Application of Excitation Sculpting in the Quantification of Conjugated Bile Acids in Bile

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INTRODUCTION: Bile acids are major components of bile and are present mostly in conjugation with glycine and/or taurine. Amide bonds formed as a result of this conjugation provide well resolved ‘NH’ signals in the down-field region of the ¹H MR spectrum of bile. However, at physiologic pH, these amide protons are in dynamic exchange with biliary water and show decreased signal intensity. Lowering the pH of bile below physiologic value has been helpful in recovering such signal loss [1]. Adjusting the pH of each and every sample, however, is tedious. Hence, alternative methods are desirable. In this study, we propose the use of excitation sculpting (ES) sequence [2] for the quantification of conjugated bile acids using their ‘NH’ signals without the need for pH adjustment.

MATERIALS & METHODS: Bile samples were obtained from patients undergoing endoscopic retrograde cholangiopancreatography (ERCP) examination for various cholestatic diseases. We edited the ¹H-homodecoupling sequence from TOPSPIN pulse-sequence library to include ES for the suppression of water signal [2]. 1D ¹H MR spectra with ¹H-decoupling were obtained using conventional PRESAT and ES sequences on standard bile acids [glycochenodeoxycholic acid (GCDCA); glycodeoxycholic acid (GDCA); glycocholic acid (GCA); taurochenodeoxycholic acid (TCDCA); taurodeoxycholic acid (TDCA); taurocholic acid (TCA)], and bile samples using a 600 MHz Avance spectrometer (Bruker Biospin). Spectra were recorded at different pH values (in the range 5.0 – 9.0) to study the effect of pH on the signal intensity of the amide (NH) peak. The bile acids were quantified from the peak areas of their characteristic amide signals in the region 7.8 – 8.05 ppm. The peak areas of NH-signals were obtained by deconvolution (TOPSPIN software), using 3-(trimethylsilyl)propionic-2,2,3,3-d₄ acid sodium salt (TSP) as an external standard.

RESULTS & DISCUSSION: Bile acids in bile are conjugated to glycine and taurine, generally in the ratio 3:1 [3]. Figure 1 (solid line) depicts the ¹H MR spectrum of human bile showing the amide proton (NH) signals of predominant glycine- and taurine-conjugated bile acids present in bile. Previously, we have reported that these amide NH signals could be used for the quantification of individual conjugated bile acids in human bile [4], provided the pH of the bile is adjusted to 6.0 ± 0.5 [at physiologic pH (7.5 – 8.5), the amide protons are in dynamic exchange with biliary water and do not represent their true intensity]. In this study, we are presenting an alternative methodology for the suppression of water resonance in bile by making use of ES (a method for the selective excitation/removal of a resonance based on the use of ‘double pulsed field gradient spin-echo’) which selectively removes resonance due to water without affecting other resonances including those which are in dynamic exchange with water [2].

CONCLUSION: We have tested the feasibility of using ES sequence for the quantification of exchangeable amide protons in human bile. We conclude that ES sequence can be utilized for the above purpose and can serve as an alternative to the current method which requires pH-adjustments.

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