ELSEVIER

Journal of Hepatology 42 (2005) 752–759

Journal of Hepatology

www.elsevier.com/locate/jhep

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

Heinz-Peter Wilhelm Schlemmer[1], Tanja Sawatzki[2], Steffen Sammet[3], Ines Dornacher[2], Peter Bachert[3], Gerhard van Kaick[4], Rüdiger Waldherr[2], Helmut Karl Seitz[2,*]

[1]*Department of Diagnostic Radiology, University Hospital, Eberhard-Karls University, Tübingen, Germany*
[2]*Laboratory of Alcohol Research, Liver Disease and Nutrition, Department of Medicine, Salem Medical Center, Zeppelinstrasse 11-33, D-69121 Heidelberg, Germany*
[3]*Department of Medical Physics in Radiology, German Cancer Research Center (DKFZ), Heidelberg, Germany*
[4]*Department of Radiology, German Cancer Research Center (DKFZ), Heidelberg, Germany*

***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 ± 0.22; $P=0.002$) and non-cirrhotic ALD patients (1.01 ± 0.13; $P=0.006$) compared to healthy controls (0.68 ± 0.04), while PE/PC and PME/PDE were significantly elevated in cirrhotic ALD patients compared to controls (1.68 ± 0.60 vs. 0.97 ± 0.31; $P=0.02$, and 0.38 ± 0.02 vs. 0.25 ± 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.**

***Keywords*: Phospholipid; Alcoholic liver disease; Cirrhotics; Phosphorus MR spectroscopy**

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

Received 11 June 2004; received in revised form 25 October 2004; accepted 1 December 2004; available online 11 March 2005

* Corresponding author. Tel.: +49 6221 483 200; fax: +49 6221 483 494.

E-mail address: helmut_karl.seitz@urz.uni-heidelberg.de (H.K. Seitz).

0168-8278/$30.00 © 2005 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jhep.2004.12.032

including folate, vitamin B_{12}, and vitamin B_6 deficiencies as well as decreased formation of *S*-adenosylmethionine (SAM), the active methylating compound [7,9–13].

Phosphorus-31 magnetic resonance spectroscopic imaging (^{31}P 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 ^{31}P MR spectrum of the human liver in vivo shows intense resonances of (a) phosphomonoesters (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 (P_i), and (d) adenosine 5′-triphosphate (ATP). In previous ^{31}P 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 ^{31}P 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 ^{31}P MRSI ($\{^1H\}$–^{31}P 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 compostition 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 ^{31}P MR spectroscopic imaging ($\{^1H\}$–^{31}P MRSI)

Patients were examined 4–20 days after their last alcohol consumption. $\{^1H\}$–^{31}P MRSI was performed using a clinical 1.5-Tesla MRI system (Magnetom Vision; Siemens, Erlangen, Germany), which was equipped with two radiofrequency systems enabling proton-decoupling during ^{31}P signal detection. A double-tuned (^{1}H, ^{31}P) planar surface coil with two concentric loops (14 cm diameter of ^{31}P-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 ^{1}H-loop was used for MR imaging, adjusting the magnetic field homogeneity (shim), and decoupling. The ^{31}P-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 ^{1}H-loop. The linewidth at half height of the water resonance was in the order of 20–50 Hz.

Localized ^{31}P 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 mm^3 = 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 ^{31}P MR spectra. Additionally, a single prepulse was applied at ^{1}H frequency of tissue water to enhance the phosphorous signal intensity (nuclear Overhauser effect) [28–30]. Broadband proton-decoupling was achieved by a series of composite pulses irradiated at ^{1}H frequency during ^{31}P 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 Magnetom 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 $\delta = 7.1$ ppm), PC ($\delta = 6.5$ ppm), GPE ($\delta = 3.5$ ppm), GPC ($\delta = 2.9$ ppm), PCr ($\delta = 0$ ppm, endogenous chemical shift reference), anorganic phosphate (P_i, $\delta \cong 5$ ppm, depending on intracellular pH), and α-, β- and γ-ATP ($\delta = -7.6$, -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