High resolution \(^1\)H NMR to study biliary cholesterol stability

J P M ELLUL, SUZANNE SEQUEIRA, H G PAREKS, G M МУРФИ (Gastroenterology Unit, UMDS, Guy’s Campus, Guy’s Hospital, London SE1 9RT and NMR Unit, Department of Chemistry, Birckbeck College, NW1) Cholesterol (CH) is thought to deposit from unstable cholesterol-phospholipid vesicles (CH-PL) in bile. Conventional methods, however, to separate biliary vesicles from mixed PL-CH bile acid micelles are unreliable. We (a) studied the effect of CH on \(^1\)H NMR spectra of vesicles, (b) determined bile PL by integration of \(^1\)H NMR spectra and enzymatic measurement (cholesterol oxidase), (c) compared the results with the nucleation time (time taken to detect cholesterol microcrystals in the biliary isotropic phase).

Small unilamellar CH/PL vesicles were prepared in TRIS buffer in mM ratios of CH:PL (5:5; 5:10; 5:15). Bile was obtained from 10 patients (H NMR spectra were obtained on a JEOL GX500 NMR spectrometer operating at 500 MHz and 11.5 Tesla at 37°C. PL peaks at 1.25 ppm (CH₃ tail) and at 3.25 ppm (N(CH₃)₃) were broad at equilibrium concentrations of CH and PL but became sharper with a decreasing vesicular CH:PL ratio. There was a strong correlation (p<0.001) between the concentration of PL as measured by (N(CH₃)₃) peak integration and the enzymatic method. The quantitative difference between the biliary PL determined from the integration of the \(^1\)H NMR (N(CH₃)₃) PL and that measured enzymatically ranged from 0-21% (n=5, mean= ±4%) in bile with nucleation time >10 days and 29-55% (n=5, mean= ±39%) in bile with nucleation time <5 days.

It is concluded that molecular motions of biliary PL and their detection by \(^1\)H NMR are restricted by saturation of vesicles with CH. The difference between the total biliary PL and that measured by integration of the (N(CH₃)₃) resonance peak gives direct non-invasive support for the hypothesis that the cholesterol in gall sludge is deposited from vesicles with C:PL >1:0.

Comparative studies of human intrahepatic biliary and gall bladder epithelial cells in vitro

R JOPLIN, L WALLACE, J M NEUBERGER, А J STRAIN (Liver Unit, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TU) Previous studies have shown that intrahepatic biliary epithelial cells (IHBE) can be isolated from both normal and diseased human liver tissue. These cells proliferate in response to hepatocyte growth factor (HGF) and maintain markers of biliary phenotype. Here, we have isolated and cultured normal adult human gall bladder epithelial cells (GBEC). Cells were prepared by immunolocalisation and cultured in 25 cm² tissue culture plastic flasks. Initial cell isolates were found to be morphologically similar to IHBE and to stain positively with HEA125 and cytokeratin 19, both markers specific for adult biliary phenotype. Using culture medium containing serum, hormones, and growth factors including HGF, which supports growth of IHBE for 10-12 weeks and up to seven passages, GBEC survived for only a few days and showed no evidence of proliferation. These findings show important differences in the growth properties of GBEC when compared with IHBE and add further evidence to the view that epithelial cells from different parts of the biliary tree show phenotypic heterogeneity.

High prevalence of histological abnormality in asymptomatic hepatitis C infection with persistently normal liver enzymes

C J HEALEY, K A FLEMING*, R W G CHAPMAN (Department of Gastroenterology, John Radcliffe Hospital, *Nuffield Department of Pathology and Bacteriology, Oxford University, Oxford) Previous studies have shown a poor correlation between raised transaminases and histological damage in hepatitis C infection. The aim of this study is to assess the liver histology of asymptomatic cases with normal biochemistry but positive for both anti-HCV and HCV RNA.

Asymptomatic cases who were positive for HCV by second generation ELISA were identified. If initial liver enzymes were normal (AST<45 IU/l, ALT<45 IU/l) serum was tested for viral RNA by the polymerase chain reaction method. If present, liver biopsy was performed. Histology was assessed by KAF (as above).

Fourteen cases were given a biopsy (mean AST=25-9 IU/l, mean ALT=31.9 IU/l). No liver biopsy was normal. Seven of 14 showed minimal changes. Comparing between the two groups (CHP vs normal) there was a significant difference in the mean ALT activity, 39±3 vs 24±4 (p<0.03) but not in the AST. In conclusion, 50% of asymptomatic HCV cases who were positive for viral RNA but who had ‘normal’ liver function had significant pathology. More severe changes were reflected in higher ALT activities, although still within the normal range. In HCV infection biopsy is needed for the accurate assessment of disease stage despite persistently normal liver enzymes.

Synaptosomal glutamate transport in thioacetamide induced hepatic encephalopathy in rats

K OPPONG, K BARTLETT, C О RECORD, H AL MARDINI (Department of Medicine, The Medical School, Framlington Place, University of Newcastle Upon Tyne) A dysfunction of excitatory glutamatergic neurotransmission has been postulated as a causative factor in hepatic encephalopathy (HE). Brain microdialysis studies in animal models have established an increase in extracellular glutamate levels in HE but the mechanisms of this are unclear. In this study we have investigated calcium dependent glutamate release and high affinity, sodium dependent glutamate reuptake in synaptosomes prepared from rats with thioacetamide (TAA) induced HE. Previous in vivo and in vitro studies of glutamate release have failed to differentiate the calcium dependant release of neurotransmitter glutamate from the calcium independent release from the cytoplasm.

There was no significant difference in synaptosomal glutamate release, total synaptosomal glutamate content or the Ka for glutamate uptake between the TAA group and the normal saline control group. There was a noticeable decrease in the Vmax for glutamate uptake in synaptosomes from rats with HE (31%, p<0.01). The results of this study provide evidence of impaired neuronal glutamate transport in rats with HE. This supports the hypothesis that decreased glutamate uptake may play a role in the neurodegeneration seen in HE.

Serum hyaluronic acid in extrahepatic biliary atresia (EHBA) before portoenterostomy identifies patients who will require early liver transplantation

A DHAWAN, P TRIVEDI, P CHEESEMAN, A P MOWAT (Department of Child Health, King’s College Hospital, Denmark Hill, London SE5 8RS) Although portoenterostomy has greatly improved the long-term survival of patients with EHBA, 10-20% still rapidly develop hepatic fibrosis and cirrhosis and require early liver transplantation. As hepatic fibrogenesis may be accompanied by increased serum concentrations of connective tissue matrix components, we have measured one of these, hyaluronic acid (HA), at diagnosis in 40 infants with EHBA to see if it can be used to identify patients with a poor prognosis.

Serum HA was significantly higher (p<0.0001; 95% confidence intervals for difference=145 ± 437 microg/ml) in the 15 patients who died (mean age at death 9.8±1.5 months) and the six who required OLT before 3 years of age than in the five who required OLT after three years, and the 14 who are alive without liver transplantation at five years (mean (SEM) HA=547 ± 256 (41)). ROC curves showed that a value of serum HA greater than 540 microg/ml was optimal for assessing prognosis, predicting death or the need for early liver transplantation with 80% sensitivity, 89% specificity, 89% positive predictive value, and 80% negative predictive value. Neither serum bilirubin, AST, GGT, INR, or albumin were of any value in assessing prognosis.

We conclude that serum HA may be a useful complementary test in assessing patients with EHBA, identifying even before portoenterostomy those who will require early liver transplantation.

Hepatitis C in children: role of serology in diagnosis

S M DAVISON, J S SKIDMORE, W L IRVING, D A KELLY (Liver Unit, Children’s Hospital, London)
Transjugular intrahepatic portosystemic shunts (TIPS): results at two years

J ROSE*, M HUDSON, A TURBULL, O W JAMES, M BASSENDINE (Departments of Radiology and Medicine, Freeman Hospital, Newcastle Upon Tyne NE7 7DN) The insertion of TIPS was evaluated in 32 consecutive patients with acute or recurrent variceal bleeding (n=20), three with associated portal gastropathy, two of whom had colitis, resistant ascites (n=11), and preoperative portal decompensation (n=1). All 32 patients had underlying cirrhosis (alcohol n=20, PBC n=7, HBV virus n=2, cryptogenic n=3), one of whom had superimposed HCC. Three of 32 had isolated portal vein, one of which was caused by neoplastic infiltration.

Successful TIPS insertion was completed in 29/32 and there were no procedure related deaths. Thus (median 9) mean CMV, 9 mm (n=2), and 10 mm (n=16). Pre TIPS mean portosystemic pressure gradient was 28 mm Hg (range 17-55), post TIPS this was reduced to 11 mm Hg (range 6-10). Complications of the TIPS procedure included worsening renal failure (n=4) and deteriorating hepatocellular function (n=1). In the group with variceal bleeding 8/20 had variceal embolisation during the TIPS insertion procedure. Eighteen of 20 patients had no further admissions with bleeding after TIPS. Both rebleeds were associated with stenosis of TIPS at eight and nine months after procedure.

All patients have been reassessed between three to six months after procedure and 40% have required balloon dilatation of the stent. At 12 months, the reintervention rate is 25%. Of the 32 patients, four have had liver transplantation, five have died, the remaining patients have survived an average of 5-3 months (range 1-16).

TIPS is effective in lowering portal pressure, and thus controlling variceal bleeding, portal gastropathy, and colopathy, and resistant ascites. Our experience would suggest that stents should be directly changed at three months to prevent subsequent complications.

Chronic hepatitis C virus infections: predictive value of genotype and level of viraemia on disease progression and response to interferon α

J C L BOOTH, G R FOSTER, U KUMAR, R GALASSINI, R D GOLDIN, J L BROWN, H C THOMAS (Departments of Medicine and Histopathology, St Mary's Hospital Medical School, London) We have studied the effect of hepatitis C virus genotype and viraemia on disease outcome in 29 patients with chronic hepatitis C virus infection. The genotype of the virus was assessed by sequence analysis of the 5′-noncoding region, and the technique of competitive polymerase chain reaction was used to assess the level of viraemia. There was a trend of increasing disease severity and response to interferon during the first three months of treatment, however, predicted a failure to derive long-term benefit from the current IFN regimen. Hence pretreatment variables cannot be used to determine which patients will benefit from interferon, but the continued presence of HCV RNA after three months of treatment is always associated with relapse even though treatment is continued for one year. In these patients consideration should be given to changing the interferon dosing regimen or using alternative treatments.

Expression of HBV polymerase in HBV related hepatocellular carcinoma

M R THURZ, G R FOSTER, M J MCGARVEY, H C THOMAS, R D GOLDIN (Departments of Medicine and Histopathology, St Mary's Hospital Medical School, London) Hepatitis B Virus (HBV) is a common cause of hepatocellular carcinoma (HCC). The mechanism of malignant transformation by HBV is unknown. Expression of HBV polymerase and the HBV genome to the cells' growth by changing its response to interferon and may thereby play a part in the pathogenesis of hepatocellular carcinoma. We have developed a polyclonal antiserum that recognises the C-terminal fragment of HBV polymerase and have used this to stain liver biopsy specimens using immunohistochemical techniques. We examined specimens from patients with positive HBV serology (HBsAg positive) and HCC group 1 to positive HBV serology and dysplasia (group 2), and negative HBV serology (HBsAg and anti-HBc negative) and HCC (group 3).

Five of six patients in group 1, one of four in group 2, and 0/20 in group 3 stained positively for HBV polymerase. The pattern of staining in HCCs (nuclear and cytoplasmic) differs from that in chronic hepatitis (nuclear), which may represent differing mechanisms of protein processing. To our knowledge this is the first report of polymerase expression in HCC.

Differential effect of transforming growth factor β on expression of 6-3 and 1-5 Kb hepatocyte growth factor mRNA transcripts in MRC-5 cells

P M HARRISON, A BOMPORD*, ROGER WILLIAMS*, P FARZANEH (Molecular Medicine Unit and Institute of Liver Studies, King's College School of Medicine and Dentistry, London) Distinct mRNA transcripts are produced from the hepatocyte growth factor (HGF) gene, by alternative exon splicing, and of these the 6-3 Kb HGF mRNA is translated into the most potent ligand. The 1-5 Kb HGF mRNA encodes a truncated protein, which is an antagonist at the HGF receptor. Thus the influence of the HGF gene on liver regeneration might be determined by the comparative levels of these HGF mRNA transcripts. Transforming growth factor β 1 (TGF β1) is an important inhibitor of liver regeneration. We investigated the effect of TGF β1 on levels of HGF mRNA transcripts in MRC-5 cells (a human skin fibroblast cell line) using northern blot analysis. TGF β1 considerably reduced the expression of the 6-3 Kb HGF mRNA but it had no effect on levels of the 1-5 Kb HGF mRNA. In RNA stability studies, TGF β1 reduced the half-life of the 6-3 Kb mRNA, although it had little effect on the stability of the 1-5 Kb mRNA. In addition to a direct effect on hepatocytes, TGF β1 might inhibit liver regeneration by modulating the expression of the HGF gene to favour production of the antagonist protein.

Fucosylated retinol binding protein (FBP) in hepatocellular carcinoma (HCC): a new diagnostic test

S N ZAMAN, S D RYDER, ROGER WILLIAMS (Institute of Liver Studies, King's College Hospital, London SES 9RS) Retinoids play an important part in growth and differentiation and abnormalities of retinol and its transporter proteins are involved in neoplasia. Abnormally glycosylated proteins have previously been studied as markers for HCC.

We aimed at studying retinol binding protein (RBP) concentrations and the proportion present in fucosylated form in HCC and cirrhosis.

Serum was used from 15 patients (13/15 cirrhotic) with HCC, 10 patients with cirrhosis without HCC, and 10 healthy controls. RBP concentrations were measured by radial immunodiffusion. The proportion of RBP fucosylated was determined by affinity purification using Sepharose 4B gel and α-methyl-D-mannnoside. FBRP was identified by dot blot analysis and quantified by RI. Percentage of fucosylated RBP present in fucosylated form was calculated.

Mean total RBP was lower in both cirrhosis (19 μg/ml) and HCC (15 μg/ml) than healthy controls (75 μg/ml) (p<0.001) but there was no difference between the cirrhotic group and the HCC group (p=0.8). FBRP was however significantly greater in HCC (29%) than both controls (13%, p=0.002), and the cirrhotic group (15%, p=0.03). Using 3 SD above mean control value as cut off, sensitivity of FBRP for HCC was 87% and specificity 84%.

In conclusion, FBRP is a specific and sensitive serological marker of malignant change in cirrhosis, and these changes in the carbohydrate structure of RBP may directly participate in hepatic neoplasia.
factor (hHGF) is synthesised as a single chain precursor, which is proteolytically cleaved to give the biologically active heterodimeric form. HGF has structural homology to plasminogen and is activated in vitro by plasminogen activators. The aim of the study was to investigate this relation in patients with fulminant hepatic failure (FHF). Serum hHGF, measured by ELISA, was significantly increased (median 6.7 ng/ml, range 1.2–2.7, n = 39, p < 0.001) when compared with normal controls (0.38–30 ng/ml, 0.28–0.77, n = 20, p < 0.001). Plasma plasminogen concentration was significantly decreased in FHF (median 9% of control, range 0.7–3.5%), but plasma free tPA activity was not significantly different from normal and neither parameter correlated with serum hHGF. Plasma D-dimer, produced by the action of plasmin on fibrin, and the haemodynamic abnormalities of the blood coagulation system in FHF, but the serine proteases involved are not clear.

L-mono-methyl-arginine in the treatment of hypotension resulting from fulminant hepatic failure

J WENDON, P M HARRISON, R SMITH, J F MARTIN, S MONCADA, ROGER WILLIAMS (Institute of Liver Studies, Department of Medicine, King’s College Hospital, London, UK, Welcome Research Laboratories, Beckenham, Kent, UK, and the Wellcome Labouratoires, Sydney, Australia).

Infra-arterial lipidol targeted treatments for unresectable hepatoma: a report on 95 patients

S BHATTACHARYA, J R NOVELL, G M DUSEIKO*, A J W HILSON**, R DICK, K E F HOBBs (Departments of Surgery, Medicine, Radiology, and Nuclear Medicine*, Royal Free Hospital and University College Hospital Medical School**, London NW3 2QG). Lipidol (iodised poppy oil) injected into the hepatic artery localises selectively in hepatocellular carcinomas (HCC).

Hepatocellular carcinoma in primary biliary cirrhosis

J D COLLIER, H MITCHEISON, P KELLY*, M F BASSEDINE, O F W JAMES (Department of Medicine and Medical Statistics†, University of Nottingham, NE2 4HH, UK). Hepatocellular carcinoma (HCC) has been considered a rare complication of primary biliary cirrhosis (PBC) unlike most other types of cirrhosis. To examine whether this apparent rarity is associated with any diagnosis of PBC, the presence of HCC was recorded in 1144 (24%) patients with PBC and the possible contribution of histological cirrhosis to the development of HCC was retrospectively analysed.

Transin release by rat lipocytes in primary culture

S K YIAS, J GENTRY, M J J P JAMES (University of Southampton, Southampton, UK). Previous studies by our group have shown that cultured hepatocytic lipocytes synthesise gelatinase A which degrades native type IV collagen and gelatin. In this study we investigated their synthesis of transin, a metalloprotease with specificity for a broader range of matrix proteins.

Primary cultures of rat hepatic lipocytes were used. Zymography of serum free culture conditioned media showed caselician activity corresponding to the known gelsidase and ungelsidase proenzyme forms of transin (Mr 60 kDa and 57 kDa). This inhibited the absence of EDTA but not PMSF or NEM. Specific activity for transin was shown by Western blotting of lipocyte conditioned serum free medium against a polyclonal anti-transin rabbit antiserum. Continued protein activity was seen in 3, 7, 14, and 21 days cultures by zymography but quantitatively, net C-casein degradation is a significant viral load was seen in anti-HIV positive and in patients after liver transplant.

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(per μg DNA) was reduced sevenfold in prolonged culture probably due to the coexpression of tissue inhibitors of metalloproteinases (TIMPs). In conclusion, we have shown that cultured rat lipocytes release net transin activity during the early proliferative phase of lipocyte culture. This may contribute to the remodelling of the ECM during lipocyte proliferation in response to hepatic injury, favouring the accumulation of fibrillar proteins that occurs in liver fibrosis.

Analysis of DNA repair in cirrhosis of different aetiologies

J D Collier, G N Major, A D Burt*, M F Basendine (Departments of Medicine and Pathology*, University of Newcastle Upon Tyne, NE2 4HH) O°-methylguanine DNA methyltransferase (O°-MT) repairs the promutagenic DNA base lesion O°-methylguanine, which results from exposure to environmental alkylating agents. Failure to repair O°-methylguanine results in a G to A mutation capable of activating oncogenes and inactivating tumour suppressor genes. In a preliminary study we have shown levels of O°-MT are low in cirrhosis suggesting one mechanism for cirrhosis being a risk factor for hepatocellular carcinoma (HCC). The comparative risk of developing HCC is related to the aetiology of the underlying cirrhosis; the aim of this study was to measure O°-MT levels in cirrhosis of different aetiologies (n=39) to find out if it accounts for these differences.

Mean (SD) enzyme levels (in fmol/mg protein) were lower in alcoholic cirrhosis (836 (314); n=16) viral (hepatitis B and C) cirrhosis (827 (481); n=9), and autoimmune cirrhosis (1101 (346); n=14) compared with normal liver (2364 (222); n=5). Within the subgroups of cirrhosis, however, a difference was only seen when comparing the alcohol and autoimmune group (p=0.038).

These findings confirm preliminary results of low levels of O°-MT in cirrhosis particularly alcoholic cirrhosis and lend support to in vitro studies showing alcohol and its metabolite acetaldehyde inhibit O°-MT, but they do not account for the increased risk of HCC in cirrhosis of viral aetiology.