

Potential of ^{31}P Magnetic Resonance Spectroscopy of Bile in the Detection of Cholestatic Diseases

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INTRODUCTION: Phosphatidylcholine (PC) is a predominant phospholipid present in bile protecting bile ducts from harmful effects of bile acids. In our earlier studies on the analysis bile from cholestatic patients using ^1H MRS, we had observed the hydrolysis of phosphatidylcholine in some patients [1]. Since phosphatidylcholine and its hydrolysis products – glycerophosphocholine (GPC), and phosphocholine – have similar chemical shift values for their $-\text{N}^+(\text{CH}_3)_3$ signals, it will be difficult to detect the biochemical changes using ^1H NMR spectroscopy. In this study, we have tested if ^{31}P magnetic resonance spectroscopy can be used for their differentiation.

MATERIALS & METHODS: Bile samples ($n = 16$) were obtained from patients with various hepatopancreaticobiliary diseases [primary sclerosing cholangitis (PSC) = 1; cholangiocarcinoma = 1; papillary cancer = 1; chronic pancreatitis = 3; pancreatic cancer = 4; and other benign biliary diseases such as common bile duct (CBD) stones = 6] undergoing endoscopic retrograde cholangiopancreatography (ERCP). MRS experiments were performed on a 360 MHz Avance Bruker spectrometer (^{31}P frequency: 145.86 MHz). ^1H -decoupled ^{31}P MR spectra were obtained using an inverse-gated sequence with WALTZ-16 composite pulse. The following acquisition parameters were used: number of scans = 500, 90° pulse = 8.15 μs , number of points in the time domain = 32k, spectral width = 5,952 Hz, acquisition time = 2.75 s and line broadening for exponential window function = 3 Hz.

RESULTS & DISCUSSION: Bile is an important biofluid which performs various physiological functions in the digestive system. Bile acids are one of the major lipid components present in bile and help in the emulsification of fatty food. Bile acids such as glycochenodeoxycholic acid and glycodeoxycholic acid are toxic in nature, and impart toxic effect on the hepatobiliary system (intra-/extra-hepatic bile ducts, gallbladder). However, the hepatobiliary system is protected from this effect by the presence of PC in bile which forms mixed micelles/vesicles with bile acids and minimizes the toxicity of bile acids. Previously, we had observed the absence of PC in bile samples of some cholestatic patients [1]. This hydrolysis is believed to be mediated by the presence of pancreatic enzymes in bile [2]. As a result, the bile composition is altered and the bile ducts are exposed to the toxic bile acids. Although we have tried to differentiate PC and its hydrolysis products (GPC or phosphocholine) in bile by 1D ^1H MRS using their $-\text{N}^+(\text{CH}_3)_3$ signals, it was difficult due to the similarity in their chemical shifts (PC: 3.24 ppm; GPC: 3.21 ppm; phosphocholine: 3.20 ppm) and the extensive broadening of signals resulting from micellar/vesicular nature of bile components. Hence, we have augmented our studies with 2D ^1H - ^1H COSY experiments. However, 2D experiments require longer experimental time which may not be desirable in clinical settings. As a result, in this study, we have tested the possibility of using ^{31}P MRS for the differentiation of various phospholipids and their hydrolysis products in bile. Khan et al. had also previously suggested using ^{31}P MRS for the detection of hepatopancreaticobiliary diseases [3]. In their study, they have observed decreased levels of PC in the patients with hepatopancreaticobiliary malignancies.

Figure 1 shows ^{31}P MR spectra of bile from a control and a patient with cholestatic disease (primary sclerosing cholangitis, PSC). We can see from Figure 1(a) that the control bile shows presence of phosphatidylcholine (-0.84 ppm) along with small amounts of other phosphorus containing biochemicals (inorganic phosphorus (Pi), lyso-PC, phosphatidylethanolamine). Figure 1(b) depicts the ^{31}P MR spectrum of bile from a PSC patient showing the absence of PC and the presence of elevated levels of GPC. We made this observation in 4 out of 16 patients (PSC, cholangiocarcinoma, papillary cancer and one patient with CBD-stone with cholangitis). However, PC was not hydrolyzed in patients with pancreatitis, pancreatic cancer and all other CBD-stone patients. The absence of PC in cholestatic patients is due to its hydrolysis possibly by the action of pancreatic enzymes [2]. Khan et al. have attributed the decrease in the PC levels to an altered cellular metabolism [3]. However, in this study, we confirmed that hydrolysis is one of the reasons for the decrease levels/disappearance of PC in bile samples. The absence of PC in bile has been considered as a potential risk factor for malignant transformations of bile ducts with a history of inflammatory diseases such as PSC [1], and this information could be helpful in the proper management of these patients.

CONCLUSION: Phospholipid metabolism in pathologic conditions can be better studied by ^{31}P MRS; and ^{31}P MRS of bile could serve as a valuable tool in understanding the etiology of hepatopancreaticobiliary diseases.

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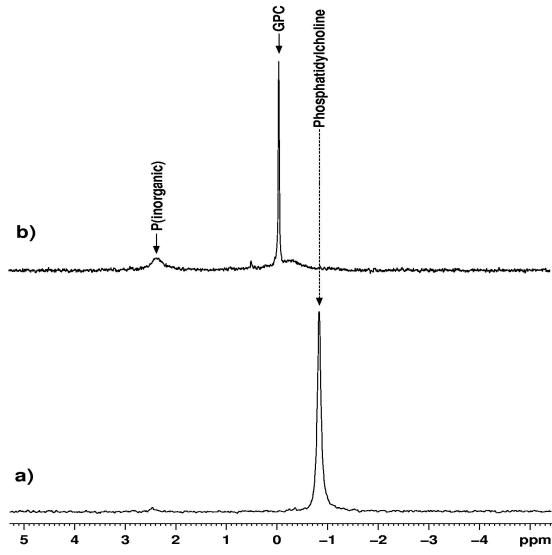


Figure 1: ^1H -Decoupled ^{31}P MR spectra of human bile obtained from (a) control subject and (b) primary sclerosing cholangitis patient, showing conversion of PC to GPC.