The acyl chain specificity of biliary phosphatidylcholine is independent from its biosynthetic origin

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change of operative strategy occurred. With respect to the standard of reference. the additional information from IOUS proved to be correct in 8/9 cases. The incorrect information concerned a patient with several dubious lesions of uncertain nature in addition to two colorectal hepatic metastases on CT. Since IOUS could not reproduce these uncertain lesions, the planned resection, i.e. right hemihepatectomy, was carried out. On histology both metastases were confirmed. However, a third metastases was found precisely where one of the dubious lesions had been suspected. Apart from additional information on intrahepatic anatomy, IOUS was documented to facilitate the resection by providing helpful three-dimensional insight in 12 patients.

In conclusion, IOUS does provide additional information over 3Ph-CT, leading to an altered operative strategy in ~15% of cases. Moreover, it is considered an invaluable tool in facilitating intrahepatic anatomical insight. Its routine use is, therefore, still advisable.

Interferon–Ribavirin therapy for chronic hepatitis C with and without cirrhosis: a meta-analysis of individual patient data

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To describe the results of Interferon–Ribavirin(IFN–Riba) combination therapy versus Interferon(IFN) monotherapy in various patient types of chronic hepatitis C (particular focus on cirrhosis), randomized controlled studies of patients with chronic hepatitis C utilizing an IFN–Riba combination arm were identified by screening the abstracts of major liver meetings in Europe till 1997. The principal investigators of each study (6) agreed to participate in combining the studies into one large database, known as the Eurohep (European Concerted Action on Antiviral Therapy of Hepatitis) IFN–Riba database. A total of 473 individual patient data were collected. A subset of 344 patients — of which 75 (22%) with cirrhosis — were included in this study; 197 received IFN + Riba (3MU 3×/week IFN + 1000–1200 mg/day Riba for 6 months) and 147 IFN monotherapy (3MU 3×/week for 6 months); excluded were patients receiving placebo or Riba monotherapy. Follow-up after therapy was 6 months. Primary outcome was sustained response defined as ALT normalization and HCV-RNA negativity 6 months after therapy. Multivariate logistic regression techniques were applied. Patients receiving IFN–Riba had a significant higher chance of response than those receiving IFN monotherapy (odds ratio IFN–Riba versus IFN: 5.8, 95% CI: 2.9–11.8); previous IFN non-responders had a significant lower chance of a sustained response ($P = 0.001$) than patients without previous therapy (naive patients), while patients with a relapse by a previous IFN therapy (relapsers) did not differ from naive patients ($P = 0.24$). Patients with a genotype 2 or 3 responded more frequently than patients with genotype 1 (odds ratio genotype 2&3 versus 1: 4.0, 95% CI: 2.0–7.9). The presence of cirrhosis was not a significant factor for patients with previous IFN therapy ($P = 0.26$ and 0.96 for previous non-responders and relapsers respectively), but tended to be so in naive patients ($P = 0.056$). The probability of a sustained response in naive patients without cirrhosis after IFN–Riba was about 33% (95% CI: 18%–51%) for genotype 1 and 65% (95% CI: 47%–79%) for genotype 2–3, rates two–three fold higher than those following IFN monotherapy. In naive patients with cirrhosis, a sustained response rate of 24% (95% CI: 4%–73%) can be expected for genotype 2–3, and 7% (95% CI: 1%–41%) for genotype 1, clearly higher than the response rates with IFN monotherapy. Responses to IFN–Riba combination therapy with cirrhosis now appear to be similar to those obtained with IFN monotherapy during 6 months in non-cirrhotic patients with hepatitis C. The outcome of this study suggest that clinically relevant response rates are obtainable with IFN–Riba combination therapy in cirrhotics, in particular for patients with genotype 2–3.

Upregulation of the multidrug resistance genes *mrp1* and *mdr1b* and downregulation of the organic anion transporter mrp2 and bile salt transporter spgp in endotoxemic rat liver

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Endotoxin-induced cholestasis is mainly caused by an impaired canalicular transport. Mrp2, the canalicular multispecific organic anion transporter, is strongly down-regulated in this situation and also canalicular bile salt secretion is reduced. We hypothesized that other adenosine triphosphate-binding cassette (ABC) transporters may compensate for the decreased transport activity to protect the cell from cytokine-induced oxidative damage.

Therefore, we examined the expression of ABC-transport proteins in membrane fractions of whole liver and of isolated hepatocytes of endotoxin-treated rats and performed RT-PCR on mRNA isolated from these livers. In addition, the localization of these transporters was examined using confocal scanning laser microscopy.

Already 6 h after endotoxin-administration, we found a clear increase of mrp1 mRNA and protein, whereas mrp2 mRNA and protein were decreased. This was confirmed in isolated hepatocytes. In addition, mdr1b mRNA was strongly increased, whereas mdr1a and mdr2 mRNA did not change significantly. Both the mRNA and protein levels of the sister of P-glycoprotein (spgp), the recently cloned bile salt transporter, decreased. After endotoxin-treatment, the normally sharply delineated canalicular staining of mrp2 and spgp had changed to a fuzzy pattern suggesting localization in a subapical compartment.

We conclude that cytokine-induced cholestasis is caused by decreased mrp2 and spgp levels as well as an abnormal localization of these proteins. The simultaneous upregulation of mrp1 and mdr1b may confer resistance to hepatocytes against cytokine-induced metabolic stress.

The acyl chain specificity of biliary phosphatidylcholine is independent from its biosynthetic origin

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Bile phospholipids are comprised mainly of phosphatidylcholines (PC), which themselves have a highly specific acyl chain composition: bile PC predominantly contains $C_{16:0}$ as acyl chain at the *sn1*-, and $C_{18:1}$, $C_{18:2}$ or $C_{20:4}$ as acyl chains at the *sn2*-position, which differs from cellular PC. The liver can synthesize PC *de novo* from choline via the CDP-choline pathway, and from phosphatidylethanolamine (PE) via the phosphatidylethanolamine *N*-methyltransferase (PEMT)-controlled pathway. It is not clear whether either of these biosynthetic routes preferentially contributes to biliary PC, or is responsible for the unique acyl chain specificity of bile PC. We investigated the capacity of the two PC biosynthetic pathways to provide PC for biliary secretion. We investigated bile composition in control mice and in mice in which the gene for PEMT was inactivated (*pemt* –/–), either on a choline-supplemented (control) diet or on a choline-deficient diet (causing strong inhibition of the CDP-choline pathway). The concentrations of bile salts, phospholipids and cholesterol in gallbladder bile of normal mice and PEMT-deficient mice fed a choline-supplemented diet were

similar. Dietary choline deficiency had no significant influence on bile acid concentration in the bile of normal or PEMT-deficient mice. Yet, the choline-deficient diet induced a reduction of phospholipid and cholesterol concentrations in gallbladder bile of normal mice (–32% and –39%, $P < 0.05$; respectively), and even a more pronounced reduction in *pemt* –/– mice (–42%, $P < 0.05$; and –71%, $P < 0.001$; respectively). In either of the four groups of mice studied, the major species of phospholipid found in gallbladder bile was PC, with $C_{16:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{20:4}$ as the predominant acyl chains. Mass spectrometric analysis of gallbladder bile revealed the predominance of PC species with one C16 and one C18 fatty acid in all four groups of mice.

Conclusions The results of this study unambiguously demonstrate that both PC biosynthetic pathways can supply the pool of PC secreted into bile. Even under the extreme metabolic pressure imposed by genetic PEMT deficiency and dietary choline deficiency. PC remains the dominant phospholipid in murine bile, while also its acyl chain specificity is maintained under these conditions.

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Platelets induce endothelial cell apoptosis upon reperfusion following cold ischemia in rat liver

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We have recently shown that sinusoidal endothelial cell apoptosis plays a pivotal role in hepatic ischemia-reperfusion (I/Rp) injury, only occurring after reperfusion. During liver transplantation up to 55% of circulating blood platelets are sequestrated in the graft upon reperfusion. Although some studies have shown that platelets may aggravate graft injury the underlying mechanisms remain unclear. Experiments were designed to study the role of platelets in I/Rp injury using an established isolated perfused rat liver (IPRL) system.

Livers were procured from male Wistar rats (175–250 g) and flushed with UW solution at 0–4°C. After cold storage for 24 h in UW solution the livers were reperfused for 1 h in a pressure controlled, oxygenized IPRL system. The system was Teflon® coated, preventing platelet adhesion and activation during 3 h in control experiments without a liver. The perfusate consisted of Krebs–Henseleit buffer with isolated red blood cells (hematocrit 8–10%), 2% bovine serum albumin and 1 g/l dextrose (control group, n = 4). In the experimental group ('platelet perfused', n = 4) unactivated purified platelets, obtained by a modified method of gel filtration of fractionated blood from 2 male Wistar rats, were added. Levels of aspartate amino transferase (AST) were determined in the perfusate and expressed as IU/liter/gram liver weight. Platelets counts were measured using an automated blood analyzer at 10 min intervals. After 1 h reperfusion tissue sections were stained by H&E and TUNEL, a marker for apoptosis. TUNEL positive cells were counted per high power field (hpf; 400×). Statistical significance was determined using the Mann–Whitney non-parametric test.

The AST levels in the platelet perfused group were higher compared to the control group at 1 h of reperfusion (mean 2.77, median 2.94 versus mean 1.97, median 2.16 IU/l/g, $P = 0.08$). Platelet counts dropped about 46% within the first 20 min of reperfusion. A significantly higher number of TUNEL positive endothelial cells was found in the platelet perfused group (mean 4.9, median 3.3/hpf), compared to the control group (mean 0.6, median 0.6/hpf; $P < 0.05$). No hepatocytes stained positive in either group.

Conclusions Platelets contribute to I/Rp of cold preserved rat liver. Platelets are shown to play a role in inducing endothelial cell apoptosis after reperfusion of the cold-stored ischemic rat liver.

Regulation of the heterogeneous expression of carbamoylphosphate synthetase in the liver

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Carbamoylphosphate synthetase (CPS), the first and flux-determining enzyme of the ornithine cycle, is highly expressed in the hepatocytes surrounding the afferent portal veins, whereas the protein is absent in the cells surrounding the efferent central veins. To understand the regulation of this expression gradient, the rat CPS gene and its regulatory elements have been characterized. A relatively simple 80 bp enhancer was found to confer hepatocyte-specific, periportal expression *in vitro* and *in vivo* (in transgenic mice) and to depend for its activity on the cooperative interaction of two hepatocyte-enriched transcription factors, *viz.* Hepatocyte Nuclear Factor (HNF3) and CCAAT/Enhancer-Binding Protein (C/EBP), and the ubiquitous Glucocorticoid Receptor (GR) only. Activated C/EBP and GR have been associated with histone acetylation, that is with weakening of the nucleosomal structure, while HNF3 has been implicated in nucleosome (re)positioning. These data imply that hormone-induced changes and regional differences in gene expression are associated with, or governed by local differences in chromosomal organization.

Although C/EBP, HNF3 and GR are shared among several periportally expressed genes, the gradient in cellular enzyme content along the porto-central radius is unique for each enzyme. We devised a model to account for these gradients of expression and their gene-dependent variation. In addition to a porto-central signal gradient, two gene-specific variables, *viz.* the affinity of the DNA-response elements for their corresponding transcription factors and the degree of cooperativity in their collective binding properties, were found to be important determinants. These parameters determine the occupancy of the DNA response elements, and thereby the expression level, along the porto-central radius. We postulate that glucocorticoids and glucagon account for the signal gradient, that the sequence variation of the DNA-response elements (resulting in differences in affinity for their corresponding transcription factors) determine the gene-specificity of the expression gradients, and that interactive binding of the transcription factors at the response unit induces cooperative binding properties. The relatively simple 80 bp enhancer of the CPS gene is ideally suited to test the relation between transcription factor binding and gene expression in the liver experimentally. To test the hypothesis, the effect of increasing or decreasing the affinity of each of the DNA-response elements on the occupation of the response unit by its transcription factors and on the level and distribution of reporter gene expression will be assessed in the liver of transgenic mice. Binding of GR, C/EBP and HNF3 to DNA can be assessed *in situ* by *in vivo* footprinting. The latter assay will be carried out in transgenic mice which also express Green Fluorescent Protein (GFP) and its yellow variant (YFP) in their periportal or pericentral hepatocytes, respectively. Nuclei from the respective hepatocyte populations can be isolated with an unprecedented degree of purity by Fluorescent-Activated Cell Sorting (FACS).

The isolation of regulators of early liver development utilizing an in vitro assay

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Liver organogenesis in the developing mammalian embryo starts with the formation of the liver diverticulum which arises as a regional thickening of the ventral foregut epithelium. Hereafter, endodermal cells from the liver diverticulum proliferate and migrate into the surrounding mesenchyme of the *septum transversum* where they differentiate into hepatocytes. This leads initially to the formation of