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 ^{1}H 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 ^{1}H-homodecoupling sequence from TOPSPIN pulse-sequence library to include ES for the suppression of water signal [2]. 1D ^{1}H MR spectra with ^{1}H-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-d_4 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 ^{1}H 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].

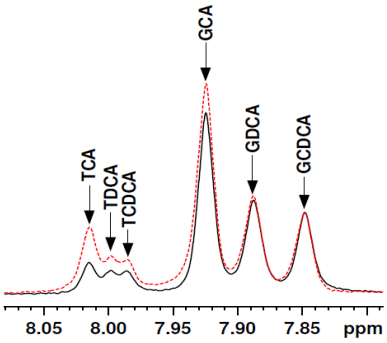

Figure 1: ^{1}H-Decoupled ^{1}H MR spectra of a human bile (pH = 8.79) obtained by PRESAT (solid line) and excitation sculpting (ES) pulse-sequences (dotted line).

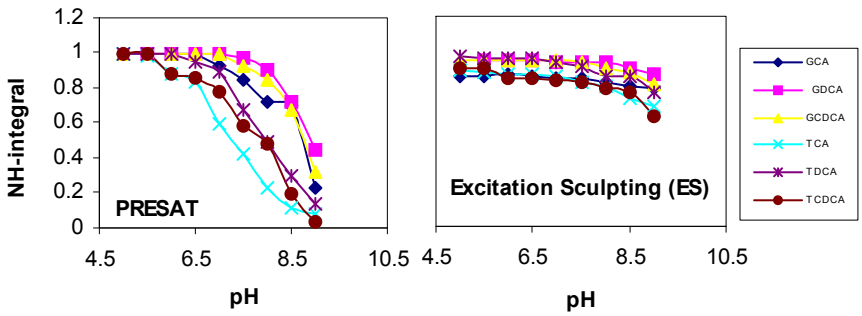

Figure 2: Plots of the peak areas of amide NH-signals as a function of pH for various conjugated bile acids present in bile, measured using PRESAT and ES sequences. It should be noted that there is more significant decrease in signal intensity at higher pH values in PRESAT compared to ES.

Figure 1 (dotted line) shows recovery of signal loss observed in the conventional PRESAT sequence. It is interesting to note that in the PRESAT experiment, the NH-signals due to taurine-conjugated bile acids are attenuated more than glycine-conjugated bile acids indicating amide protons in taurine-conjugates are more labile than in glycine-conjugates. In order to determine the exact signal attenuation/gain in PRESAT/ES sequence, we studied the effect of pH on the signal intensity of amide protons. Figure 2 shows the comparison of PRESAT and ES sequences in the measurement of peak areas of amide signals revealing a minimal signal loss in ES sequence compared to the PRESAT. Although, ES-sequence is advantageous over PRESAT in the quantification of signals arising from exchangeable protons, it does not show 100% signal intensity. We calculated the % signal in using ES-sequence and observed that TCA, TDCA, TCDCA, GCA, GDCA, and GCDCA show a maximal signal intensity of 76, 85, 75, 82, 91, and 89% respectively. With the application of appropriate correction factors in each case, the exact amount of individual bile acids in bile can be determined using ES sequence. Unlike PRESAT method, it does not require any pH adjustment to minimize the exchange phenomenon. Moreover, bile salts such as GDCA precipitate at lower pH (≤ 5.5); such problems can be overcome in the present methodology. The present method could be easily automated for the simultaneous quantification of various conjugated bile acids in human bile.

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.

ACKNOWLEDGEMENTS: We would like to thank Ms. Jothi Jangam for her help during the study.

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