In vivo ^{31}P MRS Characterisation of ANIT-induced Hepatobiliary Dysfunction

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Introduction

Cholangiocytes are pivotal in the hepatic secretion of bile. Dysfunction of these cells, resulting in cholestasis, characterises a number of human diseases affecting the biliary tree. Cholestasis can be induced in animals by chronic administration of alpha-naphthylisothiocyanate (ANIT). In this study, the induction of biliary dysfunction by chronic ANIT feeding was investigated using *in vivo* hepatic ^{31}P MRS. This modality allows the non-invasive study of certain metabolites including those with a central role in cell membrane metabolism and cellular energetics[1].

Protocol

Sprague Dawley rats (160-170g, Charles River UK Ltd) were obtained and divided into five equal groups (n=4 per group). Two groups were fed a diet supplemented with 0.1% (70-80mg/kg) and 0.05% (35-40mg/kg) ANIT for 14 days and are referred to as the high dose (HD) and medium dose (MD) groups respectively. Controls were pair fed on a normal diet according to the food intake of the HD and MD animals, or fed *ad libitum*, which are referred to as PFC_HD, PFC_MD and control groups respectively. At baseline animals were fasted and then anaesthetised for MRS on a 4.7T Varian system using a dual surface coil, tuned to ^{31}P and ^{1}H frequencies. Initially, MRI scout images were obtained for correct positioning of the liver followed by unlocalised hepatic ^{31}P MRS. MR spectra were acquired using a simple pulse-acquire sequence with the pulse optimised to give a 90^{o} excitation in the liver region. 544 ^{31}P data collects were averaged for each rat at a TR of 5s. This was repeated at 1 and 2 weeks after the supplemented diet was begun for all animals.

Results

Mean metabolite ratios before and during chronic feeding are shown in Figures 1 - 3. At baseline, no significant variations were seen in PDE:PME (Figure 1), or PDE and PME relative to total phosphorus (total P) (Figures 2 and 3) across all the groups. The PDE:PME ratio in the HD group was significantly higher than in its pair-fed control group (PFC_HD, 0.28 ± 0.10 vs. 0.86 ± 0.12, P< 0.05) after 2 weeks of chronic ANIT feeding (Figure 1). This increase could be explained by the generally elevated levels of PDE and lower levels of PME, relative to the total phosphorus signal, in comparison to pair-fed controls (Figure 2). However, the ^{31}P MR spectra from the group treated with the medium dose of ANIT showed a different response profile. The MD group showed a significant rise in PDE relative to the total phosphorus signal from baseline, after 1 week on the treated diet (P < 0.05), but did not have a significantly higher PDE:PME relative to their pair-fed controls.

Figure 1 Figure 2 Figure 3

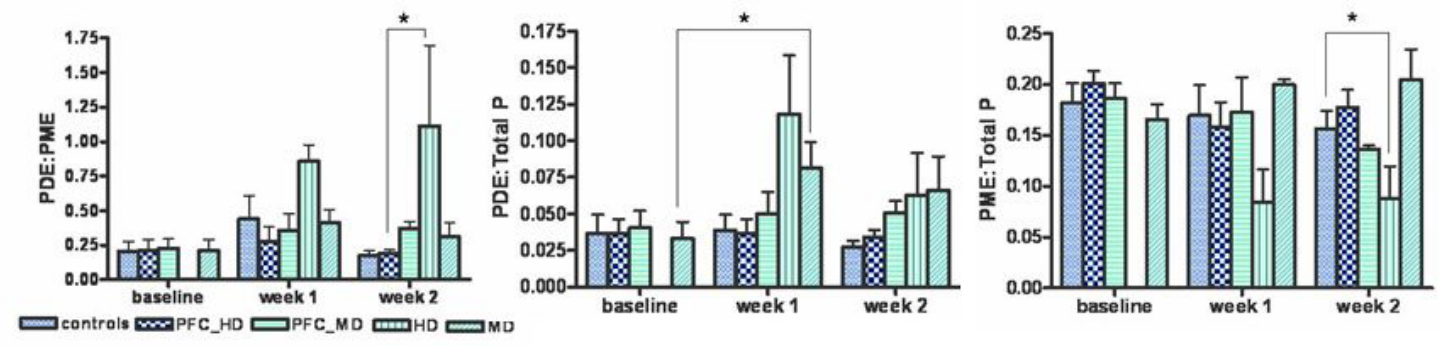

Figure 1: Ratio of PDE:PME at baseline and 1 and 2 weeks post dose(PD) for control, pair fed control for high dose (PFC_HD) and medium dose (PFC_MD), high dose (HD) and medium dose (MD) groups.

Figure 2: The ratio of PDE:total phosphorus for all groups. Figure 3: The ratio of PME:total phosphorus for all groups.

[* is statistically significant at the P < 0.05 level.]

Discussion

The PDE detected arises from phospholipids produced by cell membrane degradation and those present in bile. Bile duct obstruction has been observed to result from the sloughing of necrotic biliary epithelial cells into the duct lumen following ANIT treatment[2]. Thus, an elevation in hepatic PDE levels following 2 weeks of chronic ANIT administration is consistent with increased cell degradation following ANIT-induced functional hepatic decompensation. The generally increased level of PDE was accompanied by a significant decrease in the PME level in the HD group, suggesting concomitant increased consumption of PME for cell membrane synthesis. This finding is consistent with known cholangiocyte proliferation subsequent to ANIT-induced cholestasis[3]. However, this effect is not demonstrated in the MD animals indicating the response to ANIT treatment is dose-dependent. Thus, by ^{31}P MRS we were able to study cellular dynamics non-invasively, following the administration of an experimental biliary toxin, supporting the use of this methodology in the study of acute and chronic hepatobiliary disease.

References

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