EVALUATION OF CELL PROLIFERATION IN VIVO AND IN VITRO IN REGENERATING LIVER AFTER PARTIAL HEPATECTOMY: A COMPARISON OF DIFFERENT TECHNIQUES.

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Actually different techniques are available to evaluate hepatocyte proliferation in histologic specimens. The determination of labelling index (LI) by the injection in vivo of 3H-Thymidine (3H-T) and Bromodeoxyuridine (BrdU), which are incorporated into the nuclei during the S phase of cell cycle, is widely employed in experimental animals. Since this method cannot be used in humans for clinical purposes, LI after incubation of biopsic specimens in vivo with these substances has been evaluated with controversial results. On the other hand nuclear proteins, such as PCNA and Ki-67, which become appreciable only in late G1, S and early G2 phases of cell cycle, have been proposed as markers of proliferation. Aim of this study was to assess the reliability of these different techniques in the liver.

Our study was performed in 15 Fisher (F-344) male rats which underwent 70% hepatic resection and were sacrificed after 24 hours. Five rats received 3H-T and 5 BrdU intraperitoneally 1 hr before the sacrifice for LI in vivo, while liver samples from the remaining 5 animals were incubated in presence of the substances for LI in vitro. The expression of proliferating cellular nuclear antigen (PCNA) was evaluated in all 15 hepatocarotized rats.

Our results are reported in the table:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N (Liver)</th>
<th>Tissue:</th>
<th>SU per 10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>3H-T</td>
<td>5/5</td>
<td>22.5 ± 3.8 S/12.6</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>BrdU</td>
<td>5/5</td>
<td>19.8 ± 4.0 S/6.8</td>
<td>&lt; 0.01**</td>
</tr>
<tr>
<td>PCNA</td>
<td>15</td>
<td>42 ± 9.4</td>
<td></td>
</tr>
</tbody>
</table>

In conclusion BrdU or 3H-T LI after the incubation of biopsies does not represent in our experience a reliable technique for the study of cell kinetic in the liver because their diffusion is limited to the peripheral area of the sections. Despite PCNA expression is not specific of S phase of cell cycle, we believe that it actually represents the only reliable marker of cellular proliferation in biopsic samples. In fact, even if it gave higher values in comparison to 3H-T and BrdU, nevertheless it expressed in our study a uniform measurement of hepatocyte regenerative state in all samples.

DO BILE ACIDS AFFECT STRUCTURE AND FUNCTION OF HUMAN HEPATOCYTE MITCHONDRIA?

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It has been suggested that liver damage in primary biliary cirrhosis (PBC) is due to retention of toxic hydrophobic bile acids and that the therapeutic effect of the hydrophilic bile acid ursodeoxycholic acid (UDCA) is due to the reduction of this damage. UDCA also reduces serum levels of specific mitochondrial enzymes and anti-mitochondrial antibodies. However, the mechanism(s) involved in mitochondrial damage and protection are unclear. Our aim was to examine the effect of hydrophobic and hydrophilic bile acids on structure and function of human hepatocyte mitochondria. Mitochondria were prepared using sucrose gradient ultracentrifugation from wedge liver biopsies obtained from patients without liver disease. To investigate structural effects, we measured mitochondrial protein solubilisation by CDCA and UDCA (0-10 mM). To investigate functional effects, we studied mitochondrial electron transfer by measuring the activity of succinate cytochrome C reductase. Protein solubilisation by CDCA was detected at 0.5mM bile acid concentration and reached a maximum of 50% with UDCA it was detected at 2mM and reached a maximum of only 20%. CDCA markedly inhibited mitochondrial electron transfer by comparison to UDCA for all given bile acid concentrations. At 1 mM bile acid concentration, CDCA achieved an inhibition of 80%, whereas UDCA only achieved a maximal inhibition of 30% as a concentration of 10 mM. A combination of CDCA plus UDCA (up to 0.5mM of each) gave the same effect as UDCA alone (hepatoprotective effect). We have demonstrated that hydrophobic bile acid damage hepatocyte mitochondrial structure and function and that addition of UDCA is protective. We speculate that hydrophobic bile acid damage to mitochondria may contribute to the disease process in PBC and that the beneficial effect of UDCA may be related to the improvement in mitochondrial oxygen utilisation and ATP formation.


A device has been developed for the focused, extra-corporeal ablation of liver tumors by means of high intensity, thermal ultrasonic effects. We experienced the device in hepatic tumor-bearing animals. The device is a 1 MHz piezo-composite ultrasound (US) transducer connected with an amplifier, a radiofrequency generator and a PC computer; transducer displacements are operated by an x-y-z micropositioner and monitored by a US probe. 18 NZ rabbits received 20mg VX-2 tumor in the liver. 12 days later, 12 animals were treated (6 controls) with 1000 W/cm² energy deposition at the focal point. Target was defined as a cubic volume surrounding the tumor. Animals were sacrificed 2 days after treatment. In a first group, tumor destruction was characterized by light microscopy and quantified by stereomicroscopy and planimetry. In the second group, tumor from treated and control animals was re-implanted in the thigh of non-tumor-bearing animals. In group 1, tissue ablation was a homogeneous coagulation necrosis and the percentage of tumor ablation was 94±3%. A thin rim of normal liver parenchyma was ablated around the tumors. In group 2, none of the treated tumors induced tumor growth in the thigh of normal rabbits, in contrast with 100% of control tumors. We conclude that the device has a potential for radical extra-corporeal liver tumor destruction and suppresses tumorigenicity in this animal model. We are now investigating long term effects of high intensity ultrasound (survival and metastatic spread).
An abnormal systemic and regional tissular oxygen uptake has been described in cirrhosia. This disturbance is higher in those patients with more advanced hepatocellular failure and has been related to the hyperkinetic circulation and the opening of precapillary arterioles.

Aim and methods: To know the role of arteriogenous shunts in the abnormal tissular oxygenation, the femoral arteriogenous difference of oxygen content (CAO2) has been measured and the degree of pulmonary shunting of precapillary territory determined by using 99m Tc human serumalbumin microspheres of 305±25 μm in diameter as described by Rhodes et al. Briefly, dispersed microspheres suspensions were used as intratissular injection. Counts were recorded from both lungs every 30 sec by a gammacamera. Counts obtained after intravenous injection of the microsphere solution was considered to represent 100% shunting. Nine cases: 2 healthy age matched subjects, 1 cirrhotic patient without ascites and 6 cirrhotic patients with ascites were studied.

Results: The femoral arteriogenous difference of oxygen content was 62.5±10.9 mL in the uncomplicated cirrhotic patients, 62.6±10.9 in the compensated cirrhotic patient and 61.3±10.9 in the healthy subjects (M±SEM); this difference did not reach significance because the small size of the sample. However, CAO2 inversely and very closely correlated with the degree of shunting estimated by microspheres (r=0.98;p=0.0001), with the prothrombin index (r=0.57), UNaV (r=0.58) and SvO2 (r=-0.87; p<0.001) but not with SaO2 (r=0.08).

Conclusions: Our data indicate that the opening of arteriogenous precapillary connections may underlie the abnormal tissue oxygenation in advanced stages of cirrhosis. The disturbance is more severe as the hepatic function deteriorates.

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LACTICID VESICLES VANCOMYCIN IN THE TREATMENT OF ACUTE HEPATIC ENCEPHALOPATHY: A DOUBLE BOUND, RANDOMIZED TRIAL P. Blanch (1), M. Coulazer, (1), P. Peray (2), J. Liardet (1) D. Lamery (1), H. Michel (1), Liver Unit (1) and Statistics Unit (2), Montpellier France

Lacticid vesicles is effective as lactulose in the treatment of hepatic encephalopathy (HE), but is better tolerated. Recently, vancomycin has been shown to be more efficient than lactulose in the treatment of chronic HE. To date, the efficacy of vancomycin in the treatment of acute HE remains unknown.

The aim of the work was to compare the efficiency of lacticid versus vancomycin in the treatment of acute HE.

Patients: 60 cirrhotic patients (40 men, 20 women, mean age 57 yrs) with acute HE were randomized blindly in two groups. The 29 patients of group I received 30 g lactid/day whereas the 31 patients of group II received 2 g vancomycin/day, for 5 days. The severity of HE was assessed before treatment, at 3rd and 5th day of treatment, according to Conn’s porto-systemic encephalopathy index.

Results: Both groups of patients were similar for age, sex, cause of cirrhosis and HE. Child-Pugh score and porto-systemic index. The course of HE was not statistically different in both groups, with the 5th day: in group i: 20 patients recovered, 2 remained unchanged, 1 worsened, 4 died. 2 were lost for the study; in group ii, 21 patients recovered, 3 remained unchanged, 1 worsened, 4 died. Conclusion: lacticid is as effective as vancomycin in the treatment of acute HE. But is less expensive. Lacticid should be preferred for the treatment of acute HE.
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PORTAL COLOPATHY
ENDOSCOPIC, HEMODYNAMIC AND HISTOMORPHOMETRIC STUDY

Although it is widely accepted that congestive gastropathy is a well recognized manifestation of portal hypertension, the influence of the latter on the lower gastrointestinal tract has not been adequately studied. Therefore, we evaluate prospectively, by total colonoscopy, 30 consecutive cirrhotic patients after their varices having been obliterated by endoscopic sclerotherapy. All of them were far from any bleeding episode and all had varying degrees of congestive gastropathy, identified by UG endoscopy at the time of the study. Additionally, colonic mucosal perfusion was assessed by means of laser-Doppler flowmetry at 4 defined points on the transverse colon and two mucosal biopsy specimens were obtained from the same area for histology and morphometric analysis of mucosal capillaries. Seven non cirrhotic patients who were subjected to colonoscopy for various reasons but were without findings, served as controls.

Twenty-eight patients (rate 93%) were found to have multiple vascular ectasias of different degrees of severity. These vascular-looking lesions were mainly located at the right colon and rectosigmoid. Less frequent lesions were non-specific mucosal edema, sparse mucosal veins and rectal varices. The statistical evaluation of mucosal perfusion revealed a highly significant decrease of blood flow in cirrhosis (p=0.0001). Morphometric analysis revealed a significantly higher mean number of capillaries (p=0.0012) and a higher mean cross-sectional vascular area per field in cirrhosis than in controls (p=0.005).

It is concluded that portal hypertension affects lower gastrointestinal tract giving an endoscopic, microcirculatory and histopathologic pattern quite similar to that of gastric mucosa.

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Liver cell proliferation is a complex process that can be affected by a large number of factors such as bile acids which have been associated to the etiology of liver cancer. Taurocholic acid (TC) has been reported to modify (methyl-CH2)3thymidine (T) incorporation into DNA by isolat in "silenced" perfused rat livers during regeneration that follows partial hepatectomy. In the present study we further investigate this point by comparing the effect of different bile acids (TC; dehydrocholic acid, DHC; taurodeoxycholic acid, TDC and ursocholic acid, UDC) on the hepatic uptake, metabolism, biliary excretion and incorporation into DNA of T. Two-thirds hepatectomy was carried out 24h before perfusion of the livers with a recirculating erythrocyte-free Krebs-Henseleit solution. Livers received bile acid infusion at 25 mmol/ml/kg liver. Trace amounts of T were added to the perfusate at min 30. At the end of the experiments (min 60) the livers were processed to determine radioactivity in whole tissue, in DNA and in non DNA-related fractions. TC and, to a lesser extent, TDC and DHC, but not UDC, were found to reduce 1C incorporation into DNA. This was not due to changes in the content of 1C in whole regenerating liver tissue. None bile acid assayed had effect on T uptake; moreover, the proportion of 1C found in bile was negligible. However, bile acid-induced modification in the fate of intracellular T was observed. In regenerating livers receiving no bile acid, the 1C carried by T metabolites accounted for ~10% of that found in whole liver tissue. TC markedly increased the incorporation up to 80%. Reversal phase HPLC revealed that most of this 1C (~80%) was released in the elution time corresponding to T metabolites rather than to DNA precursors. In summary, these results suggest that bile acids either induce an enhancement in T catabolism that reduces its incorporation into DNA and/or an inhibition in the process of DNA synthesis itself, leading to a subsequent increase in the metabolism of DNA precursors. Moreover, from the diversity in this property for bile acid species it might be inferred that changes in the composition and size of the bile acid pool during liver regeneration or even carcinogenesis may play a role in the modulation of proliferative processes.

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PROGNOSTIC VALUE OF AMINOACID BREATH TEST IN ALCOHOLIC LIVER CIRRHOSIS. COMPARISON WITH CHILD-TURCOTTE-PUGH SCORE. Y.Mits, E. Makhoul, D. Urbain and J.R. Ham. Dept. Gastroenterology and Nuclear Medicine, Saint-Pierre Hospital, Free University of Brussels, Belgium.

While the prognostic value of Aminobetaine Breath Test (ABT) in liver cirrhosis has been shown repeatedly, recent studies suggested that it was just equivalent to the simple clinical Child-Turcotte-Pugh (CTP) score. The use of ABT, which is a rather expensive procedure, for assessing prognosis of these patients is therefore questionable.

The aim of this study was to evaluate, in 190 cirrhotic patients, whether the ABT could improve four years survival prediction once patients have been stratified into the 3 classes provided by the CTP score. Among these patients, 113 died less than four years after the inclusion date. Complete follow up was available in the remaining 77 patients.

According to the CTP classification, patients were distributed as follows: class A: 35 patients, class B: 93 patients and class C: 42 patients. Log-rank test is indicative that the four year survival rate is significantly higher in group I compared to group II and III (p<0.01). No statistical difference was found between groups II and III. The visual comparison of the survival curves using ABT stratification and those using CTP stratification indicated that the prediction capability of the ABT was not superior than that of the CTP score.

As the individual result of the ABT was not perfectly correlated to the CTP score, the prognostic value of the ABT was re-examined once patients had been stratified according to the CTP score.

The results indicated that in CTP class B patients, further stratification using ABT results provided a better prognostic evaluation. The 4 years survival rate was better in group I (59 %) and II (50 %) than in group III (23 %). Log-rank test indicated that these differences were statistically significant (p<0.01 between groups I and II and p<0.05 between groups II and III). The limited number of patients in some of the Aminobetaine Breath Test groups within the C-T-P classes A and C did not allow statistical analysis.

In conclusion, our results suggest that once patients are stratified into the 3 classes defined by the CTP score, the ABT is useful for a further prognostic evaluation in patients with CTP class B in whom the prognostic is the most difficult to forecast.

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EXPRESSION OF INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) AND ICAM-2 IN LIVER DISEASE. A. Marui, M. Inoto, Y. Fukuda, Y. Koyama, I. Nakano, F. Urano, M. Kanda, K. Isobe, M. Katanos. Second Department of Internal Medicine, Nagoya University School of Medicine, Nagoya, Japan.

We have investigated the distribution of ICAM-1 and ICAM-2 in the liver by immunohistochemistry. Methods: 34 liver biopsy specimens were used for this study. Three cases with normal liver, and 18 with chronic hepatitis, 18 with liver cirrhosis and 6 with hepatocellular carcinoma. Three sections with normal liver were used for the control study. Frozen sections were applied to the immunoperoxidase procedure using mouse monoclonal anti-ICAM-1 (British Biotechnology) and anti-ICAM-2 (gift from Dr. Gahngborn). Results: ICAM-1. In normal liver, ICAM-1 was weakly expressed on the endothelial cells of portal vessels and in sinusoidal lining cells (SLC). No staining was found in the parenchyma. Enhanced ICAM-1 staining on SLC was found in chronic hepatitis. In the area of spotty necrosis, strong ICAM-1 staining was detected on the surface of the hepatocyte. In addition, ICAM-1 created a dendritic staining pattern in the lymph follicle of the portal tracts. In liver cirrhosis ICAM-1 was observed on SLC and on the surface of hepatocytes which constituted a bile duct pattern. ICAM-1 was expressed on carcinomatous sinusoidal endothelial cells and carcinoma cells in 4 of 6 carcinomas. ICAM-2. In normal liver, ICAM-2 was detected in Kupffer cells and a part of SLC. In chronic hepatitis, ICAM-2 was found in increased Kupffer cells and in SLC. ICAM-2 was stained in a dendritic pattern in lymph follicles of enlarged portal tracts. ICAM-2 expression in liver cirrhosis was similar to that in chronic hepatitis. ICAM-2 was not expressed on hepatocytes either in normal or chronic diseased livers. In hepatocellular carcinoma, ICAM-2 was detected on carcinomatous sinusoidal endothelial cells and carcinoma cells in 2 of 4 cases. Conclusions: These results indicate that the distribution of ICAM-1 and ICAM-2 in the liver is different though both are expressed, and that their expression is increased in the area of inflammation.