loop. The linewidth at half height of the water resonance was in the order of 20–50 Hz.

Localized ³¹P MR spectra were obtained with two-dimensional spectroscopic imaging (repetition time [TR] = 1100 ms; field-of-view [FOV] = 250 mm, slice thickness = 50 mm. 8 × 8 phase encoding steps; voxel size = 30 × 30 × 50 mm³ = 45 ml). The number of excitations was 20 resulting in a total measurement time of 23.5 min for a single SI data set comprising 64 localized ³¹P MR spectra. Additionally, a single prepulse was applied at ¹H frequency of tissue water to enhance the phosphorus signal intensity (nuclear Overhauser effect) [28–30]. Broadband proton-decoupling was achieved by a series of composite pulses irradiated at ¹H frequency during ³¹P signal detection (WALTZ-8 with 128-ms length of pulse train) [31]. The equipment for double resonance (second radio frequency transmitter, double-tuned surface coil, pulse sequences) was purchased from Siemens and was certified by the manufacturer which also ensured that the specific absorption rate in decoupling experiments was always below the recommended limits.

The total examination lasted on average 60 min including positioning of the patient, acquisition of the images, shim, and data acquisition.

2.3. Spectroscopic data analysis

Spectroscopic data were processed using a commercial program (LUISE; Siemens) available at the Magnemot Vision scanner. To evaluate spectroscopic signal exclusively from the liver, the voxel grid was superimposed on MR scout images and then carefully shifted until one voxel was located adjacent to the center of the coil, and completely within the liver (Fig. 1). The correct positioning of the voxel entirely within the liver was verified by the weak phosphocreatine (PCr) signal in the MR spectrum, because PCr is absent in the liver parenchyma and originates from muscles in the abdominal wall only. Contamination from neighbouring voxels to the VOI is inherent to the applied SI technique particularly due to the relatively large voxel size.

Signal postprocessing included zero-filling to 2k data points, line-broadening (by multiplication with an exponential function), and Fourier transformation, followed by interactive phase and baseline correction. The resulting Fourier spectra were analyzed using the least-squares fit algorithm in LUISE and assuming a Lorentzian lineshape for each peak.

Signal intensities of PE (chemical shift δ = 7.1 ppm), PC (δ = 6.5 ppm), GPE (δ = 3.5 ppm), GPC (δ = 2.9 ppm), PDE (δ = 0 ppm), endogenous chemical shift reference), anorganic phosphate (Pᵢ, δ = ±5 ppm, depending on intracellular pH), and γ-ATP (δ = −7.6, −16.0, −2.4 ppm, respectively) were obtained by integration of the fits of the resonances (Fig. 1). The sum of the intensities of PE and PC as well as of GPE and GPC were defined as signal intensity of PME and PDE, respectively.

2.4. Statistics

Statistical analysis of signal intensity ratios of the various resonances was performed by means of ANOVA (ANalysis Of Variance) and unpaired
3. Results

In most examinations of this study, the PME and PDE resonance bands could be resolved into PE, PC, GPC, and GPE, respectively, with the exception of PME in six patients and in two healthy controls and PDE in three patients. Fig. 1b shows the fit of a $^{31}$P MR spectrum of the liver of a healthy volunteer with well-resolved signals and only small PCr signal contamination from the abdominal wall.

Fig. 2a shows a representative $^{1}$H–$^{31}$P MR spectrum of a patient with alcoholic hepatic fibrosis, Fig. 2b a spectrum of a patient with alcoholic cirrhosis of the liver. The ratio PE/PC was found to be elevated in cirrhotics as compared to non-cirrhotics. Reduced $^{31}$P signal-to-noise ratios were observed in patients with advanced ALD.

Fig. 3 shows a Box-and-Whiskers-Plot of the signal intensity ratio PME/PDE. The ratio PME/PDE was significantly elevated in C as compared to HC and NC ($P = 0.002$ and $P = 0.0002$, respectively), while NC and HC cannot be differentiated using this spectral parameter.

GPE/GPC in C and NC were significantly increased compared to HC ($P = 0.002$ and $P = 0.006$, respectively), with no significant difference in C compared to NC (Fig. 4).
Signal intensity ratios of PE/PC were significantly elevated in C compared to HC and NC (P < 0.02 and P < 0.05, respectively) without significant difference of this parameter between HC and NC (Fig. 5).

No significant correlation of hepatic fat content (<30% vs. 30–60% vs. >60%) with PE/PC ratio (1.32 ± 1.41 vs. 1.46 ± 1.06 vs. 1.15 ± 1.92, n.s. (not significant) and with GPE/GPC ratio (1.23 ± 1.01 vs. 0.88 ± 0.96 vs. 1.12 ± 1.19, n.s. (not significant) was noted.

For all signal intensity ratios the data range was broader in C compared to HC.

4. Discussion

The data presented here show for the first time that {1H}–{31P} MRSI permits assessment of relative signal intensities of GPE, GPC, PE and PC in patients with ALD.

Fig. 1. {1H}–{31P} MRSI of the liver of a 22-year-old female healthy control. (a) Axial T1w MR image of the liver obtained with a 14-cm-diameter surface coil; overlay: grid of {31P} MRSI (voxel size: 3.1 × 3.1 × 5.0 cm³). (b) Fit of resonances and difference of in vivo {1H}–{31P} spectrum and fit. Resonances of PE, PC, P, GPE, GPC, PEP (phospho-enol-pyruvate, according to [27]), residual PCr (from the muscle layer between coil and liver) and α-, β-, and γ-ATP are resolved. The low signal intensity of the PCr resonance indicates good spatial localisation of the voxel (rectangles) within the liver.

Fig. 2. (a) {1H}–{31P} MRSI spectrum and corresponding hepatic histology of a 55-year-old female patient with alcoholic liver fibrosis. The biopsy shows extensive perportal and parasinusoidal fibrosis in addition to typical ballooning of hepatocytes. (b) {1H}–{31P} MRSI spectrum and corresponding hepatic histology of a 49-year old male patient with cirrhosis of the liver. Biopsy shows micronodular alcoholic cirrhosis with typical pseudolobules. Compared to spectra of healthy controls (Fig. 1) and the patient with non-cirrhotic liver disease (Fig. 2a) increased PE/PC and compared to HC increased GPE/GPC ratios can be observed. The position of the observed voxel is indicated in orthogonal slices. [This figure appears in colour on the web.]
With this technique it could be clearly demonstrated, that phospholipid metabolism is severely disturbed in ALD. $^{31}$P MRSI has already been used in the past to study changes in high-energy phosphates, such as ATP, and changes in PME and PDE [24–26]. Proton decoupling enables further analysis of these phosphoesters. Proton decoupling enhances the spectral resolution and leads to a separation of the GPE and GPC, and PE and PC resonances, respectively. In addition, it achieves an approximately 30% enhancement of the signals by the nuclear Overhauser effect [28,29]. Therefore, this technique seems to be the method of choice to determine various PME and PDE in vivo. It is important to note, that no direct information from the lipid-soluble constituents of the membranes can be obtained. However, the MRS-detectable precursors (PE, PC) and degradation products (GPE, GPC) of phospholipids reflect the balance of anabolic and catabolic pathways.

The data reported here show an increase of the intensity ratios GPE/GPC in ALD and of PE/PC in cirrhotics, which mainly may result from either a decreased production of choline-containing compounds or an increased accumulation of ethanolamine-containing components or both within the hepatic membranes. These data confirm recent reports in the baboon which show a decrease in hepatic PPC after chronic alcohol consumption [3,6]. The pathogenesis of a relative decrease in hepatic GPC and PC and/or a relative increase in hepatic GPE and PE following chronic alcohol consumption and in ALD may involve various mechanisms.

Besides the fact that the availability of methyl groups and also the activation of methionine to SAM following alcohol ingestion is limited [7,9–13], an important observation seems to be the inhibition of PEMT by acetaldehyde [7,8]. PEMT is an enzyme that catalyses the stepwise methylation of phosphatidylethanolamine to phosphatidylcholine. Phosphatidylcholine can be synthesized by two pathways. The major route for phosphatidylcholine synthesis in most cells is via the cytidinediphosphocholine pathway. However, in the liver an alternative pathway, namely phosphatidylethanolamine-$N$-methylation, is responsible for approximately 30% of phosphatidylcholine synthesis and this process can be stimulated when methionine is provided [32]. Besides the fact that an inhibition of PEMT by acetaldehyde leads to a decreased synthesis of GPC and therefore to morphologic and functional changes in biomembranes, PEMT also contributes to the control of hepatocyte cell division since its inactivation is associated with several types of hepatic proliferation [33] including carcinogenesis [34,35]. It is noteworthy that PEMT is permanently inactivated in liver cancer induced by the Solt and Faber model [36], and it has been shown that the loss of PEMT activity may contribute to malignant transformation of hepatocytes [36]. Thus, if indeed the changes in phospholipid metabolism observed in our study are...
Most recently, an increase in PME/PDE ratios in chronic trations were found to be increased and could even be endoplasmic reticulum. In alcoholic hepatitis PME concentrations in liver tissue. Subsequently, attempts should be made to evaluate the sensitivity and specificity of the technique for its clinical application.

References


