Can Regurgitation of Pancreatic Juice into the Biliary Tract be Detected by ^{1}H MR Spectroscopy?

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INTRODUCTION: Regurgitation of pancreatic juice into the biliary tract and the subsequent mixing of pancreatic juice with bile is frequently observed in patients with pancreaticobiliary maljunction (PBM), which is a potential risk factor for the carcinogenesis of bile duct and gallbladder [1]. The mixing of pancreatic juice with bile is generally detected by measuring the amylase activity in bile [2]. Recently, this phenomenon has also been observed in some patients without PBM [2]. In this study, we analyzed bile samples from patients with various cholestatic diseases to investigate if any of these patients show mixing of their bile with pancreatic juice.

MATERIALS AND METHODS: Bile samples were obtained from patients (n = 33) undergoing endoscopic retrograde cholangiopancreatography (ERCP) examination/surgery for various cholestatic conditions. Pancreatic juice was also obtained during ERCP examination from patients with chronic pancreatitis (n = 17). ^{1}H MR spectra of bile and pancreatic juice were obtained with simple one-pulse and CPMG sequences (total echo-time: 480 ms) on a 360 MHz spectrometer (Bruker Instruments) using 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP) dissolved in D_2O as a chemical shift reference. Neat bile and pancreatic juice samples were mixed *ex vivo* in the ratio 1:1, and incubated for 72 hours. ^{1}H MR spectra were obtained at different time intervals (0, 24, 48, 72 hours).

RESULTS & DISCUSSION: Regurgitation of pancreatic juice into the biliary tract is usually detected by measuring amylase activity in bile. However, this activity is affected by experimental conditions such as pH and temperature and also by interfering molecules such as bilirubin (a bile component). We therefore tested the possibility of detecting the reflux of pancreatic juice into bile using ^{1}H MR spectroscopy. Figure 1 depicts the ^{1}H MR spectra (CPMG) of neat samples of human bile and pancreatic juice showing their metabolic profiles. The CPMG-^{1}H MR spectrum of bile is characterized by the presence of lipids- taurine-conjugated to bile acids, glycine-conjugated bile acids, phosphatidylcholine and other low-molecular-weight metabolites such as lactate, acetate, acetone, choline and glucuronic acid. The CPMG-^{1}H MR spectrum of pancreatic juice shows the presence of lactate, alanine, acetate, acetone, glutamate, glutamine, choline, glycine, formate and the aromatic amino acids- tyrosine, histidine and phenylalanine.

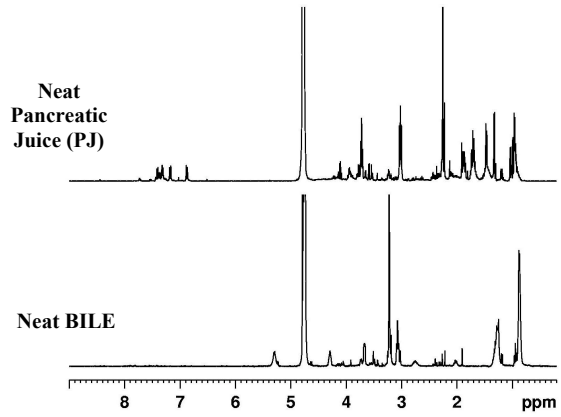

Figure 1: ^{1}H MR spectra of neat samples of human bile (lower plot) and pancreatic juice (PJ) (upper plot) obtained using CPMG pulse-sequence.

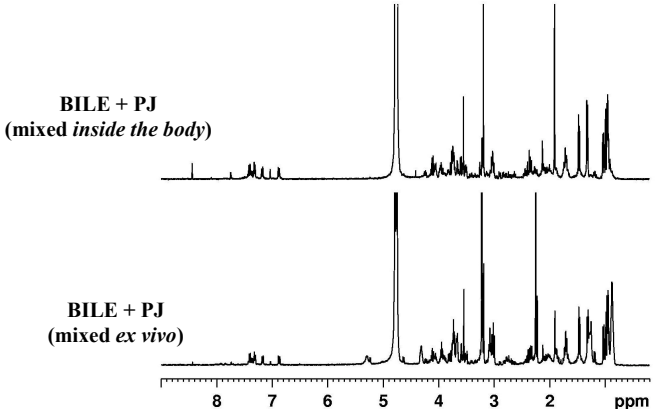

Figure 2: CPMG-^{1}H MR spectra of bile mixed with pancreatic juice, *ex vivo* (lower plot) and of a bile sample mixed with pancreatic juice mixed inside the body (upper plot). There are similarities in the spectral patterns of both samples.

Figure 2 shows the ^{1}H MR spectra of bile and pancreatic juice mixed *ex vivo* in the ratio 1:1 and incubated for 48 hrs at 37°C (lower plot), and of a bile sample which was mixed with pancreatic juice *inside* the body (upper plot). The two show comparable spectral patterns. 5 of the 33 bile samples analysed showed spectral patterns indicative of mixing with pancreatic juice. We monitored the changes in the composition of bile mixed with pancreatic juice (*ex vivo*) as a function of incubation time, and observed a gradual decrease in the levels of phosphatidylcholine (PC). PC completely disappeared after 48 hours. We also found that PC was absent in all of the 5 bile samples which were mixed with pancreatic juice *inside* the body. The disappearance of PC in bile samples mixed with pancreatic juice could be attributed to the hydrolysis of PC by pancreatic enzymes (such as pancreatic lipase). Since PC is an important component of bile protecting hepatocytes and biliary epithelium from harmful effects of various bile acids, its absence could be cytotoxic to both hepatocytes and cholangiocytes. In patients with cholestatic diseases such as primary sclerosing cholangitis, the absence of PC in the bile has also been considered as a risk factor for the progression towards bile duct cancer (cholangiocarcinoma) [3]. Moreover, 3 out of the 5 patients who showed this phenomenon also showed elevated levels of plasma-bilirubin indicating underlying cholestasis. All these observations support the hypothesis that reflux of pancreatic juice into the biliary tract or gallbladder could be a potential risk factor for the development of cholestatic conditions and ultimate progression towards malignant transformations as observed in patients with PBM. These results show that pancreaticobiliary reflux can be reliably detected by ^{1}H MR spectroscopy.

CONCLUSION: Reflux of pancreatic juice into the biliary tract/gall bladder can easily be detected by ^{1}H MR spectroscopy of bile, and this method may prove to be an alternative to the measurement of amylase activity in the rapid detection of pancreaticobiliary reflux. Recently, the feasibility of *in vivo* spectroscopy of bile has been demonstrated and using such an approach it should be possible to detect the reflux of pancreatic juice into the gallbladder non-invasively.

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