Use of $^1$H NMR spectroscopy ($T_2$ relaxation times) to examine the effects of conjugated ursodeoxycholic acid on phospholipid fluidity of human gallbladder bile

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An essential element of altered bile chemistry in cholesterol gallstone disease is supersaturation with cholesterol. Cholesterol is solubilised in bile by aggregation with other biliary lipids. The major aggregates in human bile are micelles and vesicles containing phospholipids, cholesterol and bile acids. Although the precise nature of the non-micellar aggregate phase is a matter of some dispute [1,2] it is generally accepted that patients with cholesterol gallstones have a higher proportion of their biliary cholesterol in the non-micellar phase.

Ursodeoxycholic acid (UDCA) has been used for many years as a medical treatment for cholesterol gallstones, and there is agreement, that in UDCA treated patients, the non-micellar lipid aggregate fraction is more stable than that in untreated gallstone patients [3,4]. The molecular basis of this enhanced stability is not known. It has been shown, however, that UDCA therapy prolongs the time necessary for the first deposition of cholesterol microcrystals from gallbladder biles (the nucleation time) even when biliary supersaturation with cholesterol is unchanged. This is achieved without drastically altering the distribution of cholesterol between the micellar and non-micellar fractions [4,5]. We have recently shown that gallbladder biles from patients with cholesterol gallstones and rapid nucleation times have a reduced phospholipid fluidity as measured by $^1$H NMR when compared with biles from patients without stones and of prolonged nucleation times [6].

Here we report the use of $^1$H NMR to examine the effects of UDCA and its conjugates on bile phospholipid fluidity. $^1$H NMR spectroscopy was performed on a JEOL G5000 NMR spectrometer operating at 500 MHz and 11.75 Tesla as previously described [6]. Human gallbladder bile was obtained from 5 patients undergoing cholecystectomy. In each case the bile sample was freeze dried and reconstituted in an equivalent volume of $^2$H$_2$O or 100mM bile acid in $^2$H$_2$O. The bile acids used were: glycodeoxycholic acid (GDCA), glycochenodeoxycholic acid (GCDCA), glycoursodeoxycholic acid (GUDCA), taurochenodeoxycholic acid (TCDCA), taurocholic acid (TCA) and taoursodeoxycholic acid (TUDCA). $^1$H NMR spectroscopy was then used to examine the effect of the various bile acids on the $T_2$ (spin-spin) relaxation time of the choline head group in the five bile samples.

Addition of bile acid invariably increased the $T_2$ relaxation time of the phospholipid choline head group. Within the taurine and glycine groups the $T_2$ values increased in proportion to the hydrophobicity index of the bile acid added. The effects of the conjugates of UDCA on the fluidity of the choline head group were less than those of the conjugates of cholic, chenodeoxycholic and deoxycholic acids (Fig 1).

Total bile acid, phospholipid and cholesterol concentrations were measured and the biliary cholesterol saturation index (CSI) calculated following the addition of 100mM bile acid. Increases in $T_2$ relaxation time were associated with decreases in CSI ($r = 0.97$).

![Fig 1 $T_2$ relaxation measurements following addition of bile acids to whole bile](image1)

![Fig 2 Correlation of CSI and $T_2$ relaxation in the five bile samples following addition of glycine conjugated bile acids](image2)

These results show that dilution of bile with UDCA and its conjugates results in an increase in bile phospholipid fluidity which is less than that produced by the more hydrophilic bile acids.

Clearly UDCA-induced changes in the stability of the non-micellar fraction of biliary cholesterol are not necessarily reflected by changes in the phospholipid fluidity of the whole bile.

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References