Detection and Quantification of D-Glucuronic acid (GlcUA) in Human Bile by using 1H MRS

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INTRODUCTION: D-glucuronic acid is synthesized in the liver from UDP-glucose mediated by UDP-glucosedehydrogenase and is involved in a number of key detoxification pathways by removing a variety of non-polar drugs, environmental toxins and carcinogens from the body [1]. It is also responsible for the removal of bilirubin, a major end-product of heme catabolism. In the liver, D-glucuronic acid is conjugated to bilirubin, forming bilirubin diglucuronide, and is excreted into the bile [2]. The levels of bilirubin diglucuronide in bile in healthy individuals or patients with biliary obstruction are almost undetectable by 1H MRS. However, bilirubin diglucuronide could be hydrolyzed in the presence of β-glucuronidase, and as a result, free bilirubin and D-glucuronic acid may be released into the bile. Bilirubin is reabsorbed into the body, but glucuronic acid is retained in the bile and could be analyzed. A previous study has reported that D-glucuronate can be detected in bile by 1H MRS after its enzymatic hydrolysis [3]. In this study, we have analyzed bile samples from various patients with biliary obstruction (controls), chronic pancreatitis and pancreatic cancer by using 1H MR spectroscopy. We report here a simple 1H MRS method for the detection and quantification of D-glucuronic acid in human bile samples.

MATERIALS AND METHODS: Bile samples from patients with biliary obstruction (control group, n=10), chronic pancreatitis (n=3) and pancreatic cancer (n=4) were collected during an ERCP examination. The samples were obtained by deep cannulation of the common bile duct (CBD). One-dimensional (1D) 1H MR spectra (using one-pulse and CPMG sequences) were obtained for all bile samples on a 360 MHz spectrometer (Bruker Instruments). D-glucuronic acid in bile was identified by making use of two-dimensional (2D) DQF-COSY and TOCSY experiments, and finally confirmed by spiking the bile samples with standard D-glucuronate.

RESULTS & DISCUSSION: Figure 1 shows 1H MR spectra of a neat bile sample (from a pancreatic cancer patient) obtained by both single- and CPMG pulse sequences, showing the proton signals of D-glucuronic acid along with other biliary biochemcials. Since the signals due to D-glucuronate in bile were overlapping with the broad lipid signals, it was difficult to observe these signals using a single-pulse experiment. Therefore, we used a CPMG pulse sequence with a relatively long echo-time (480 ms). The α- and β-anomeric proton signals of D-glucuronic acid appear at 5.24 and 4.64 ppm respectively, and other 1H signals resonate in the region 3.05 - 4.1 ppm. The presence of D-glucuronate in bile was confirmed by 2D DQF-COSY and TOCSY experiments. Finally, the identity of D-glucuronic acid was confirmed by spiking the bile with standard D-glucuronate. Moreover, the existence of D-glucuronate in its free form was ascertained by derivatizing it with phenylhydrazine [4].

The quantification of D-glucuronic acid was achieved by measuring the peak area of α-CH resonating at 5.24 ppm. The peak area was obtained by deconvolution relative to the peak area of the TSP signal, and the quantity of D-glucuronate was calculated making use of the following equation,

\[ [GlcU](mM) = \frac{W_{i}(TSP)_{mg} \times \text{PeakArea(GlcU)} \times \text{No.of Prot}(TSP) \times 1000}{\text{Volume}(Sample) \times \text{A} \times \text{B}} \quad \text{Eq. (1)} \]

where A (=100/54.5) and B (=100/78.19) are two correction factors introduced to overcome the loss in signal intensity due to the anemic effect and the use of a CPMG sequence. Due to the close structural resemblance of glucuronic acid with D-glucose, D-glucuronic also exhibits an anemic effect and shows only 54.5% signal intensity for the α-CH signal, which is used here for the quantification. The correction factor ‘A’ overcomes the signal loss due to the anomeric effect. Similarly, the use of a longer echo-time (480 ms, which was necessary for the attenuation of the most abundant lipid signals in bile) in the CPMG sequence resulted in a signal loss of ~22%, and the correction factor ‘B’ was introduced to overcome this loss. Standard addition experiments were performed to test the accuracy and precision of the method. Bile samples from patients with control group, chronic pancreatitis, and pancreatic cancer were tested for the presence/absence of D-glucuronic acid. Both control and pancreatitis patients showed an absence/negligible amount of D-glucuronic acid, but the pancreatic cancer patients showed elevated levels of D-glucuronic acid. This could be attributed to the hydrolysis of bilirubin diglucuronide in bile by β-glucuronidase released from the pancreas into the bile during the course of malignancy [5]. The amount of D-glucuronic acid in bile from pancreatic cancer patients determined by the present method was 0.91 ± 0.58 mM (mean ± SD).

CONCLUSION: D-glucuronic acid could be conveniently detected and quantified by 1H MRS, and the presence of elevated levels of D-glucuronic acid in pancreatic cancer patients may have diagnostic implications in the detection of pancreatic cancer.

REFERENCES: