NMR lipid profiles of hepatic bile collected from the patients undergoing liver transplantation (OLTx)

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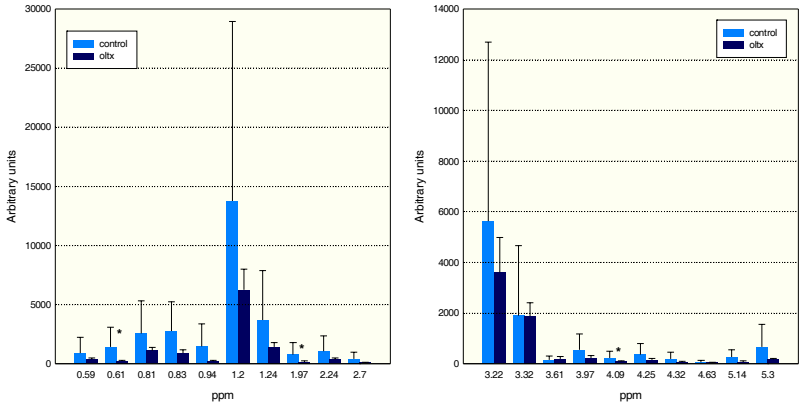
Introduction

Nuclear magnetic resonance spectroscopy provides information on the lipid metabolism. This method offers also an opportunity to assess condition of liver. We hypothesize that NMR spectroscopy in vitro of human bile may provide information about the status of hepatocytes. It may also be an additional tool in assessment of the graft following liver transplantation.

Material and method

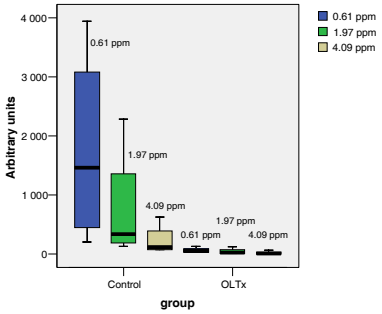
The bile samples (n=4) were obtained from the bile duct at the end of liver transplantation just before completion of haemostasis and surgical closure of the wound. Control samples (n=4) were obtained from a patient during cholecystectomy, when revision of bile ducts was necessary. Bile samples (1.5 ml) were frozen at –80°C until analysed. Extraction of lipids from hepatic bile was performed according to Bligh and Dyer method. Lipid extracts were studied by ^{1}H NMR spectroscopy at 9.4 T. Quantification of NMR signals was done using phosphatidylcholine signal intensity calibrated in raw bile ^{1}H NMR spectra vs. 1mM TSP standard. Statistical analysis was done for the signal intensity.



Results

Signals from twenty lipid functional groups were analysed. Intensities of almost all analysed signals of bile extract were lower in the transplanted liver (OLTx) patients group (Fig.1) as compared to the control group. Mann-Witney test shows significant differences between corresponding signals of two groups ($p<0.05$): for cholesterol –C(18)H_3 (δ=0.61), for –CH_2HC= in fatty acyl chain (δ=1.97) and in glycerol backbone of phospholipids and triglicerydes –C(1)H_2 (δ=4.09) (Fig.2). For all other analysed signals no significant differences of intensities was observed.

Fig. 1 Mean magnitudes of analysed NMR signals of bile extracts. The chemical shift of resonances are assigned as follows: 0.59ppm: unassigned, 0.61 ppm: –C(18)H_3 , 0.81 ppm: -CH_3 in fatty acyl chain, 0.83 ppm: –C(21)H_3 in free cholesterol, 0.94 ppm: –C(19)H_3 in esterified cholesterol, 1.2 ppm: $(–CH_2–)_n$ in fatty acyl chain, 1.24 ppm: =CH–$CH_2$$(–CH_2)_n$ in fatty acyl chain, 1.97 ppm: =CH–CH2- in fatty acyl chain, 2.24 ppm: –CO–CH_2– in fatty acyl chain, 2.7 ppm: =CH–CH_2–CH= in fatty acyl chain, 3.22 ppm: –$N^+(CH_3)_3$ in phosphatydylcholine head group, 3.32 ppm: unassigned, 3.61 ppm: –CH_2–$N^+(CH_3)_3$ in phosphatydylcholine or sphingomyelin head group, 3.97 ppm: –C(3)H_2 in glycerol backbone of phospholipids, 4.09 ppm: –C(1)H_2 in glycerol backbone of phospholipids and triglycerides, 4.25 ppm: –CH_2–CH_2-$N^+(CH_3)_3$ in phoshatydylcholine or sphingomyelin head group, 4.32ppm: –C(1)H_2 and –C(3)H_2 in glycerol backbone of triglycerides, 4.63 ppm: – unassigned, 5.14 ppm: –C(2)H in glycerol backbone of phospholipids and trigicerydes, 5.3 ppm: –HC=CH– in fatty acyl chain



Discussion

Decreased lipid secretion from transplanted liver suggests altered liver function. This analysis shows that NMR spectroscopy in vitro could be used for analyzing the function of the graft and early signs of rejection. More sophisticated data analysis will be carried out on a greater number of samples together with medical data.

Fig2. Signal intensities showing significant differences between groups.

Acknowledgement: this work was supported by grant 2 PO5C 076 28 from State Committee of Scientific Research