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Falk Workshop

THE GUT AND THE LIVER

Bonn (Germany)
January 28 – 29, 2010

Scientific Organization:
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P.A. Knolle, Bonn (Germany)
J. Nattermann, Bonn (Germany)
T. Sauerbruch, Bonn (Germany)
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Session I

Immune function
Lymphocyte homing to the gut and liver

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The liver and gut axis is central to our ability to absorb, process and metabolize nutrients, but it requires tight immunological control to prevent the induction of immune responses to harmless food antigens whilst maintaining vigorous and effective immunity to pathogens. Translocation of gut antigens to the portal circulation means that antigens that are not eliminated directly in the gut are brought to the liver, where further levels of immune regulation operate. This constant exposure to gut antigens might explain why the human liver contains more than $10^{10}$ lymphocytes including memory T cells with specificity for persistent viruses. It is possible that some memory lymphocytes that are activated in the gut recirculate through the liver to allow memory of mucosal antigens to be shared across both sites. Although there is currently no definitive evidence that mucosal memory T cells show enhanced trafficking through the normal liver there is evidence that under certain inflammatory conditions mucosal T cell are preferentially recruited to the liver. Such studies include graft versus host disease and our own studies of patients with hepatic complications of inflammatory bowel disease. In these latter patients mucosally activated $\alpha^4\beta^7^+CCR9^+$ T cells compromise 20% of the hepatic infiltrate. The existence of such shared pathways could help to explain the association between autoimmune liver disease and IBD. Lymphocytes that are destined to traffic to the gut are primed to mucosal antigens by DCs in mesenteric lymph nodes and Peyer’s Patches which have the unique ability to imprint responding T cells with the gut homing receptors CCR9 and $\alpha\beta^7$ allowing the lymphocyte to respond to the gut-restricted chemokine CCL25 and the $\alpha\beta^7$ ligand, Mucosal Addressin Cell Adhesion Molecule-1 (MAdCAM-1) restricted to mucosal vessels.

But what occurs if tissue specificity is lost and MAdCAM-1 and CCL25 are aberrantly expressed? In such a scenario mucosal lymphocytes could be recruited to extraintestinal sites causing inflammation outside the gut. Most extra-intestinal manifestations of IBD are driven by the inappropriate recruitment of mucosal effector lymphocytes to extra-intestinal tissues and resolve once the bowel inflammation is treated. However the autoimmune liver diseases autoimmune hepatitis and PSC develop when bowel inflammation is quiescent or after the bowel has been removed. We have shown that these diseases are associated with the aberrant expression of CCL25 and MAdCAM-1 on hepatic endothelium which results in the recruitment of $\alpha^4\beta^7^+CCR9^+$ mucosal T cells to the liver. Once recruited to the liver other chemokine receptors position the mucosal T cells at the biliary epithelium by allowing them to respond to chemokines secreted by bile ducts. Evidence that these T cells are of mucosal origin is provided by experiments in which we used DCs isolated from gut or normal and inflamed human liver to induce the gut homing phenotype in responding lymphocytes. We found that imprinting and plasticity of gut homing human CD8 T-cells requires primary activation or reactivation by gut DCs and is retinoic acid-dependent. The inability of liver DCs or hepatic stellate cells to efficiently imprint gut-tropicism suggests that liver-infiltrating $\alpha^4\beta^7^+CCR9^+$ T cells in PSC are primed in the gut.
This still leaves the unresolved question of what induces aberrant MADCAM-1 expression on hepatic endothelium in PSC? Liver endothelium expresses a cell surface enzyme, AOC-3 or vascular adhesion protein-1 (VAP-1), which is also an adhesion molecule. We have shown that activation of VAP-1 by substrates including methylamine, an aliphatic amine ingested from food and wine, drives NFkB-dependent activation of ICAM-1 and VCAM-1. In recent unpublished work we have shown that VAP-1 enzyme activity can synergise with proinflammatory signals to drive MADCAM-1 expression on liver endothelium. We detected low levels of MAdCAM-1 mRNA and protein in untreated primary human hepatic sinusoidal endothelial cells (HSEC) but levels were too low to support lymphocyte adhesion. However, treatment with the AOC-3 substrate methylamine and TNFα induced expression of functional MAdCAM-1 that supported lymphocyte adhesion under conditions of physiological flow. Blocking studies confirmed this adhesion was α4β7-dependent. Thus we propose that increased methylamine in the portal blood of patients with IBD together with proinflammatory cytokines might act as an environmental factor to drive hepatic MAdCAM-1 expression resulting in the recruitment of disease-associated mucosal T cells in PSC.

Thus we propose a novel model to explain the extra-intestinal complications of inflammatory bowel disease in which long-lived mucosal memory T cells undergo entero-hepatic recirculation between the liver and gut. Under normal conditions this will provide immune surveillance across both sites. However, aberrant expression of gut adhesion molecules in the liver as a consequence of bowel inflammation can amplify the recruitment of mucosal lymphocytes resulting in sustained hepatitis and liver injury. Although the circumstantial evidence for this model is strong it will be important to prove its relevance in vivo and we are currently establishing murine models to allow us to do this. In addition the development of effective CCR9 and α4β7 inhibitors will allow us to carry out proof of concept studies in patients with PSC.

References:


Septic immune paralysis – Cross-talk between gut, liver and spleen

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A well-known consequence of gram-negative sepsis is septic shock, a severe health condition, which is caused by recognition of bacteria by TLR4 and the uncontrolled production of pro-inflammatory cytokines. Much less is known about the role of gut derived gram-negative bacteria on the systemic immune response. Here we show that bacteria entering the peritoneum from the gut rapidly distribute systemically and that mice surviving septic shock or mice after clearing bacteria from the peritoneum are no longer able to mount an adaptive CD8 T cell response against viral or bacterial challenge infections. This suppression of adaptive immunity was dependent on bacteria reaching the spleen as mock treated control mice were not suppressed although these mice showed high titers of bacteria in liver and lung, but not in spleen. Interestingly, mice exhibiting bacterimia showed only suppression of systemic infections, but nearly normal adaptive immunity against local infections in the lung. This means that only bacteria reaching the spleen cause immune suppression and that immune suppression is not general but restricted to the systemic circulation. In the spleen suppression was caused by recognition of E. coli by TLR4, as only in the absence of TLR4, but not of TLR2 or 9 (also expressed by E. coli), suppression was prevented. Suppression was further dependent on the combined action of MyD88 and TRIF as immune suppression in single KO mice was dependent on the presence of either molecule. The importance of type I IFN was demonstrated by the dependence of suppression on IFNAR and IRF3 and/or 7.

As in the absence of TLR4, MyD88/TRIF, IRF3/7 and IFNAR normal adaptive immunity could be induced, our results suggest that knocking out the TLR system (e.g. siRNA against TLR4, MyD88 and TRIF) for a short period (during surgery) could prevent severe pathology, i.e. septic shock and septic immune paralysis.
Plasticity of dendritic cell function – Influence of gut-derived products

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Hepatic DC subsets are constantly exposed to microbial products that induce unresponsiveness to LPS (endotoxin tolerance) and other TLR ligands. At the molecular level, commensal bacterial products appear to stimulate expression of IRAK-M (IL-1R-associated kinase), a negative regulator of TLR signaling, that may underlie the refractory state of liver DC. DNAX-Activating Protein of 12KDa (DAP12) is a homodimeric Immunoreceptor Tyrosine-based Activation Motif (ITAM)-bearing transmembrane adaptor protein, that integrates signals through regulatory receptors, including Triggering Receptor Expressed on Myeloid Cells (TREM)-1 and -2 in NK and myeloid cells, including DC and macrophages. Liver myeloid DC (mDC) express DAP12, and loss of DAP12 enhances their phenotypic and functional maturation, increasing their T cell stimulatory capacity and diminishing IL-10 secretion in MLR. Loss of DAP12 also correlates with increased basal levels of STAT3 phosphorylation and diminished IRAK-M expression in liver mDC, resulting in loss of endotoxin tolerance. Agonistic anti-TREM-1 mAb decreases IL-6, but enhances IL-10 production by liver mDC. These data highlight, for the first time, a regulatory role for DAP12 in hepatic DC function.

Nucleotide-binding Oligomerization Domain (NOD)2/CARD15 protein, that senses muramyl dipeptide (MDP), a product of bacterial peptidoglycan, appears to play an important role in regulating intestinal immunity. Although the liver is exposed to gut-derived MDP, the influence of NOD2 ligation on hepatic DC is unknown. Freshly-isolated mouse liver and spleen plasmacytoid DC (pDC) expressed higher levels of NOD2 message than conventional mDC. Following MDP stimulation in vivo, liver pDC, but not mDC, upregulated expression of IFN Regulatory Factor 4 (IRF4), a negative regulator of TLR signaling, and induced less allogeneic T cell proliferation and IFNγ production. Their adoptive transfer failed to prime allogeneic T cells. By contrast, splenic DC IRF4 levels and T cell stimulatory activity remained unchanged. Liver pDC from MDP-stimulated mice also displayed greater IκBα, cell surface B7-H1, and B7-H1 relative to CD86 than control liver pDC. No similar effects were observed for liver mDC or spleen DC. B7-H1−/− liver pDC reversed the inhibitory effect of MDP. After ex vivo stimulation with LPS or CpG, liver pDC but not mDC from MDP-treated animals secreted less IL-12p70, IL-6 and TNFα, and induced weaker allogeneic T cell proliferation than those from controls. Moreover, CpG-stimulated liver pDC from MDP-treated mice secreted less IFNα than splenic pDC. These findings suggest that differential effects of NOD2 ligation on liver pDC may play a role in regulating hepatic innate and adaptive immunity.
Session II

Biliary system
Bile acids as mediators of the gut-liver interaction: Role of nuclear receptor

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Cholestasis is a liver disorder characterized by impairment of bile flow with consequent hepatic retention of toxic bile acids. The absence or reduction of bile acid flow in the gut impairs the intestinal epithelial function and barrier with bacterial overgrowth and translocation that can result in the vicious cycle of systemic infection/inflammation. Thus, therapies aiming at reducing hepatic bile acid overload and restoring intestinal mucosa fitness are required. In this context, the nuclear bile acid receptor (farnesoid X receptor, FXR:NRH4), the master regulator of BA homeostasis that is expressed in the enterohepatic system, is a suitable target. Activation of FXR by bile acids in the gut-liver axis reduces their levels in a feedback way by repressing the expression of cholesterol 7α-hydroxylase, the rate-limiting enzyme in the conversion of cholesterol to bile acids, via a synergistic mechanism involving the hepatic orphan nuclear receptor small heterodimer partner (SHP:NR0B2) and the intestinal hormone fibroblast growth factor 15/19 (Fgf15/FGF19). Although systemic activation of FXR has already been shown to protect, to some extent, against cholestasis, these studies focused exclusively on FXR in the liver. We thus sought to gain further mechanic insights on the role of intestinal FXR in cholestasis. To this end, we first generated a transgenic mouse model expressing a constitutively active form of FXR only in the intestine (iVP16FXR) under the control of the villin promoter. Then, we challenged iVP16FXR mice with different models of cholestasis (obstructive via bile duct ligation; intrahepatic via ANIT administration; genetic via bred-cross with MDR2KO:PFIC3 mice). Using gain and loss of function in vivo models, we show that selective activation of intestinal FXR is sufficient to protect against cholestasis by reducing size and hydrophobic index of the total bile acid pool via Fgf15. Finally, activation of FXR in the intestine also protects from intestinal bacterial overgrowth and translocation across the mucosal barrier. Thus, strategies aiming at intestinal FXR activation and FGF19 secretion might bona fide represent the future pharmacological therapy of cholestatic disorders.
Is FGF19 signaling from gut to liver disturbed in NAFLD?

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Fibroblast growth factor 19 is a secretory protein that is produced in the terminal ileum upon activation of the farnesoid X-receptor (FXR) by bile acids. Via the portal blood FGF 19 reaches the liver where it binds to its receptor FGFR4. FGFR4 is a receptor tyrosine kinase that upon activation decreases hepatic forkhead transcription factor 1 (FoxO1) activity through phosphatidylinositol (PI) 3-kinase-dependent phosphorylation (Shin and Osborne. J Biol Chem. 2009; 284: 11110–11120). The decrease of FoxO1 leads to a suppression of CYP7A1, the rate determining enzyme in bile acid biosynthesis. This may be independent of the small heterodimer partner SHP (Kong et al. AASLD 2009 abstract #107), although this issue has not been fully resolved yet. However, other pathways may be involved in the signaling cascade of FGF19. We have shown that only the fully glycosylated form of FGFR4 is active in transducing the signal from FGF19, showing that glycosylation of the receptor is partly responsible for efficient signalling. β-Klotho binds the inactive core glycosylated form that chaperones the receptor to be degraded by the proteasome. On the other hand, expression of hepatic FGFR4 is reduced during fasting and increased by insulin. This indicates that FGF19/FGFR4 plays a more general role in liver metabolism.

It has now been firmly established that FGF19 not only affects bile acid synthesis but also has effects on lipid and carbohydrate metabolism. FGF 19 inhibits insulin-stimulated fatty acid synthesis (Bhatnagar et al. J Biol Chem. 2009; 284: 10023–10330). It does this by suppressing insulin stimulated expression of fatty acid synthase, sterol regulatory element binding protein SREBP-1c and acetyl coA carboxylase thereby inhibiting lipid synthesis and activating fatty acid oxidation. In addition, FGF19 also inhibits phosphoenolpyruvate-carboxykinase an enzyme that controls gluconeogenesis in the fasted state. Via these actions of FGF19 the entry of food in the gut causes a signal to the liver to stop producing bile acids, lipids and carbohydrates.

The question arose if this system might be disturbed in non-alcoholic fatty liver. In this condition the liver keeps producing fat despite excessive intake. We undertook a study in human volunteers and patients with NAFLD wherein we gave these subjects a fatty test meal and studied its effect on FGF 19 production and bile acid synthesis. In normal volunteers FGF 19 in the blood peaks at 3-4 hours after a meal. Patients with NAFLD show the same response. Serum levels of 7a-hydroxy-4-cholesten-3-one, the first committed metabolite in the conversion of cholesterol to bile acids, is appropriately decreased in “insulin sensitive” but not in “insulin resistant” NAFLD patients. This suggests a degree of FGF19 resistance in insulin resistant NAFLD patients. This was further investigated in HEPG2 cells. Incubating HEPG2 cells with human recombinant FGF19 results in the down regulation of CYP7A1 mRNA and protein. However after loading HEPG2 cells with palmitate such a reduction was abrogated. This indeed suggests that in steatosis hepatocytes display FGF19 resistance. At the same time, palmitate causes ER stress (Akazawa et al. AASLD 2009 abstract #148 and Malhi and Kaufman, AASLD 2009 abstract #1883) that could lead to an altered or hindered glycosylation of FGFR4, leading also to FGF19 resistance under similar conditions.
In conclusion our results show that in NAFLD, signalling from gut to liver may be disturbed by FGF19 resistance. This may explain the unabated hepatic fat synthesis despite excessive intake.
The role of the intestine in the formation and prevention of gallstones

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For decades, cholelithiasis has been regarded as hepatobiliary disease, with hepatic hypersecretion of cholesterol or bilirubin and consecutive gallbladder inflammation representing the key underlying defects. During the past years, a third organ has been proposed as an important player in gallstone pathogenesis, the intestine (van Erpecum & van Berge-Henegouwen 1999). The intestine may affect (i) gallbladder motility as well as (ii) absorption, (iii) enterohepatic circulation and (iv) hepatic metabolism of biliary lipids.

Gallstone patients display disordered migrating motor complexes in the fasting state, dissociated gallbladder contraction, and abnormal release of the hormone motilin in the interdigestive state (Stolk et al. 2001). Prolonged intestinal transit could increase gallstone risk by enhancing formation deoxycholate, and data from patients with gallstones, acromegalics on octreotide and animal models suggest that deoxycholate increases biliary cholesterol secretion and destabilizes cholesterol-rich vesicles in bile (Shoda et al. 1995, Berr et al. 1996). Recently, cholecystokinin and fibroblast growth factor 19 (FGF-19) have been shown to control gallbladder contraction and filling, respectively (Choi et al. 2006). The release of FGF-19 from ileal enterocytes is regulated by bile salts via the nuclear receptor 1H4 (FXR), and upon binding to FGFR4, FGF-19 suppresses hepatic bile salt synthesis. However, systematic analysis does not support a major role of genetic variation of genes controlling gallbladder motility in cholelithiasis.

In contrast, a common genetic variant of the intestinal and hepatobiliary cholesterol efflux pump \textit{ABCG5/G8} has been identified as genetic risk factor for gallstones in genomewide association and family studies, consistent with inbred mouse studies (Wittenburg & Lammert 2007). Rare variants of the Niemann-Pick C1 like 1 (NPC1L1) could also affect intestinal cholesterol absorption. Whereas mouse models point to a role of cholesterol hyperabsorption in gallstone formation, subgroups of gallstone patients might display high and low cholesterol absorption (Lammert & Wang 2005).

Cholesterol gallstone formation might be prevented by lifestyle changes, in particular by reducing total caloric intake, but randomized controlled trials are yet to be performed. For specific conditions that are associated with an increased gallstone risk such as rapid weight loss, octreotide treatment or total parenteral nutrition, gallstones can be prevented efficiently by ursodeoxycholic acid or early enteral nutrition, respectively. Administration of cholecystokinin or motilin agonists such as erythromycin might also have protective effects in the latter setting. An exciting new concept in the prevention of gallstone formation is the stimulation of nuclear receptors that regulate the metabolism of biliary lipids, as shown by the efficient prevention with synthetic FXR agonists in mouse models (Moschetta et al. 2004). Since distal intestinal infection with a variety of enterohepatic Helicobacter species is essential to nucleate cholesterol supersaturated bile in the inbred mouse model.
(Maurer et al. 2009), further studies should investigate whether chronic inflammation in the intestine has a direct pathogenic role in cholesterol gallstone disease.

Bile salt loss increases the risk of cholesterol gallstones, but if severe, induces colonic absorption and enterohepatic cycling of unconjugated bilirubin (Brink et al. 1999). This mechanism contributes to the increased gallstone prevalence in patients with Crohn disease and cystic fibrosis (Wasmuth et al. 2006). Attempts to decrease the enterohepatic circulation of bilirubin included the administration of agents that trap unconjugated bilirubin in the intestine by absorption to non-absorbable solids, or by forming insoluble salts with calcium or zinc. Recently, the lipase inhibitor orlistat has been demonstrated to enhance fecal bilirubin excretion and to decrease serum bilirubin level (Hafkamp et al. 2007), but its stone protective effects have yet to be studied.

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Inflammatory bowel disease (IBD) is associated with a number of hepatobiliary diseases, among which steatosis, drug-induced liver injury, primary sclerosing cholangitis (PSC), and autoimmune hepatitis (AIH). PSC is the only one of these disorders which primarily affects the intra- and extrahepatic bile ducts. It is a chronic fibrosing cholangitis, which is slowly progressive without treatment leading to development of biliary fibrosis and cirrhosis (1, 2). PSC has a prevalence of 9–14/100,000 in Northern Europe and the U.S. The typical patient is a young man (70%) at an age of 25–40 years at the time of diagnosis, but children, adolescents and elderly adults can also be affected. More than 70% of PSC patients have an IBD, clearly more often (4:1) an ulcerative colitis (UC) than Crohn’s disease (CD), and about 5% of patients with UC suffer from PSC. Patients with PSC develop without treatment after 10–18 years complications of cirrhosis (1, 2). A cholangiocarcinoma is observed in 8–13% of PSC patients during long-term follow-up (3) and gallbladder carcinoma in about 2%. The risk to develop colon carcinoma is higher (OR > 4) in patients with PSC and UC than in patients with UC only (4).

The etiopathogenesis of PSC is unclear (1, 2). Genetic factors play an important role as documented by a higher risk in families of PSC patients to develop PSC. Enhanced intestinal mucosal permeability with invasion of bacterial products into the portal circulation and stimulation of cholangiocytes to secrete cyto- and chemokines are held responsible to contribute to PSC (1, 2). Aberrant homing of intestinal mucosal T cells in the liver caused by aberrant expression of gut adhesion molecules and chemokines by liver sinusoidal endothelial cells may also play an important role (5). However, none of these hypotheses has been convincingly proven so far.

The diagnosis of PSC is made when serum markers of cholestasis (alkaline phosphatase, gamma GT) are elevated and characteristic stenoses and dilatations of intra and/or extrahepatic bile ducts is demonstrated by magnetic resonance cholangiography of high quality (MRC) or endoscopic retrograde cholangiography (ERC), particularly in patients with IBD (1). Liver biopsy often shows noncharacteristic inflammation of portal fields and portal fibrosis, but sometimes characteristic onionskin-like sclerosis around the bile ducts. The histological stages of PSC are [1] portal inflammation, [2] portal and periportal inflammation, [3] septal fibrosis, and [4] cirrhosis (1, 2).

Treatment of PSC includes medical, endoscopic and surgical approaches. Treatment with ursodeoxycholic acid (UDCA; 13–20 mg/kg/day) improves serum liver tests and prognostic surrogate markers as shown by randomized, placebo-controlled studies (6-11). It is still unclear whether UDCA improves the long-term prognosis of patients with PSC (1, 10). Pilot studies showed higher effectiveness of higher doses (≥ 20 mg/kg/d) (10), but at least late-stage patients develop more complications when high doses of 30 mg/kg/d were administered over a period of 5 years in a recent randomized, placebo-controlled trial (12). Thus, UDCA should be administered at doses not higher than 15–20 mg/kg/d in PSC (1). UDCA improves impaired secretory function of hepatocytes and cholangiocytes, exerts antiapoptotic effects and detoxifies bile (13). UDCA might beneficially affect development of cholangio
carcinoma (14) and dysplasia/carcinoma of the large intestine in patients with PSC and CU (15), but further data are needed to confirm these preliminary findings. Immunosuppressive treatment of PSC has shown disappointing results (1). Only young patients with a histology-proven overlap syndrome of PSC and AIH and patients with IgG4-associated cholangitis (IAC), an important differential diagnosis of PSC mainly in male patients 60 years or older, seem to benefit from immunosuppressive therapy.

In patients with dominant strictures of the extrahepatic bile ducts, endoscopic dilatation therapy under antibiotic treatment should be considered (balloon dilatation, transient short-term stenting) (16, 17).

Liver transplantation is the only effective treatment of advanced PSC and should be considered in patients with decompensated cirrhosis, but also those in an advanced stage who suffer from recurrent bacterial cholangitis or extensive dominant strictures of the bile ducts. One- and 5-year survival after liver transplantation are above 85–90% in many large centers (18).

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Session III

Metabolism (1)
Bile acid signaling and control of metabolism

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Bile acids are natural detergents, facilitating the intestinal absorption of dietary lipids and fat-soluble vitamins. In addition to this function, bile acids have emerged as important signaling molecules that can coordinate diverse metabolic pathways. A number of these bile acid-mediated effects involve the activation of the nuclear hormone receptor farnesoid X receptor (FXR) and include the transcriptional control of the synthesis and recycling of bile acids as well as the regulation of hepatic lipid and glucose production. Recently, we found that bile acids can also exert metabolic effects via an FXR independent pathway thereby protecting mice against diet-induced obesity. Bile acid-mediated increase in energy expenditure was shown to be mediated by the membrane receptor TGR5, also known as GPR131, and involves the induction of the thyroid hormone activating enzyme deiodinase 2. We also provide evidence that bile acids improve glucose tolerance through the stimulation of the release of the incretin, glucagon like peptide 1, from enteroendocrine L cells. Together our data suggest that bile acids are important signaling molecules, with activities, which clearly extend beyond nuclear receptor activation and involve activation of the GPCR, TGR5.
The intestine and fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) is recognized as a clinicopathological condition of emerging importance. In the majority of cases NAFLD is associated with obesity, type 2 diabetes, dyslipidaemia or hypertension, e.g. conditions, which have insulin resistance as the common factor and cluster to form the metabolic syndrome. There is emerging evidence that the liver is not only a "target" of the metabolic syndrome but NAFLD directly promotes insulin resistance, and herewith, affects other organs. Conversely, one has to consider, that in addition to the liver the metabolic syndrome pathophysiologically affects several other organs, and herewith, may additionally induce and promote hepatic injury. The gut-liver interaction appears as an important example of such a mutual pathophysiological interaction of two organs, which in summary promotes hepatic injury.

Thus, similarly as in alcoholic steatohepatitis, products of intestinal bacteria, particularly LPS, have been identified to promote disease progression from simple steatosis to non-alcoholic steatohepatitis (NASH). Noteworthy, diabetic subjects reveal intestinal bacterial overgrowth, and genetically obese mice display enhanced intestinal permeability and increased portal endotoxemia. Combining experimental models of NASH and chronic intestinal inflammation we observed enhanced hepatic inflammation and fibrosis in mice. Further, we observed that the intestinal barrier is additionally impaired by a NASH inducing diet in mice. Together, these findings suggest that also in obese and/or diabetic patients increased intestinal permeability is a critical factor that contributes to hepatic inflammation and fibrosis. Herewith, the bacterial flora and an intact intestinal barrier appear as promising therapeutic targets to inhibit the development and progression of NASH.
Gut barrier dysfunction and alcoholic liver disease

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A significant body of evidence indicates that endotoxemia and endotoxin-mediated cell injury play a crucial role in the pathogenesis of alcoholic liver disease. Disruption of intestinal epithelial barrier function and increased intestinal permeability to luminal bacterial toxins are the main cause of endotoxemia in alcoholic liver disease. Increased intestinal permeability to macromolecules has been demonstrated in patients with alcoholic liver disease as well as in experimental models of alcoholic liver damage in rats and mice. The mechanism of ethanol-induced disruption of intestinal epithelial barrier function is an emerging area of interest in the studies toward understanding the pathogenesis of alcoholic liver disease. Recent evidence indicates that intestinal microflora and metabolism of ethanol into acetaldehyde are essential factors involved in the mechanism of ethanol-induced intestinal barrier dysfunction. Therefore, it is crucial to understand the mechanisms involved in ethanol-induced barrier dysfunction to seek insight into the pathogenesis of alcoholic liver disease.

Our recent studies showed that acute or chronic ethanol administration leads to a disruption of epithelial tight junctions and increase in paracellular permeability in colon. Acute ethanol (0.1–0.5%) administration to isolated intestinal loops dose-dependently increased inulin permeability in the colon, but not in the ileum. Chronic ethanol (5% in Lieber Dicarli liquid diet) feeding for 4 weeks resulted in a disruption of epithelial tight junctions and adherens junctions in the distal colon, but not in the proximal colon or ileum. Disruption of tight junction in colon was associated with a redistribution of occludin, ZO-1, E-cadherin and β-catenin from the intercellular junctions and reduction in the levels of these proteins. Ethanol feeding also increased the tyrosine-phosphorylation of claudin-3 in distal colon, but not in ileum or proximal colon.

To understand the mechanisms associated with ethanol-induced tight junction disruption we conducted studies in Caco-2 cell monolayers, a cell culture model of the intestinal epithelium. Ethanol up to 1.0% failed to affect the tight junctions and paracellular permeability in Caco-2 cell monolayers. However, acetaldehyde, the metabolic product of ethanol, effectively disrupted tight junctions and increased paracellular permeability by a phosphorylation and protein phosphatase 2A (PP2A)-dependent mechanism. Expression of alcohol dehydrogenase 1B (ADH1B) primed the Caco-2 cell monolayers for ethanol-induced barrier dysfunction, while the over expression of aldehyde dehydrogenase 2 (ALDH2) attenuated acetaldehyde-induced barrier disruption. Ethanol dose-dependently potentiated the effect of acetaldehyde on the tight junction disruption by a Src kinase and myosin light chain kinase (MLCK)-dependent mechanism. Inhibition of Src kinase and MLCK attenuates the ethanol-mediated sensitization of acetaldehyde-induced tight junction disruption. Disruption of tight junctions by acetaldehyde is associated with a Thr-dephosphorylation of occludin and Claudin-4 by a protein phosphatase 2A (PP2A)-dependent mechanism. Our recent study showed that phosphorylation of occludin on...
Thr-403 and Thr-404 facilitates its assembly into tight junctions. The expression of T403/404D mutant occludin (mimics phosphorylated occludin) significantly attenuated acetaldehyde-induced disruption of tight junctions and barrier dysfunction.

In summary, ethanol induces gut barrier dysfunction predominantly in the distal colon in mice by disrupting the tight junctions and adherens junctions. Ethanol and acetaldehyde synergistically disrupts tight junctions by activating distinct intracellular signaling pathways. PP2A and dephosphorylation of occludin on specific threonine residues plays a role in acetaldehyde-induced tight junction disruption, while Src kinase and MLCK activations are involved in ethanol-induced sensitization of barrier disruption by acetaldehyde.
Session IV

Metabolism (2)
Drug metabolism: The gut and the liver

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The routes of elimination for the 200 drugs most often prescribed ("top 200") in the United States according to the RxList (http://www.rxlist.com) indicate that the vast majority were found to be subject to hepatic elimination by either metabolism or excretion into bile. Metabolism via cytochrome P450 (CYP)-mediated reactions represented the absolute majority of hepatic biotransformation processes. Members of the CYP3A family contributed to the metabolism of 37% of the drugs, followed by CYP2C9 (17%), CYP2D6 (15%), CYP2C19 (10%), CYP1A2 (9%), and CYP2C8 (6%). CYP2B6 and other CYP isoforms (CYP2A6 and CYP2E1) participated in the metabolism of 4% and 2% of the drugs, respectively. Isozymes of families CYP1, CYP2 and CYP3 are collectively responsible for most phase I biotransformations of drugs and other xenobiotics in human liver. In contrast to CYPs of families CYP4 to CYP51, which are involved in endogenous metabolic pathways of steroids, fatty acids, prostaglandins, etc., the CYP1, 2 and 3 isozymes have broad and overlapping substrate specificities which usually provide for a robust elimination of lipophilic xenobiotics.

The extremely variable expressions and functions of CYP isozymes, typically exceeding 100-fold in a large population sample, lead to unforeseen drug responses, including over-reaction, toxicity, or lack of response in a considerable fraction of treated patients. The major sources of interindividual and intraindividual variability in CYP activity are environmental influences, including inhibition or induction by concomitant medications (drug-drug interactions, DDI), biological factors including sex and physiological determinants, such as hormonal status, disease, and circadian rhythms, and genetic polymorphisms in CYP genes and their regulators. There are large differences between the individual CYP isoforms regarding their susceptibility to these mechanisms and their expression in human liver and/or gut. CYP1 family enzymes are commonly inducible by polycyclic aromatic hydrocarbons through the Ah-receptor/ARNT pathway, and those of families 2 and 3 are generally inducible, but to various extents, by a diverse class of structurally unrelated xenobiotics which are usually ligands of the orphan nuclear receptors pregnane X receptor (PXR) and constitutively active receptor (CAR). Sex differences in hepatic CYP expression are a controversial but intensely debated topic, and some CYPs, in particular CYP3A4, show significant sex differences in humans. The impact of disease has been particularly well investigated in the context of inflammation, which generally leads to transcriptional repression that affects some isoforms more than others. The most clinically well-established CYP polymorphisms of CYP2C9, CYP2C19, and CYP2D6 are involved in approximately half of these top 200 drugs. In particular, these include NSAIDs metabolized mainly by CYP2C9, proton-pump inhibitors metabolized by CYP2C19, and anti-cancer drugs (e.g., tamoxifen), beta blockers and several antipsychotics and antidepressants metabolized by CYP2D6.
Moreover several studies have shown that drug metabolism in the gut wall substantially contributes to the overall first-pass metabolism of a large number of therapeutic drugs. In particular, CYP 3A4-mediated biotransformation in enterocytes from human small intestine has been extensively characterized and seems to play a major role in the variable and low oral bioavailability of many CYP3A4 substrates. Apart from intestinal and hepatic first-pass metabolism, incomplete oral absorption may also reduce drug bioavailability. It has been suggested that the function of CYP3A4 and the efflux transporter P-glycoprotein in enterocytes is complementary, forming a coordinated intestinal absorption barrier against xenobiotics. For instance there are data available indicating that neither duodenal CYP3A4 protein content nor catalytic activity correlated with hepatic CYP3A4 activity (as measured by the erythromycin breath test), suggesting that CYP3A4 is not coordinately regulated in these tissues. The content of CYP3A4 protein is about 3 times and that of P-glycoprotein is about 7 times higher in enterocyte specimens than in the liver specimens. No intraindividual correlations between the intestine and liver with respect to CYP3A4 protein content or CYP3A4-dependent catalytic activities (verapamil N-dealkylation and N-demethylation) were reported. Likewise, there is no relationship between intestinal and hepatic P-glycoprotein expression. Many DDI involving induction or inhibition of CYP enzymes, in particular CYP3A, have been proposed to occur substantially at the level of the intestine rather than exclusively within the liver, as originally thought. CYP3A and CYP2C represent the major intestinal CYPs, of total immunoquantified CYPs, but CYP2J2 is also consistently expressed in the human intestine. An overwhelming proportion of clinically relevant DDI where the intestine has been suggested as a major contributor to first-pass metabolism involve drugs that undergo CYP-mediated biotransformation. In summary the first-pass contribution of the intestine and liver should be successfully decoupled.
Regulatory mechanisms involved in HFE hereditary hemochromatosis

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As an essential nutrient and a potential toxin, iron poses an exquisite regulatory problem in biology and medicine. Disturbances of the delicate balancing systems for systemic and/or local iron homeostasis are emerging as underlying causes of common hematological, metabolic and neurodegenerative diseases. Our research aims to understand the physiological regulation of genes involved in iron metabolism and its disturbances in human disease.

One major research focus of the lab is to understand molecular mechanisms involved in hereditary hemochromatosis (HH), the most prevalent genetic disorder in the western world. The disease is mainly caused by mutations in the HFE gene, which codes for a MHC class I like molecule. Work from our lab and others demonstrated that HFE is required for appropriate hepatic expression of the iron hormone and antimicrobial peptide hepcidin: expression of this negative regulator of duodenal iron absorption is decreased and cannot be adjusted in response to elevated hepatic iron levels in Hfe-deficient mice and HH patients. These findings further our understanding of the molecular mechanism of Hfe function and suggest that the primary locus of Hfe function is the liver and not the duodenum, as was previously hypothesized. Indeed, analysis of tissue specific Hfe knock-out mice has recently unambiguously demonstrated that local Hfe expression in hepatocytes serves to maintain physiological iron homeostasis, answering this longstanding question in medicine. Additionally, HFE controls hepcidin expression in response to inflammatory stimuli. This links HFE to the immune system and to the anemia of chronic diseases (ACD), which results in iron redistribution in response to inflammation, infection and malignancy.

An important challenge is now to understand how signalling pathways in general and the hemochromatosis-associated proteins, specifically, regulate hepcidin expression. To identify cis-acting elements and trans-acting factors for hepcidin expression under steady state conditions and in response to experimental (e.g. inflammatory) stimuli, we established a cell-based assay system and luciferase reporter vectors containing the human hepcidin promoter. Applying these tools we were able to show that a STAT-binding motif at position -64/-72 of the hepcidin promoter and STAT-3 are critical determinants for the control of hepcidin mRNA expression both under inflammatory and steady state conditions. In addition we uncovered two distinct, multi-functional BMP-responsive elements (BMP-RE) that control hepcidin promoter activity in response to the HH proteins as well as its inflammatory response to IL-6. These data uncover a point of cross-talk for hepcidin regulation by HH proteins and inflammatory stimuli.
We are currently moving towards network/systems-based analysis of iron metabolism by integrating DNA microarray approaches, mouse models and high through-put siRNA screens. The overall aim is a more detailed understanding of regulatory mechanisms involved in iron homeostasis and the identification of novel regulators of iron metabolism.
Gut-liver-brain axis: Consequences of liver dysfunction

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Multiple interactions between gut, liver and the brain exist. One example is the regulation of hepatic glucose production by upper intestinal lipids, which involves vagal afferent and efferent nerves (Wang et. al. Nature 2008; 452: 1012–1016). Another example is the hepatorenal reflex, which is activated by the portal amino acid load, which triggers a decrease in glomerular filtration through activation of hepatic vagal afferences and sympathetic efferences to the kidney (Lang et al. Hepatology 1991; 14: 590–590). This latter reflex was considered to play a role in the development of kidney dysfunction in liver disease. The gut-liver-brain axis is involved in the pathogenesis or hepatic encephalopathy in liver disease. Under these conditions gut-derived ammonia is not sufficiently cleared by the liver due to a derangement of a sophisticated structural and functional organization of ammonia and glutamine metabolism in the liver acinus and reaches the brain. Here, ammonia induces astrocyte swelling and triggers an oxidative/nitrosative stress response through a NMDA receptor and Ca^{2+}-dependent activation of NADPH oxidase isoenzymes and neuronal NOS. Ammonia and swelling induced NMDA receptor activation involves induction of glutamate exocytosis through activation of an indometacin-sensitive and prostanoid-dependent Ca^{2+} signalling pathway. There is a positive regulatory feed forward loop between astrocyte swelling and oxidative stress (Häussinger & Schliess. Gut. 2008; 57: 1156–1165). On the one hand swelling induces oxidative stress and on the other oxidative stress triggers astrocyte swelling. Functional consequences of ammonia- and swelling-induced oxidative/nitrosative stress are the induction of protein tyrosine nitration (PTN), activation of transcription factors and the oxidation of RNA in neurons (Görg et al. Hepatology. 2008; 48: 567–579). Increased levels of PTN and RNA oxidation were also found in post mortem human brain from HE-patients. In vitro, ammonia-induced oxidative stress activates the transcription factor SP-1 in astrocytes in a Zn^{2+}-dependent way, thereby augmenting the expression of the peripheral benzodiazepine receptor. Ammonia-induced oxidative stress also induces RNA oxidation in neurons. Such oxidized RNA species are found in the Nova-2 positive RNA-containing granules along the dendrites, which may be used for local synaptic protein synthesis. As a consequence one may speculate about an ammonia-induced impairment of local synaptic protein synthesis, which is known to play a role in memory formation, thereby providing a potential link between oxidative stress and cognitive defects in HE. Disturbances of synaptic plasticity may underly the disturbances of oscillatory networks in the brain, which account for HE symptoms.
Session V

The gut as entry site for microorganisms
Cirrhosis leads to a disruption of the functional layers of the gut mucosal barrier. Patients and experimental models of cirrhosis with ascites show increased passage of enteric bacterial products to the systemic circulation, whether detected through an increase in lipopolysaccharide (LPS) binding protein (LBP) or bacterial DNA fragments in serum, or culture or bacterial DNA in mesenteric lymph nodes. The immediate consequence of repeated passage of enteric bacterial products to the circulation is an increased risk of spontaneous bacterial infection. Indeed, patients with cirrhosis and increased passage of enteric bacterial products to the circulation, as identified by a high LBP level or bacterial DNA in serum, show an increased risk of bacterial infections and a poor disease outcome in the short-term, respectively. Besides, the translocated bacterial products drive a pro-inflammatory response of the host immune system. Cirrhosis should be envisaged as a disease in which immune-mediated inflammation plays a prevalent pathogenic role. Experimental and clinical data have shown that in cirrhosis immune cell activation initiates in the mesenteric lymph nodes and is greatly promoted by gut bacterial translocation. Thereafter, activated monocytes and T cells gain access to the peripheral blood by recirculation, leading to systemic inflammation. Intermittent, yet continuous, interaction of gut bacteria or their products with immune cells leads to activation of the inflammatory immune system at the systemic level. Indeed, patients and animal models with advanced cirrhosis show a marked increase in TNF-alpha-secreting monocytes in peripheral blood, accompanied by polarization of the T-helper cell compartment toward a Th1 pattern of activation, and increased serum levels of proinflammatory cytokines.

The intense LPS-driven proinflammatory cytokine release observed in animals with bacterial translocation and in patients with high LBP levels or bacterial DNA in serum plays a role in the hemodynamic derangement of cirrhosis. Circulatory deterioration is more intense in cirrhotic rats with bacterial translocation to mesenteric lymph nodes and patients with ascites and high LBP. The contribution of the enteric bacterial stimulus to this hemodynamic derangement is further supported by the attenuation after norfloxacin of the hyperdynamic circulatory state, nitric oxide-mediated vasodilation and endogenous vasoactive system activation. Thus, a link exists in cirrhosis among bacteria or bacteria-delivered endotoxin, the release of pro-inflammatory cytokines, and endothelial nitric oxide overproduction, which worsens peripheral vasodilation and prognosis.
Gut liver communication – The role of bacterial translocation in hepatic and systemic disease

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It becomes increasingly clear that the intestinal tract and the liver have more in common than the fact that they are the key organs for nutrient absorption and metabolism. Many intestinal diseases manifest in the liver and vice versa, which often results in a vicious circle of disease progression and clinical complications. Here we review the latest experimental and clinical advances in understanding the role of intestinal commensals and endotoxins in the complex crosstalk between the gut and the liver. Increased intestinal permeability in patients with chronic liver disease promotes translocation of luminal bacteria, endotoxins and other pathogen associated molecular patterns (PAMPs) into the blood of the portal vein and the peritoneum. Upon translocation to the liver, PAMPs activate pattern recognition receptor (PRR) expressing immune cells like Kupffer cells. Interestingly, a number of liver resident non-immune cells also express functional PRRs. A recent study demonstrates a key role of Toll like receptor 4 (TLR4) activation on hepatic stellate cells by the bacterial cell wall component LPS during the pathogenesis and progression of liver fibrosis. We discuss the underlying mechanisms and potential therapeutic implications of this newly described pathway.

Notably, patients with chronic liver disease not only display higher endotoxin levels due to decreased intestinal barrier function, but they also suffer from enhanced susceptibility to sepsis and importantly, elevated sepsis-associated mortality. New data from our laboratory sheds light on the role of the liver in regulating innate inflammation during polymicrobial sepsis caused by intestinal perforation. We provide evidence that liver derived acute phase proteins represent crucial regulatory elements of the innate immune response during sepsis.

Taken together these new findings highlight the complex communication between the liver, intestinal microbes and the immune system. Detailed understanding of this relationship may yield exciting new targets of treatment for patients with chronic liver disease.
Intestinal flora in liver cirrhosis: A therapeutic tool?

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The ecologic intestinal system fulfills a myriad of functions and correspondingly produces multiple agents and gases, e.g. ammonia, ethanol, acetaldehyde but also phenols, benzodiazepines etc. all of which are more or less metabolized by the liver. Moreover, there is continuous interaction and communication between the flora and the gut-associated lymphatic tissue determining many aspects of host immunity and metabolism. In respect to human liver cirrhosis, scientific knowledge on any of these issues is limited.

However, it is well-established that cirrhosis is accompanied by multiple alterations in intestinal flora including bacterial overgrowth in the small intestine as well as mucosal barrier dysfunction leading to “pathologic” increases in bacterial translocation (BT). In this scenario, indepent of the development of any overt bacterial infection gut-derived bacterial products as well as the BT-associated pro-inflammatory cytokine response may have severe clinical consequences in advanced liver cirrhosis. Particularly considering the well-known priming of mononuclear cells to produce pro-inflammatory cytokines associated with a marked decrease in clearance capacity in advanced cirrhosis along with an enhanced susceptibility of the splanchnic circulation for further hemodynamic derangement points towards the potent clinical impact of any bacterial stimuli. In this respect, not only endotoxins but also bacterial DNA has been shown to induce a marked inflammatory response and nitric oxide overproduction (1–3). TNF in mesenteric lymph nodes were found to be higher in cirrhotic patients than in controls, particularly in those with ascites (4). Finally, presence of bacterial DNA in serum and ascites in absence of any overt infection has recently been demonstrated to decrease survival in advanced cirrhosis (5). Therefore, it is tempting to speculate that BT becomes “motor of a vitious circle” triggering and/ or exacerbating hemodynamic disturbances, hepatic dysfunction, hepatic encephalopathy and/or hepato-renal syndrome (Fig. 1).

In fact, presence of BT and endotoxinemia associates with pronounced arterial vasodilation (6) and selective gut decontamination has been shown to ameliorate the hyperdynamic circulation in experimental as well as human cirrhosis (7, 8). Moreover, pro- and synbiotic modulation of the gut flora has been demonstrated to lower systemic endotoxinemia and to improve liver function in liver cirrhosis (9–12). In addition, pilot studies indicate that modulation of intestinal flora potentially can ameliorate portal hypertension (13). Moreover, norfloxacin in high-risk cirrhotic patients delays also the development of hepatorenal syndrome most likely via inhibition of pathological BT (14). By the same approach, prophylactic use of selective gut decontamination has been shown to prevent the development of spontaneous bacterial peritonitis being associated with improvement in survival (15, 16). In respect to hepatic encephalopathy, targeting intestinal flora is of therapeutic benefit, e.g. recently via utilizing new non-absorbable antibiotics such as rifaximin (17). Finally, experimental data even support a pathophysiological role of intestinal flora and BT for the development of hepatopulmonary syndrome (18).
Therefore, the potential clinical use of modulating the intestinal flora aiming at prevention and/or treatment of chronic liver disease and its complications is obvious. However, in contrast to multiple evidence in experimental models data from valid randomized controlled clinical trials are limited so far and more well-designed studies are needed.

Fig. 1

References:


Tapeworm eggs: From the gut to the liver – Pathogenesis, prevention and treatment

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Tapeworms (Cestodes) are an ancient class of highly specialized flatworm parasites. During evolution innumerable species have subtly adapted to the behaviour, diet and immunology of their hosts. Most cestodes require at least two host species to support their different stages of their life cycles. Adult tapeworms inhabit the gut of a vertebrate animal, with four species specifically adapted to humans (e.g. Taenia spp.). Proglottids and/or eggs are released into the environment and are ingested by the intermediate host. Viable eggs hatch in the stomach and small intestine, and the larva (oncosphere) becomes invasive, migrates through blood and lymph vessels into the host tissue, and develops in one of much distinctive morphology of the cestode larvae (Metacestode). The liver is the primary target organ after ingestion of tapeworm eggs of Echinococcus spp. Humans are susceptible to this intermediate stage and develop echinococcal diseases, such as cystic echinococcosis caused by E. granulosus, and alveolar echinococcosis caused by E. multilocularis. The incubation time remains undetermined, often a decade or more elapses between a possible exposure to eggs and the presentation of the liver disease. Maturation to the asexually proliferating metacestode includes several steps, and is modulated by the immune system. The fully developed metacestode of E. granulosus is typically unilocular, spherical in shape, and fluid-filled. This stage is often referred to as “hydatid cyst”. Its morphology by ultrasound has been classified into several categories. Treatment strategy follows the WHO-classification of liver cysts and encompasses minimal invasive procedures, surgery, and treatment with benzimidazoles or watchful waiting. The metacestode of E. multilocularis develops differently from that of E. granulosus. It is a multivesicular, infiltrating structure with no limiting host-tissue barrier, consisting of numerous small vesicles embedded in a dense stroma of connective tissue. The larval mass contains a semisolid matrix rather than fluid, it proliferates peripherally, and at the same time regressive changes occur centrally. A progressively enlarging mass of necrotic tissue with a relatively thin zone of viable parasite is produced. The reactive host tissue adjacent to this proliferating zone can be indirectly visualized by FDG-PET scan. The staging of alveolar echinococcosis follows the principles of tumour staging, since infiltration of neighbouring tissue or distant metastasis formation is often observed at the time of initial presentation. Curative surgery can only be applied in a minority of cases; relapses are frequent after non-radical surgery. Backbone of the treatment are benzimidazoles (mainly albendazole), given for lifelong.
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POSTER ABSTRACTS

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Endotoxin receptor CD14 gene expression in chronic hepatitis C

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Introduction: Chronic hepatitis C develops in 70%-80% of patients and ranges widely from mild hepatic inflammation to liver cirrhosis and hepatocellular carcinoma. Due to its anatomical links to the gut, the liver is constantly exposed to gut-derived bacterial lipopolysaccharides (endotoxin), which are suggested to enhance liver injury in alcoholic liver disease. Intensive studies are carried out to identify the factors that might exacerbate hepatic alterations in chronic hepatitis C. This study analyzed the hepatic and peripheral blood mononuclear cells (PBMCs) expression of the endotoxin receptor CD14 gene with regard to liver histology in chronic hepatitis C.

Methods: Liver biopsy specimens from a total of 42 German chronic hepatitis C patients, (22 men, mean age 46.9 years, range 23-68), were taken and evaluated histologically. Total cellular RNA was extracted from both homogenized liver tissue and PBMC samples and applied to quantitative real-time RT-PCR using commercially available Taq-Man Assays-on-Demand for CD14 and GAPDH. PBMCs’ CD14 transcripts from 42 healthy individuals (22 men, mean age 31.8 years, range 23–57) were quantified as controls.

Results: CD14 expression was found to be relatively similar in healthy individuals and chronic hepatitis C patients (p = 0.341). Five histological characteristics were investigated in patients’ biopsies including: hepatitis activity (mild, moderate or severe); fibrosis progression (absent, mild, moderate, marked or cirrhosis); steatosis (absent, mild, moderate or marked); portal lymphoid aggregates (absent or present); and bile duct damage (absent or present). Neither hepatic nor PBMCs' CD14 mRNA expression were found to be different between patients with low or high degrees of the studied histological features.

Discussion/Conclusion: Our data confirm and extend earlier findings by others on CD14 gene expression and liver histology in a different population. They thus argue against a possible role of CD14 expression in promoting any of the histological marks of chronic hepatitis C.
Effect of gadolinium chloride on liver regeneration following thioacetamide-induced necrosis in rats

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Introduction: Kupffer cells, as the macrophages residing in the sinusoids of the liver, are the first macrophage population to come into contact with drugs. Gadolinium chloride (GD) attenuates drug-induced hepatotoxicity by selectively inactivating Kupffer cells. The effect of GD was studied in reference to postnecrotic liver regeneration induced in rats by thioacetamide (TA).

Methods: Rats, intraveously pretreated with a single dose of GD (0.1 mmol/kg), were intraperitoneally injected with TA (6.6 mmol/kg). Hepatocytes were isolated from rats at 0, 12, 24, 48, 72 & 96 h following TA intoxication, and samples of blood and liver were obtained. Parameters related to liver damage were determined in blood. In order to evaluate the mechanisms involved in the post-necrotic regenerative state, the time course of DNA distribution and ploidy were assayed in isolated hepatocytes. The levels of cyclin D and cyclin E as well as protein p27 and PCNA were determined in liver extracts, and circulating cytokine TNFα was assayed in serum samples. TNFα was also determined by RT-PCR in liver extracts.

Results: The results showed that GD significantly reduced the extent of necrosis. The effect of GD induced noticeable changes in the post-necrotic regeneration, causing an increased percentage of hepatocytes in S phase of the cell cycle. Noticeable changes were detected in the levels of cyclin D1, cyclin E, p27 and PCNA when compared to those induced by thioacetamide. Hepatocytes increased their proliferation as a result of these changes. TNFα expression and serum level were diminished in rats pretreated with GD.

Discussion/Conclusion: Thus GD pre-treatment reduced TA-induced liver injury and accelerated the postnecrotic liver regeneration. No evidence of TNFα implication in this enhancement of hepatocyte proliferation and liver regeneration was found. These results demonstrate that Kupffer cells are involved in TA-induced liver, as well as in the postnecrotic proliferative liver states.
Antifibrotic properties of NOV/CCN3 in liver

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Introduction: Hepatic Nephroblastoma overexpressed (NOV/CCN3) is a matricellular protein of the CCN family, comprising CCN1 (CYR61), CCN2 (CTGF), CCN4 (WISP-1), CCN5 (WISP-2), and CCN6 (WISP-3). CCN proteins are involved in mitosis, adhesion, apoptosis, extracellular matrix production, growth arrest and migration of multiple cell types. Compared to CTGF which has been extensively investigated, the biological role of NOV in liver fibrosis still remains rather obscure.

Methods: We researched the hepatic expression of NOV in chronic liver injury upon long term CCl4 treatment and bile duct ligated (BDL) rat models, using siRNA technology in primary cultured hepatic stellate cells (HSC) and CFSC.

Results: In experimental fibrotic rat models, hepatic NOV/CCN3 showed significantly increased mRNA- and protein levels. NOV/CCN3 was detected mainly in the areas of tissue damage and tissue repair. In the BDL model, the pathology was localized along portal tracts, while CCL4 was concentrated in the centrilobular areas. In vitro siRNA technology derived data clearly demonstrated down regulation of NOV/CCN3 attenuated CFSC cell migration and cell proliferation, well in line with in vivo findings that various types of cells expressing NOV/CCN3 migrate into the area of actual liver injury- and regeneration. Interestingly, the CFSC Nov gene knock down showed to increase several fibrotic marker proteins, including alpha SMA, collagen type I, fibronectin, CTGF, and TIMP-1 expression.

Discussion/Conclusion: The marked increase of NOV/CCN3 in fibrotic models rather points at an interesting auto-protection process instead of driving fibrosis. Our findings manifest NOV/CCN3 to be an essential counterbalance component of CTGF induced fibrogenesis in the complex process of wound healing and tissue repair.
Association of CD14 gene variations with hepatic CD14 gene expression, serum soluble CD14 (sCD14) concentration, and liver fibrosis progression in patients with chronic hepatitis C

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Introduction: The single nucleotide polymorphism (SNP) rs2569190 within the CD14 endotoxin receptor gene was found to be associated with advanced liver fibrosis in patients with alcoholic liver disease. This association was suggested to be due to sensitization of the liver for gut-derived endogenous LPS via an enhanced T allele mediated hepatic CD14 expression. This study analysed liver fibrosis progression and CD14 expression in patients with chronic hepatitis C with regard to CD14 genetic variations.

Methods: Liver biopsy samples from a total of 144 chronic hepatitis C patients were evaluated histologically and scored according to standard criteria. All patients were genotyped at SNPs rs2569190 and rs2563298 by allelic discrimination in 5'-nuclease reactions and tetra-primer ARMS-PCR, respectively. In smaller cohorts of patients, genotyping at four variant positions (rs5744455, rs2569190, rs4914, rs2563298) was performed. Total cellular RNA was extracted from liver samples and CD14 gene expression was quantified by real time RT-PCR. Serum sCD14 concentrations were determined by ELISA.

Results: Genotype distributions for all four SNPs in all cohorts followed Hardy-Weinberg equilibrium. Analysis of histological data with regard to the SNP rs2569190 revealed no association between genotypes and the stage of liver fibrosis (p = 0.9550). Hepatic CD14 mRNA expression and serum sCD14 concentration were significantly related solely to rs2563298 genotypes (TT vs. GG+GT, p = 0.0225 and 0.0385, respectively). The analysis of histological data revealed a slight decrease of risk for severe liver fibrosis in T allele carriers at the position rs2563298 compared with GG homozygotes (p = 0.1618).

Discussion/Conclusion: We found no association of the SNP rs2569190 neither with liver fibrosis progression nor with hepatic CD14 expression and sCD14 serum concentration in patients with chronic hepatitis C. Another SNP, rs2563298, seems to be more closely associated to these parameters.
Non-alcoholic liver disease – Gut-related symptoms and liver fibrosis correlations

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is a broad spectrum disease produced in the absence of alcohol ingestion, with a growing prevalence in the general population. These patients have very few symptoms related with the liver disease until the evolution of their illness toward more advanced stages of liver fibrosis.

Methods: We present an observational/prospective study on 125-NAFLD patients with a specific focus on their gut related symptoms and correlations with the degree of liver fibrosis. Patients were evaluated using abdominal ultrasonography and biochemical tests. Meanwhile the liver fibrosis was evaluated using non-invasive available tools (Forns score, APRI, API, ASPRI, Fib 4, ASAT/ALAT, Fatty Liver Index).

Results: The study included 88 women and 37 men, age between 26–81 years (median 55.67), most of them living in the city (69%) and having a “modern” lifestyle (sedentarism, low fruits and vegetable intake, high lipids and carbohydrates diet). The symptomatology was dominated by asthenia (94%), fatigability (77%), nausea (20%), constipation (45%) and abdominal bloating/meteorism (72%). Many patients presented several important comorbidities – diabetes mellitus (40%), obesity (65%), dyslipidemia (72%), cardiovascular diseases (65%). Even though non-invasive liver fibrosis methods revealed slightly different results, in the majority of patients they confirm some degree of liver fibrosis and infirm liver cirrhosis with some significant correlations with other anthropometrical/biochemical factors.

Discussion/Conclusion: The results confirm stating that NAFLD is thoroughly related with intestinal malfunctioning and also that lifestyle/diet changing is very important for the treatment/control of symptoms.
TGF-beta inhibitor Smad7 – Ambiguous functions during hepatocarzinogenesis

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Introduction: Hepatocellular carcinoma (HCC) is one of the most common and deadliest cancers worldwide. The cytokine TGF-beta is involved in progression of chronic liver disease, which eventually ends in HCC. It is known to function as tumor suppressor in cancer development. During tumor progression it changes to be a tumor promoter. Aberrations in TGF-beta signaling regularly occur in HCC. However, TGF-beta signaling related mutations often occurring in different cancers are comparably rare in HCC.

Methods: Real Time and immunohistochemical analysis were used to determine Smad7 expression. Mutation and sequence analysis were performed for parts of the Smad7 promoter. TGF-beta response in HCC cell lines was characterized with Western Blot, cell death and proliferation assays.

Results: In an up to now limited number of matched human HCC/normal liver samples, Smad7 is elevated in 80% of tumor tissues. Interestingly, the overexpression is found in single foci within the tumorigenic tissue. No detectable alterations within the most conserved Smad7 promoter region suggest another underlying mechanism. Additionally, we found in in vitro experiments that different human HCC cell lines exhibit strongly varying Smad7 expression and thereto converse activation of Smad2. However, there is no further correlation between Smad7 levels and TGF-beta response in regard of cell death and growth arrest induction. This underlines the ambiguous nature of TGF-beta and Smad7 in hepatocarcinogenesis.

Discussion/Conclusion: We suggest that Smad7 could represent a new marker for HCC. However, up to now it is unclear whether increased Smad7 levels in specific foci within the HCC tissue fulfill tumor promoting or suppressing tasks. To understand the ambiguous nature of TGF-beta and Smad7 in hepatocarcinogenesis further investigations are planned.
Comparative analysis of phase I and II enzyme activities in 6 hepatoma cell lines identifies Huh-7 cells to have the highest potential to study drug metabolism

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Introduction: Primary human hepatocytes is the gold standard to perform human drug metabolism studies. Due to donor organ scarcity hepatoma cell lines are widely used as an alternative. Although, phase I and II drug metabolizing activities of these cell lines are substantially lower than in primary hepatocytes, their major advantage is immediate availability, standard culture conditions and unlimited life-span. Thus, aim of this study was to investigate the drug-metabolizing profile of 6 standard hepatoma cell lines (HepG2, Hep3B, Hep1.6, HCC-T, HCC-M and Huh-7) over a culture period of 10 passages.

Methods: At 70–80% confluence fluorescent-based assays for 7 different cytochrome P450 (CYP) iso-forms and 7 different phase II enzymes were performed and compared with basic activities of human hepatocytes.

Results: CYP activities were much lower in cell lines (5–15% of human hepatocytes). Only phase II enzymes activities, involved in buffering oxidative stress (e.g. glutathione-S-transferase), reached levels comparable to primary hepatocytes. Interestingly, HCC-T cells between passage 4 and 6 exhibited a drug-metabolizing profile closest to primary hepatocytes. Although being a low responder for CYP2D6, most constant results were obtained by Huh-7 cells. Induction of CYP1A1/2 or CYP3A4 by rifampicin, 3-methylcholanthrene and phenobarbital in both cell lines confirmed this observation.

Discussion/Conclusion: Our data shows that phase I and II enzyme activities in these cell lines varies strongly during culture time. Although, HCC-T cells between passage 4 and 6 showed highest enzyme activities, Huh-7 cells did not vary that strongly between the different passages and showed thus the highest potential to study drug metabolism.
Does caffeic acid phenethyl ester (CAPE) have ameliorating effects on acetic acid-induced experimental colitis model in rats?

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Introduction: Although the pathophysiology of inflammatory bowel diseases (IBDs) is not certainly identified, immunological processes and reactive oxygen species (ROS) have been proposed to contribute in development of tissue injury. Meanwhile, in this aspect, the antioxidant treatment modalities are gaining attention in IBD. Caffeic acid phenethyl ester (CAPE) is suggested to protect tissues from ROS mediated oxidative stress in ischemia-reperfusion and toxic injuries. We aimed to investigate the effects of CAPE on the antioxidant parameters of rats with acetic acid (AA) induced colitis.

Methods: Twenty one female Wistar-albino rats weighing 150–200 g were randomly divided into three groups: control group (n = 6), colitis group (n = 8), CAPE group (n = 7), respectively. Colitis was induced by intracolon enema with 4% AA. Effects of CAPE were subsequently evaluated after 3 days of CAPE administration following introduction of colitis.

Results: The increase in colonic malondialdehyde and nitric oxide level at the colitis group was reduced by CAPE (10 µmol/kg) treatment (p = 0.043, p = 0.006, respectively). Reduced glutathione activity in the colitis group was increased by CAPE treatment (p = 0.008). Treatment with CAPE did not reduce the lesion score of the colitis group at microscopic level (p > 0.05).

Discussion/Conclusion: Our data show that CAPE has positive effects on some biochemical parameters, although there was not statistically significant histologic improvement with CAPE treatment. CAPE may be beneficial in an AA-induced rat colitis model through the antioxidant and anti-inflammatory effects. Further studies are needed to investigate the exact effects of CAPE in experimental colitis models and clinical trials.
Accelerated larval growth of *Echinococcus multilocularis* in the immunodeficient host?

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**Introduction**: Human alveolar echinococcosis (AE) caused by the larval stage of the tapeworm *Echinococcus multilocularis* is a life-threatening helminthic disease, primarily affecting the liver. Slow larval growth with a prolonged incubation period of several years to decades is a characteristic feature of the disease. It is believed that accelerated larval growth occurs in immunocompromised patients. We wondered whether the use of immunosuppressants is linked to accelerated larval growth in patients with AE.

**Objectives**: Aim of this study was to retrospectively evaluate the kinetics of larval growth in immunocompromised patients treated for AE at our centre.

**Results**: Out of about 220 AE patients seen at our clinic in the last 54 month, 18 patients were immunocompromised. 10 patients had various malignancies. All showed an unremarkable clinical course of AE. Three women presented with AE in pregnancy, two of them showed accelerated growth of their liver-lesion, whereas one lesion remained stable. Another woman developed a retroperitoneal relapse of AE after 2 years of immunosuppression for liver-transplantation. Only recently an extensive AE liver-lesion was found in a patient shortly after being diagnosed with Myasthenia gravis. The further clinical course is to date unclear as albendazole treatment was delayed because of azathioprine-induced hepatotoxicity. Three patients treated with TNF-α-blockers for rheumatic diseases showed an accelerated growth of AE lesions, but after introduction of albendazole all lesions remained stable.

**Discussion/Conclusion**: At this stage our analysis does not allow definite conclusions whether larval growth is accelerated in immunocompromised patients with AE in general. But as immunosuppressive therapy is now more commonly used this needs to be evaluated further. In addition reliable criteria need to be established to assess larval growth in AE.
Impact of disturbed glucose tolerance on 90-day mortality in patients with decompensated liver cirrhosis

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Introduction: Hepatogenous diabetes is a common complication of liver cirrhosis. Several studies have assessed the prognostic value of a disturbed glucose metabolism in liver cirrhosis and showed that diabetes mellitus (DM) as well as impaired glucose tolerance (IGT) correlate with poor long-term prognosis. The aim of this study was to evaluate whether a disturbed glucose metabolism predicts short-term prognosis of patients with decompensated liver cirrhosis.

Methods: Seventy-nine patients with liver cirrhosis of different etiologies [alcohol (n = 64), hepatitis B infection (n = 2), hepatitis C infection (n = 2), other causes (n = 11)] without previous diagnosis of DM and IGT were enrolled in a prospective cohort study. DM and IGT, diagnosed by a 75-g oral glucose tolerance test (OGTT), were defined according to current World Health Organization criteria. Cumulative survival rates were calculated using the Kaplan-Meier methods, and the differences between survival curves were evaluated using the log-rank test.

Results: IGT was diagnosed in 29 subjects (37%), DM in 27 subjects (34%), and normal glucose tolerance (NGT) in 23 subjects (29%). Three-month survival analysis showed that compared to normal glucose tolerance, disturbed glucose tolerance (IGT and DM) is a predictor of poor survival (OR 3.4, p = 0.038). Short-term mortality of patients with Child C liver cirrhosis and disturbed glucose tolerance (IGT [n = 19], DM [n = 16]) was significantly higher than in cirrhotic patients with Child C stage and NGT (n = 20) (p = 0.042). Multiple regression analysis yielded IGT (HR = 2.55), MELD score (HR = 1.085 for each additional point, HR = 2.26 for a change of +10 points) and age (HR = 1.04) as the most powerful independent negative predictors of ultimate survival. For DM, however, a non-significant hazard ratio of 1.06 with a wide confidence interval (0.48–2.34) was calculated. Overall, infectious complications were the leading cause of death.

Discussion/Conclusion: Disturbed glucose metabolism, i.e., IGT and DM, in patients with decompensated liver cirrhosis correlates with poor prognosis for 3-month survival and represents an independent, negative predictor of ultimate survival.
Interleukine-18 and CD95-lymphocytes in liver cirrhosis caused by chronic hepatitis B

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Introduction: The process of positive and negative selection of lymphocytes by apoptosis is a basic for adaptive immunity in the viral infection. Apoptosis subsequently works and provides the constant limited lymphocytes amount. The important function of lymphocytes is their ability to cause the apoptosis of another cells with intracellular pathogen or presented aberrant peptides. This is the critical point for starting of functional activity of immune system. Chronic HBV infection is associated with a variety of autoimmune phenomena. Th17 is new subpopulation of T-helpers described as population responsible for pathogenesis of autoimmunity. Interleukin-18 (Il-18) is one of proinflammatory cytokines participating in the activation of IL-23 via the IL-18Rα and then in expansion of Th17. Also the attendant activity of TGF-β and IL-6 is obligatory for initiation Th17 differentiation. On the other hand, in the process of fibrogenesis TGF-β plays the key role. We aim to study the level of IL-18 in the serum and capacity of CD95+ in the patients with liver cirrhosis caused by chronic hepatitis B.

Material and methods: Forty-eight non-treatment patients with compensated chronic hepatitis B (group A) and fifty non-treatment patients with liver cirrhosis Class B (Child-Pugh) (group B) were included in this study as well as thirty age and sex matched healthy control. CD95+ was cytometrically quantified in patients in groups A and B and in health control group investigated (FaxCalibur, BD). Concentration of IL-18 in the serum was estimated by ELISA (IBL).

Results: Group A patients (123.7 ± 7.4/ml) and group B patients (167.4 ± 9.0/ml) shown a higher amount of mononuclears expressed CD95+ than healthy control (89.4 ± 4.4/ml, p < 0.05 (A) and p < 0.05 (B) accordingly). The serum levels of IL-18 in patients of two groups (156.7 ± 7.4 pg/ml (A), 189.7 ± 9.2 pg/ml (B) with the chronic hepatitis B are higher than in health group (122.5 ± 6.78 pg/ml, p < 0.05 (A), p < 0.05 (B), accordingly).

Both the amount of CD95+ lymphocytes and the level of IL-18 in the group B patients shown a higher meanings than in group A patients (p < 0.05). Moreover, serum level of IL-18 showed correlation with the amount of CD95 in the group A (r = 0.57).

Conclusion: These results indicate that IL-18 can possess of proapoptotic activity and participate in the transfer of autoimmune signal in the same time. Possible both the lymphocytes apoptosis and the autoimmune inflammatory are very important in the pathogenesis of progression HBV chronic liver disease.
Resveratrols weak toxicity on tumor cells of liver origin

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Introduction: Resveratrol (RES) is a naturally occurring polyphenol. There is increased interest in polyphenols since anti-inflammatory and antikanzerogene properties were proven in various cell types and models. RES has been under investigation on many different cell lines but only a few studies exist to investigate its potential on liver cells and cancer cells from liver origin. The present work demonstrates diverse actions of RES on primary human hepatocytes and the hepatocellular carcinoma cell line SkHep1.

Methods: For the isolation of human hepatocytes, resected liver tissues from patients with primary and secondary tumors were used. Hepatocytes were isolated using a two-step collagenase P perfusion technique, followed by a Percoll density gradient centrifugation. The cell proliferation of SkHep1 under the influence of RES was measured by XTT. Changes in cell integrity of human hepatocytes and SkHep1 were determined by measuring the release of AST and LDH. FACS analysis was used for cell cycle determination. Western blot was used to determine changes in expression of cell cycle related proteins and phosphorylation of MAPK.

Results: RES decrease the cell proliferation of SkHep1 significantly with p < 0.05, when comparing untreated SkHep1 cells with 40 and 80 µM RES treated cells. Low concentrations of RES (0–40 µM) led to a cell cycle arrest in the S phase, whereas higher concentrations of resveratrol led to an increased arrest of cells in the S phase, concomitant with an increase in the G1 phase. The cell cycle arrest in the S phase comes due to the inhibition of pAKT conjoined with an increased phosphorylation of ERK. The downstream regulation of p53 via pERK was observed in SkHep1 cells and subsequently p53 was activated during 48 hours. Regardless of p53 activation, no cell death was detected by measuring the transaminase release (AST, LDH) in SkHep1 and human hepatocytes. Only after 48 hours, SkHep1 cells treated with 40–80 µM RES, showed a modest increase of LDH in SkHep1 cells. RES showed a relative weak toxicity towards SkHep1, AKN-1 and Hep3B cells and showed no effect on primary human hepatocytes.

Discussion/Conclusion: It seems that RES distinct effect on tumor cell lines is based on the tumor origin. For example, colon cancer cells response intense on RES treatment, whereas liver cancer cell lines showed only a proliferation inhibition. Based on the literature and together with our results we suggest that RES cell cycle arrest is reversible and that RES can act only as a long time supplement human diet in preventing tumor promotion.
Analysis of the common vasoactive intestinal peptide receptor 1 polymorphism in gallstone patients

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Introduction: Gallstone disease is a common condition with both genetic and environmental background (e.g., dysmotility of the gallbladder wall). We showed that the p.D19H polymorphism of the ABCG8 cholesterol hemitransporter increases the risk of gallstones [1]. Recently, a single nucleotide polymorphism (SNP) of the vasoactive intestinal peptide receptor 1 (VIPR1) gene has been linked to late onset of achalasia, a dysmotility disorder of the lower oesophagus [2]. As VIPR1 is expressed in the gallbladder wall a well [3], here we analyse the influence of this VIPR1 SNP on cholelithiasis.

Methods: We analyzed 254 gallstone-free controls (confirmed by ultrasound, age 21–78 years, 93% women, BMI 16–43 kg/m²) and 234 individuals from 108 families with gallstones (age 24–80 years, 86% women, BMI 17–49 kg/m²). All individuals were genotyped for the VIPR1 rs437876 SNP (intron 4) with PCR-based 5'-nuclease and fluorescence detection assays (TaqMan). We performed nonparametric linkage (NPL) analysis in affected sib pairs (ASPs) and association tests.

Results: The allele frequencies did not deviate from Entrez SNP database reports, and no deviation from Hardy-Weinberg equilibrium was detected. Neither allele and genotype distributions nor NPL scores or the restriction of analysis to older individuals (age > 50 years) provided evidence for association or linkage of the VIPR1 SNP and gallstones.

Discussion/Conclusion: The VIPR1 polymorphism, previously linked to gastrointestinal dysmotility disorders does not enhance the risk of developing gallstones in the general or in an elderly population.

References:


Autotaxin and its product, lysophosphatidic acid, are potential mediators of cholestatic pruritus

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Introduction: Although pruritus is a common symptom in cholestatic disorders, the causing factors are unknown. We hypothesized that potential pruritogens accumulate in the circulation of cholestatic patients and activate sensory neurons.

Methods: Cytosolic free calcium (Ca++)i was measured in neuronal cell lines by ratiometric fluorimetry upon exposure to diluted serum samples of patients with intrahepatic cholestasis of pregnancy (ICP; n = 33), pregnant controls (PC; n = 29) patients with other causes of cholestasis (mainly PBC; n = 52) and healthy subjects (HC; n = 202). The (Ca++)i inducing factor in pruritic serum was identified by analytical techniques including quantification by HPLC-MS. Autotaxin activity, bile salts and histamine were quantified by enzymatic assays, µ-opioid activity by receptor binding assay. In mice, scratch activity after intradermal pruritogen injection was quantified using a magnetic device.

Results: Transients in (Ca++)i in human SH-SY5Y neuroblastoma cells, induced by PBC and ICP sera were higher than those of corresponding controls. Lysophosphatidic acid (LPA) could be identified as major (Ca++)i agonist in pruritic sera. Serum LPA concentrations were increased only in those cholestatic patients that suffered from pruritus. LPA injected intradermally into mice, induced scratch responses. Serum autotaxin (ATX) is the enzyme that converts lysophosphatidylcholine into LPA. ATX was markedly increased in sera of ICP patients vs. PC (p < 0.0001) and in sera from cholestatic patients with vs. without pruritus (p < 0.0001). ATX activity correlated highly with intensity of pruritus (p < 0.0001). In PBC patients who underwent nasobiliary drainage both itch intensity and autotaxin activity significantly decreased during drainage and returned to increased levels when pruritus had returned. Neither bile salts, histamine, nor µ-opioids correlated with itch intensity.

Conclusion: Our data suggest that autotaxin and its product, LPA, play a key role in cholestatic pruritus. We speculate that ATX inhibitors may be useful as antipruritic agents in treatment of cholestatic pruritus.
Polyphenoles: A class of compounds with diverse interferences in hormonal and morphogen signaling of liver and gut carcinomas

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Introduction: Uncontrolled activation of the Wnt/β-catenin signal transduction pathway has been implicated in a variety of malignant diseases, such as liver and gut carcinomas. CK-2 a member from this pathway is quantitatively elevated in proliferating tissues and tumor cells. GSK-3β, another kinase from this pathway plays also critical roles in insulin signaling and regulation of metabolism.

Methods: Insights into the molecular interactions between different kinases and their potential inhibitors could be obtained from molecular modeling and docking studies. The inhibitory potential of selected hit-compounds was determined with a radio-labeled in-vitro kinase assay and some biological activities e.g. cytotoxicity were measured. A library of about 30 chemically modified anthraquinones and 15 native flavonoids were tested on 4 kinases in silico and at least 3 kinases in vitro.

Results: Comparing emodin and its derivative 4-aminoethylaminoemodin (4-AAE) with respect to inhibition of protein kinases revealed that emodin and most of the emodin-like structures inhibit CK-2, while 4-AAE preferentially inhibits GSK-3β. 4-AAE is a potent and sensitive inhibitor of GSK-3β that is more than 10-fold more active than the standard inhibitor TDZD-8. The peculiar biological features of 4-AAE e.g. its stimulation of glycogen synthase activity, glycogen content and fatty acid synthesis, but not of β-catenin point to a specific interference with at least one other protein kinase that is absent in closely related emodin derivatives.

Conclusion: The elucidation of molecular details of specific protein-inhibitor interactions discloses further possibilities for compound modification and for target-orientated signalling pathway interference.
Bone morphogenetic protein 9 (BMP-9) plays a potential role in liver regeneration

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Introduction: It has been reported that BMP-9, a member of the transforming growth factor (TGF)-β superfamily may be involved in cell proliferation. Our recent work demonstrates that BMP-9 can indeed induce cell proliferation in cell lines of hepatocytes, cholangiocytes and hepatic stellate cells (HSC). Additionally we found that BMP-9 expression is induced in activated primary HSC in vitro. These patterns of expression of BMP-9 in the different liver cell types prompted us to delineate the role of BMP-9 in liver regeneration.

Methods: The animal model of acute liver injury was established with one single carbon-tetrachloride injection. Mice were sacrificed on day1, day2, day3 and day8 respectively. Serial liver tissue sections were prepared for detecting the immunostaining of BMP-9 and its target gene, Id1. RNA expression level was tested with real time PCR.

Results: Positive staining for BMP-9 can be observed in hepatocytes in the areas of active regeneration. Id-1 is mainly expressed in the border area of BMP-9 positive staining. During the recovery of acute liver injury, BMP-9 positive staining and RNA levels increased rapidly on day2 and day3 and disappeared until day8, a time point when liver recovery is finished. This shows that BMP-9 is transiently induced during liver regeneration.

Discussion/Conclusion: Since gut-derived endotoxins like LPS are known mediators of alcoholic liver damage and BMP-9 has a potential function in liver recovery, we are additionally investigating the role of BMP-9 in livers from mice after LPS ± alcohol treatments.
In summary our data point to a potential role of BMP-9 as mediator of liver regeneration by inducing proliferation especially in hepatocytes at sites of damage.
Giant liver hematoma complicating ERCP. A case report

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Introduction: Liver hematoma following endoscopic retrograde cholangiopancreatography (ERCP) is a rare complication with only few cases reported in the literature [1, 2]. It is mostly due to guide wire injuries of the bile duct and intrahepatic vessels which cause hemorrhagic complications. We report on a patient 59-year-old with a giant subcapsular hematoma following ERCP.

Case report: A 59-year-old man who suffered from biliary colic caused by common bile duct (CBD) stones underwent an ERCP and stone retrieval. During this setting a sphincterotomy was carried out. However, the patient developed abdominal pain and discomfort following the procedure. Subsequently a CT-scan was carried out revealing an extensive intrahepatic hematoma. Although the patient was stable with no signs of hemodynamic instability he was preventively transferred to our tertiary care department for further monitoring. Initially the hematoma decreased and we could discharge the patient for further follow up at our outpatient’s clinic. However, because of further progress the patient was readmitted and the hematoma was drained percutaneously with a CT guided pigtail catheter. It eventually resolved and we removed the drain with no further sequela.

Discussion: Intrahepatic hematoma following ERCP is an extremely rare complication which if not early recognized can result in fatal bleeding [3, 4]. Such a situation always requires close monitoring of patient’s condition and frequent diagnostic measures for a prompt recognition of the situation and a reliable assessment of the damage mostly consisting of ultrasound and CT-scan [2]. Our patient likewise most cases reported in the literature underwent a conservative treatment by means of interventional drainage placement. An early transfer of such patients to a tertiary care facility for monitoring and various treatment options (Surgery, Embolisation) in case of vital bleeding is in our experience mandatory.

Literature:


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Phosphatidylcholine antagonizes ethanol-induced changes of intestinal barrier function

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Introduction: Chronic alcohol abuse leads to damage of the mucosa of the upper small intestine thereby increasing the permeability of macromolecules such as endotoxins. The enhanced translocation of endotoxin into the portal vein has been shown to play a key role for the development of alcoholic liver disease (ALD). Since phosphatidylcholine (lecithin) has been shown to abolish ALD in experimental animals we hypothesize that it might prevent the leaky gut induced by ethanol.

Methods: Experiments were conducted in a coculture model that partly mimics physiological conditions by using layers of enterocytes (Caco-2) combined with peripheral blood mononuclear cells (PBMC). The enterocytes were treated by apical endotoxin stimulation (derived from E. coli K12) and incubated with alcohol in physiological concentrations (22 mM, 44 mM, 66 mM), different combinations of conjugated primary bile salts, phosphatidylcholine and human bile.

Results: The results prove that even small amounts of alcohol account for a decreased monolayer integrity and an elevated permeation of endotoxin through the enterocyte monolayer in a dose-dependent manner. Even in the presence of alcohol the impact of LPS challenge on endotoxin translocation was completely abolished by the addition of phosphatidylcholine or human bile. Thus the alcohol-induced decrease in monolayer integrity, enhanced permeability of endotoxin and the subsequent activation of sub-cultivated PBMC were antagonized by the addition of phosphatidylcholine or human bile.

Discussion/Conclusion: Taken together, the beneficial effect of phosphatidylcholine on the development of ALD is at least partly due to the reduction of alcohol-induced permeability of the intestinal barrier for endotoxin.
Detection of *Helicobacter rodentium*-like DNA in the liver tissue of patients with chronic liver diseases by PCR-DGGE and sequence analysis

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Introduction: Many Helicobacter species were isolated from stomach, intestinal tract, and liver of different animals. *Helicobacter pylori* is well established causative agent of stomach cancer. International Agency for Research on Cancer classified *Helicobacter pylori* infection as a type I carcinogen, making it the first bacterial agent strongly associated with human cancer.

Some of Helicobacter species were found in bile and liver tissue and were suspected to cause cancer or chronic diseases in humans. In our study we aimed at estimating the prevalence of *Helicobacter* spp. DNA in the liver tissue of patients suffering from chronic liver diseases in the population of Northern Poland.

Methods: Liver biopsies from patients with chronic liver diseases were screened for the presence of *Helicobacter* species DNA by a genus specific PCR and DGGE. Further, the chosen PCR product were sequenced to confirm the species designation.

Results: The majority of Helicobacter DNA detected was identified as *Helicobacter rodentium*-like DNA (64%). The DNA of other Helicobacter species detected was similar to *Helicobacter pylori* DNA. No correlation was found between the frequency of Helicobacter PCR-positive results and aetiology of liver diseases and severity of the disease.

Discussion/Conclusion: PCR-DGGE is a very useful screening method for assigning species heterogeneity.
Expression of the receptor for advanced glycation end products (RAGE) in hepatitis C patients with varying degrees of hepatic fibrosis

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Introduction: Advanced glycation end products (AGEs) and their receptor RAGE are associated with obesity and the metabolic syndrome inducing localized inflammation and profibrinogenic reactions. In the present study we aimed to investigate whether AGEs may induce proinflammatory or profibrogenic effects via RAGE in liver tissue of patients with chronic hepatitis C.

Methods: Human liver biopsies of 84 patients with chronic hepatitis C and of 7 healthy control patients were analyzed immunohistochemically for RAGE expression, lymphocytes, macrophages and hepatic stellate cells/myofibroblasts. Transcript levels of RAGE, inflammatory/anti-inflammatory cytokines (TNF-α, Interleukin (IL)-12, interferon-γ, IL-10), and fibrosis-related genes (CTGF, TGF-β1, procollagen-α1(I), matrix-metalloproteinase (MMP)-2, MMP-3, MMP-9, tissue inhibitor of MMPs (TIMP)-1) were measured by quantitative PCR (Lightcycler, Roche). Results were correlated with histological inflammation grade and fibrosis stage.

Results: RAGE was overexpressed in patients with chronic hepatitis C and advanced inflammation (grade 2 and 3) and fibrosis (stage 3 and 4). Co-localization studies revealed that RAGE was more expressed by mononuclear cells than myofibroblasts, hepatocytes or sinusoidal cells. IL-12 and procollagen-α1(I) as well as MMP-9 transcript levels were independent predictors of inflammation and fibrosis, respectively. There was a significant grade- and stage-dependent positive correlation of RAGE mRNA with markers of inflammation (overall: IL-12 > TNF-α > interferon-γ > IL-10) and fibrosis (overall: TIMP-1 > TGF-β, procollagen-α(I) > MMP-2 > MMP-3 > MMP-9).

Discussion/Conclusion: AGE-RAGE interactions appear to be involved in the process of inflammation and fibrosis in chronic hepatitis C patients through upregulation of inflammatory and profibrogenic gene expression.
Cyclin E1 controls the cell cycle activity of hepatic stellate cells and triggers fibrogenesis in mice

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Recent studies demonstrated that the E-type cyclins E1 (CcnE1) and E2 (CcnE2) are not essential for cell proliferation per se, but for transition of quiescent cells into the cell cycle. We recently showed that CcnE1 is a key player for hepatocyte proliferation during liver regeneration, while CcnE2 is a negative regulator of CcnE1. The aim of this study was to investigate the potential contribution of E-type cyclins for liver fibrosis.

Increase of CcnE1 expression in hepatocytes and non-parenchymal cells of human fibrotic liver suggested an important role of CcnE1 in liver fibrosis. Administration of CCl4 to WT mice for 4 weeks results in prominent fibrosis and septum formation. Importantly, CcnE1 mRNA- and protein expression were also increased. In CcnE1−/− animals, the extent of liver fibrosis after CCl4 treatment was significantly lower and in situ staining revealed reduced numbers of α-SMA positive cells. As response on CCl4 treatment, CcnE2−/− mice presented earlier start of fiber formation. Experiments in primary HSC derived from WT mice demonstrated that the CcnE1 expression peak was associated with onset of HSC proliferation and precedes transdifferentiation into myofibroblasts. In contrast, CcnE1−/− HSC showed incomplete cell cycle progression leading to S-phase arrest and strongly increased cell death. In contrast, CcnE2−/− HSC showed earlier and prolonged α-sma expression, which was also correlates with high level of CcnE1.

In summary, our results show an essential role of CcnE1 for fibrogenesis in man and mice and identify HSC as a target cell population for the pro-fibrogenic effect of CcnE1.
Doppler ultrasound evaluation in portal hypertension due to liver cirrhosis. Relationship with etiology, severity and nutritional status

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Introduction: Ultrasound is used to evaluate patients with portal hypertension. The most frequent cause of this vascular alteration is liver cirrhosis. To evaluate portal hypertension along B mode we use color Doppler, pulsed mode, etc.

Methods: The aim of this study is to evaluate relationship between hemodynamic indexes and etiology of the disease, encephalopathy, severity stage, nutritional status. 50 patients admitted in our clinic due to liver cirrhosis were part of this study. Liver cirrhosis was diagnosed before admitting the study using clinical findings, serology, abdominal ultrasound, upper gastrointestinal endoscopy. Patients were included regardless of etiology, severity stage, nutritional status. Malignancy was excluded. Severity stage used was Child Pugh score. Antropometric measurements were taken for body mass index (BMI) and tricipital skinfold thickness (TST). All patients underwent B mode and Doppler abdominal ultrasound. The examination and the measurements were performed by the same doctor using the same equipment, in the morning, à jeun.

Results: There was no difference regarding gender or environment. Repartition by severity score was uniform. The most frequent etiology was alcohol, followed by hepatic virus C and B. We found 6 malnourished using BMI and 15 patients using TST. The majority of malnourished patients had alcoholic liver cirrhosis (p < 0.005), decreased portal flow (0.0003). Patients with the ascites had lower portal vein flow (p < 0.0001). Portal vein diameter is larger in patients with advanced severity score (p < 0.03) and patients with reduced liver diameter (p < 0.01). Encephalopathy is correlated with low portal flow (p < 0.001). Congestion index of portal vein is correlated with presence of ascites (0.01) but congestion index of splenic vein did not.

Discussion/Conclusion: Severity stages were statistically correlated with portal vein diameter over 15 mm and mean portal flow under 15 cm/sec. Triceps skin fold was superior for detection of malnutrition. Malnutrition was correlated with alcoholic etiology, decreased portal vein flow and ascites. Ascites is correlated with decreased portal vein flow, portal vein congestion index, bun not with splenic vein congestion index. Encephalopathy and decreased portal vein flow were correlated.
Alterations of oro-cecal transit time in liver cirrhosis before and after the intake of a probiotic – A pilot study

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Introduction: Several studies report for intestinal motility disorders in liver cirrhosis (LC). Oro-cecal transit time (OCTT) is a potent indicator for intestinal motility and can be easily measured by hydrogen breath test (HBT). The aim of our study was to monitor the changes in OCTT in LC before and after the intake of a probiotic.

Methods: HBT with lactulose was performed before and after 7 days of probiotic intake. The study included 10 patients with LC, Child-Pugh score A (8 male, 2 female, mean age 58.5 ± 11.3, 44–78 years) with different etiology (HCV – 6, HBV – 2, primary biliary cirrhosis – 1, alcoholic liver disease – 1). All patients received daily 100 million colony forming units of the probiotic bacteria Lactocacillus reuteri protectis.

Results: Initially 7/10 patients had prolonged OCTT (≥ 120 minutes), 1/10 had small intestinal bacterial overgrowth (SIBO) and 2/10 had normal HBT. After the 7-day intake of a probiotic 2/7 patients normalized their OCTT and SIBO was corrected. In 2/10 patients with normal HBT there was a prolongation of OCTT but within referent values.

Discussion/Conclusion: HBT with lactulose is a useful method for the evaluation of OCTT in LC. The small number of patients in this study does not allow evaluation of the protective/risk role of etiology, age and sex for the development of motility disorders and SIBO in cirrhotic patients. Further studies are needed to elucidate the exact role of probiotic bacteria in the alterations of intestinal motility in LC.
Serotonin level in serum of patients with inflammatory bowel disease

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Introduction: Brain-gut axis modulates gut functions. Alterations in this axis can play a role in the pathogenesis of Inflammatory Bowel Disease (IBD). Serotonin is a key neuropeptide in brain-gut axis, also is responsible for pathogenesis of IBD and Irritable Bowel Syndrome. Abnormal serotonin secretion can cause changes in motility, secretion, immune function, and blood flow to the gastrointestinal tract – all present in active IBD.

Methods: 79 individuals were included in this study – 20 healthy controls and 59 patients with IBD (23 with Crohn’s disease and 36 with ulcerative colitis). 27 patients were in exacerbation and 32 in remission. Serotonin level in serum was measured using Serotonin ELISA. The concentration of the serotonin is presented in ng/ml. The reference values for females are between 80–450 ng/ml, for males are – 40–400 ng/ml.

Results: All patients with IBD, as well as healthy controls, showed normal serotonin levels in the serum. The results were not influenced by disease activity, nor gender.

Discussion/Conclusion: Serotonin in serum of patient with IBD is not altered. However, based on many researches, its crucial role in gut function is doubtless. Probably its level should be measured directly in gut mucosa where probably any changes will be detected in relation with IBD.
Unexpected increased oxidative burst in patients with severe liver cirrhosis

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Introduction: Bacterial infections are frequent complications and the main cause of death in patients with advanced liver cirrhosis [Nobre SR 2008]. The efficient clearance of microbes by neutrophils requires the concerted action of reactive oxygen species (ROS).

Methods: We included 13 patients (group 1) with liver cirrhosis without infections, 34 patients (group 2) with advanced liver cirrhosis and temporary infection (increased levels of CRP, microbiological proven infection), 5 patients with temporary infection without liver disease (group 3), and 16 healthy persons (control). Additional, we compared ROS in 16 patients with normal blood ammonia concentration (16.5–48.0 µmol/l) to 16 patients with higher levels (group 1, 2). Production of ROS was evaluated by flow cytometry.

Results: In granulocytes of patients with liver cirrhosis we observed increased levels of ROS when compared to control. Stimulation with E. coli revealed a fluorescence intensity (X geomean) of 47.4 (range 30.3–97.8) in controls, 88.4 (41.1–156) in group 1, 44.0 (17.2–108) in group 2, 29.7 and (20.8–44.6) in group 3. Comparable results were observed in PMA and fMLP stimulated granulocytes. In presence of strikingly increased blood ammonia concentration and stimulation with E. coli/PMA, ROS was 66.1 (23–108; E. coli)/167.2 (21.4–349; PMA) compared to patients with normal blood ammonia concentration 49.96 (20.1–156; E. coli)/148.7 (25.3–493; PMA).

Discussion/Conclusion: We hypothesized that in patients with severe liver cirrhosis and infections, ROS levels were decreased, paradoxically these patients show increased levels of ROS. One reason for this observation may be metabolic changes like high ammonia concentration and increased LPS levels, resulting in priming of granulocytes. Priming inhibits chemotaxis and can lead to higher levels of ROS in granulocytes, with sharpening tissue damage and liver cirrhosis.
Defects in bile production associated with intestinal blastocytosis

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Intestinal blastocytosis plays a major role in the development of liver lesions leading to impeded bile production and cholestasis.

Methods: The study focused on 130 UC inpatients and included stool parasites tests and biochemical blood tests, which measured the activity of alkaline phosphatase, bilirubin and its fractions, γ-glutamyl transpeptidase.

Results: Tests for parasites revealed blastocytic invasion in 56% of the cases, while the figure for healthy individuals did not exceed 5%. The patients had a higher total bilirubin level reaching 38.73 ± 1.52 mmol/l, compared with 20.35 ± 1.12 mmol/l (p < 0.05) in the control group. The rise resulted mostly from the increased level of bilirubin's direct fraction to 23.0 ± 0.66 52 mmol/l, which equaled 5.41 ± 0.32 52 mmol/l (p < 0.05) in the control group. At the same time there were no statistically valuable changes in the indirect bilirubin level.

The patients were shown to have a significantly increased alkaline phosphatase level in comparison with healthy individuals (177.7 ± 14.87 nmol/l, p < 0.05).

The level of γ-glutamyl transpeptidase in study members (503.17 ± 3.94 nmol/l) was 2.5 times higher than in healthy (186.78 ± 3.94 nmol/l) individuals.

In addition, the patients reported dyspeptic problems, abdominal pains (76.2%), diarrhea (31.4%), and occasional skin rashes (12.7%).

Discussion/Conclusion: Testing of UC patients reveals a high degree of Blastocystis hominis invasion. Blastocytic invasion in UC patients is associated with impeded bile production, which is confirmed by a significant rise in the levels of bilirubin, alkaline phosphatase and γ-glutamyl transpeptidase.
Alterations in intestinal microflora

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Hepatoma patients suffer significant alterations in intestinal microbial flora.

Methods: The study focused on bacteriological investigation of the intestinal microflora of 136 patients afflicted with hepatomas aged 27–64, among them 37 females and 99 males.

Results: Alterations in hepatoma patients' intestinal microflora were pronounced and included increased bacterial density and a higher incidence of opportunistic flora accompanied by decreased normal microflora activity. For instance, the level Bifidobacterium declined to 3.23 ± 0.30 lg KOE/g, Lactobacillus – to 2.21 ± 0.32 lg KOE/g, Bacteroides – to 3.86 ± 0.39 lg KOE/g, while the figures for the control group were 6.72 ± 0.31 lg KOE/g, 4.24 ± 0.62 lg KOE/g and 8.76 ± 0.41 lg KOE/g respectively. The values for Candida increased twice, Staphylococcus – 1.6 times, Klebsiella – 1.8 times.

Discussion/Conclusion: Hepatoma development leads to substantial and consistent alterations in intestinal microflora.
Improved differentiation of hepatocyte-like cells from adipose tissue via epigenetic changes: Possible application for cell therapies in surgery

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Question: Due to donor organ scarcity, researchers nowadays focus on cell transplantation as alternative method to orthotopic liver transplantation. For this purpose, several groups attempt to generate hepatocyte-like cells from various adult stem or precursor cells. Aim of this study was to improve hepatic function and amount of hepatocyte-like cells via epigenetic changes.

Methods: Human Ad-MSCs were isolated from 15 different patients. For hepatic differentiation several supplement combinations of 5-azacytidine, FGF-4, dexamethasone, nicotinamid, ITS, HGF and EGF were used. The generated hepatocyte-like cells were stained for glycogen, glucose-6-phosphatase and neutral lipids. We further investigated glucose and urea metabolism as well as several phase I and II drug metabolizing enzyme activities. Expression of pluripotency-, mesoderm- and endoderm-markers was analyzed by RT-PCR.

Results: After 14 days of differentiation, Ad-MSCs show similar morphological features than primary human hepatocytes and gain the ability to accumulate glycogen and express glucose-6-phosphatase. To investigate the metabolic ability urea- and glucose metabolism was analyzed. Phase I and II enzyme activities reached levels up to 70% of primary human hepatocytes. Pre-treatment with 5-azacytidine further increased both metabolic and enzymatic activities of the cells significantly. Pluripotency of isolated Ad-MSCs were tested via Oct3/4, KLF4, Sox2 and c-Myc.

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<tr>
<td>Lipidakkumulation</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+++</td>
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<td>Ureaformation</td>
<td>-</td>
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<td>++</td>
<td>+++</td>
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<tr>
<td>Glucoseproduction</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
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<tr>
<td>Phase I/II- Enzymactivity</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+++</td>
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</tbody>
</table>

+++++ = best Result   - = worst Result

Conclusion: Our work shows, inhibition of DNA-methyltransferase leads to a better hepatic differentiation of Ad-MSCs. Furthermore, a large number of AD-MSC’s can be generated. Hence, these cells may be used for alternative autologous therapies in surgery to bridge liver dys-functions.
Coexistence of colorectal cancer with reactive inflammatory changes and fatty liver. Preliminary studies

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Introduction: The aim of the study was to analyse the histopathological picture of the liver in autopsy cases of colorectal carcinoma with no metastases into internal organs.

Material and methods: Histopathological investigations of colon adenocarcinoma and liver specimens obtained at the same time (routine HE staining; in chosen cases staining for collagen fibroplasia using the Azan method, according to Gomori, by Masson’s trichome method) were conducted on 12 autopsy cases (5 men and 7 women; age of the dead 51–85 years). In 5 cases the rectum was affected, in 7 cases the cancer was localized in the sigmoid. Taking into account the histologic malignancy grade of the cancer and depth of infiltration, G1pT2 was found in 2 cases, G2pT2 in 7 cases and G2pT3 in 3 cases.

Results: Inflammatory changes of pericholangitis chronica type were detected in the liver, with accompanying variously pronounced hepatic steatosis in all the autopsy cases with variously locally advanced colorectal carcinoma. Around bile ducts of varied calibration there were profuse inflammatory infiltrations mainly of mononuclear cells (lymphocytic and plasmatic cells), many times with addition of neutrophils and eosinophils. These changes were frequently accompanied by inflammatory infiltrations in the interstitium. Also proliferation of biliary canaliculi could be observed in some portal spaces. Fatty degeneration of the liver, concomitant with these abnormalities, was usually focal (7 cases) – mixed i.e. of macrodroplet-microdroplet type. In the remaining 5 cases it was more pronounced, even diffuse, mainly the macrodroplet type. Moreover, in half of the cases morphologic indices of portal and periportal liver fibrosis were observed, with the formation of incomplete and single complete portal-portal fibrous bridges.

Conclusions: It should be assumed that the inflammatory changes and fatty degeneration of the liver represented reactions to the neoplastic process in the colon and/or its treatment. It seems useful to investigate this type of changes using a wider and more representative group of cases in order to elucidate their potential significance in the progression of the neoplastic disease.
The correlation of *Helicobacter* spp. 16S rRNA gene, IL-1 and IL-8 mRNA expression in the liver specimens in patients with chronic liver diseases

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²Department of Biotechnology, Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Kładki 24, 80-822 Gdansk, Poland

**Introduction**: The *Helicobacter pylori* role in pathogenesis of stomach cancer is proven. *Helicobacter spp.* DNA was found in bile samples, gallbladder tissue and liver specimens in patients with various hepatobiliary diseases. However, the association of *Helicobacter* spp. with chronic liver diseases (CLD) is still controversial. The aim of the study was the estimation of *Helicobacter* spp. DNA prevalence in CLD patients' liver and its influence on a host immunological response in the liver.

**Methods**: Fifty two patients (25 females, 27 males), aged 19–68 years (mean 41 ± 13) were admitted to Department of Infectious Diseases in Gdansk because of CLD. A needle biopsy was done in routine diagnosis of CLD. The Local Ethics Committee accepted the protocol of this study. Liver samples were stored in -20°C with and without RNA inhibitor. *Helicobacter* spp. DNA was detected by nested-PCR with previously described primers. Sequencing method was used to confirm species designation. The quantitative detection of IL-1 and IL-8 mRNA was done by real-time PCR with specific primers.

**Results**: Because of low DNA quality only 27/57 liver samples were analysed. *Helicobacter* spp. DNA was detected in 16/27 cases, *H. pylori* confirmed in 15/16 cases. *H. rodentium*, *H. cetorum* was also discovered. Gene expression levels of IL-1 and IL-8 were normalized with respect to β-glucuronidase gene (GUS). IL-1 gene expression mean value was twofold higher in *H. pylori*-positive than in *H. pylori*-negative patients, but without significance. IL-8 gene expression level was similar in both groups.

**Discussion/Conclusion**: Sequence analysis showed that *H. pylori* was the most prevalent species (94%) in Polish patients. *H. rodentium* and *H. cetorum* DNA was also detected. In contrast to gastric epithelial cells, the presence of *Helicobacter* spp. in the liver of patient with CLD has no influence on IL-8 and IL-1 status.
Intraperitoneal LPS enhances Kupffer cell-dependent portal pressure increase in fibrosis

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Introduction: Recent studies demonstrated an increased risk of variceal bleeding in patients with infections like spontaneous bacterial peritonitis (SBP). Kupffer cells (KC’s) produce vasoconstrictory eicosanoids upon activation by bacterial constituents. Here, we hypothesize that the pre-existence of intraperitoneal LPS escalates portal pressure increase.

Methods: Non-recirculating liver perfusion was performed 4 weeks after bile duct ligation (BDL, n = 5) or sham operation (n = 5). Zymosan for KC activation (150 µg/ml), the thromboxane (TX) agonist U46619 (0.1 µM), or leukotriene (LT)C₄ (20 nM) was infused from 40–46 minutes after starting liver perfusion in the absence (n = 5) or presence of LPS pre-treatment (E. coli 026:B6, 1 mg/kg b.w. for 3 hours i.p., BDL n = 7, sham n = 5) with or without Gadoliniumchloride (GdCl₃, 10 mg/kg b.w., n = 5), the LT receptor antagonist Ly171883 (20 µM, n = 5) or the TX receptor antagonist BM 13.177 (20 µM, n = 5). Efflux of LTC₄/D₄/E₄ and TXB₂ in the perfusate was measured by ELISA, intrahepatic expression of TLR4 and MyD88 by Western Blot. Furthermore, portal pressure was measured in vivo (Zymosan 3.2 mg/min, minute 0–6, n = 5; additionally with LPS pre-treatment, n = 7).

Results: All BDL rats showed severe liver fibrosis, ascites and elevated in vivo portal pressure. Activation of KC by zymosan resulted in a transient increase of portal perfusion pressure from 11.0 ± 1.2 cm H₂O up to 23.5 ± 2.1 cm H₂O. Pre-treatment with LPS enhanced this transient to a long lasting increase, which sustained until the end of experiments (18.7 ± 2.5 vs. 12.1 ± 1.8 cm H₂O) in BDL but not in sham-operated animals. GdCl₃, Ly171883 or BM 13.177 reduced the maximal and long lasting increase in BDL animals by about 50–60%. The increase of perfusion pressure was paralleled by long lasting LT (185 ± 23 vs. 73 ± 31 pg/min*g liver) and TX production (3800 ± 360 vs. 1130 ± 230 pg/min*g liver) following LPS pre-treatment and KC activation. However, the response to the vasoconstrictors was not altered by LPS (maximal U46619-induced portal pressure: 28.1 ± 2.7 vs. 27.3 ± 2.3 cm H₂O; LTC₄: 26.7 ± 2.2 vs. 25.7 ± 1.8 cm H₂O). Western Blot analyses revealed an increased TLR4 and MyD88 expression after LPS pre-treatment in BDL rats. In vivo experiments confirmed that LPS pre-treatment enhances KC-dependent portal pressure increase induced by Zymosan (28.1 ± 2.3 vs. 21.4 ± 1.7 cm H₂O).

Discussion/Conclusion: Upregulation of TLR4 and MyD88 expression in fibrotic livers confers hypersensitivity to LPS. This may lead to escalation of portal hypertension by production of TX and cys-LT after endotoxin-induced KC activation. LT inhibitors may therefore represent a promising treatment option in addition to early administration of antibiotics in SBP (supported by DFG STE 1022/2-1).
Aminotransferase activity under abdominal sepsis: Multiple organ dysfunction syndrome

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Introduction: Abdominal sepsis (AS) is leading in terms of diagnostic, prophylactics, treatment complexity, and mortality amongst the most spread surgical diseases associated with digestive system. While the multiple organ dysfunction/failure syndrome (MODS), including enteral dysfunction syndrome and hepatic insufficiency became even more significant in prognosis and treatment outcome, role of messenger/regulatory and metabolic changes under AS is growing. The aim of the study was to reveal changes of systemic aminotransferases activity under AS.

Methods: Alaninaminotransferase [KF 2.6.1.2] (ALT) and aspartataminotransferase [KF 2.6.1.1] (ALT) activity we assessed dynamically using Kone®-Ultra system (U/l) in 52 AS patients (aged 41.93 ± 3.47). Patients were divided into SIRS-2 (2 SIRS symptoms) – 1 group, SIRS-3 (3 SIRS symptoms) – 2nd, and SIRS-4 (4 SIRS symptoms heavy sepsis) – 3rd group. 17 patient without abdominal pathology formed control group.

Results:

<table>
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<th>Day 5</th>
<th>Day 7</th>
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<td>24.91±1.00</td>
<td>26.50±2.57</td>
<td>18.13±2.19</td>
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<tr>
<td>1 ALT *</td>
<td>21.00±9.03</td>
<td>21.00±9.53</td>
<td>29.67±14.99</td>
<td>34.33±13.14</td>
<td>23.33±2.48</td>
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</tr>
<tr>
<td>1 AST *</td>
<td>29.63±8.52</td>
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<td>20.75±5.43</td>
<td>24.08±2.43</td>
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<tr>
<td>2 AST *</td>
<td>28.37±5.03</td>
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<tr>
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<td>24.22±4.45</td>
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<td>28.33±4.12</td>
<td>31.09±4.48</td>
<td>29.67±5.61</td>
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</table>

Discussion/Conclusion: There is no doubt concerning development of hepatic dysfunction under AS. Although clinically expressed dysfunction/insufficiency was relatively rare (only 21.15%), this study shows that hepatic cytolysis syndrome under AS develops much earlier than clinical signs or laboratory marks occur.
Another vicious circle in acute hepatoenteral dysfunction syndrome: Relationship between gut microflora, antiendotoxin core antibodies (EndoCAb) and NO levels

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Bukovinian State Medical University, Chernivtsi, Ukraine

Introduction: Multiple organ dysfunction syndrome (MODS) is the leading course of hospital associated mortality in Europe. We hypothesized that acute hepatoenteral dysfunction syndrome (HEDS) may contribute into this malicious statistics by means of a vicious circle including disorders of gut microbiocenosis with associated EndoCAb and NO levels changes.

Methods: Study included 87 patients with proven HEDS, mean age – 49.06 ± 8.34. EndoCAb assessed by ELISA, NO (nitrite/nitrate) by IEA.

Results: Colonic flora changes dramatically under HEDS: significant decrease (p < 0.05) or elimination of autochthonic anaerobic microorganisms and hyperproliferation of conditionally pathogenic Enterobacteriacea: E.coli, including Hly+ – 9.31 ± 0.62 lg CFU/g against 7.39 ± 0.56 lg CFU/g in control; Klebsiellae – 5.17 ± 0.40 lg CFU/g against 3.48 ± 0.49 lg CFU/g in control, Proteus – 6.23 ± 0.35 lg CFU/g, and Serratia – 5.49 ± 0.74 lg CFU/g (not found in control). EndoCAb changes were not uniform. In patients with negative (complicated or lethal) clinical course of disease EndoCAb IgM (1.05 ± 0.02 MMU/ml) and IgG (2.51 ± 0.11 GMU/ml) were significantly lower than in control (p < 0.01). However, positive current of the syndrome, even accompanied by MODS, gives higher figures of IgM (2.98 ± 0.23 MMU/ml) and IgG (9.57 ± 0.84 GMU/ml). In most cases (83–95.4%) significant (p < 0.05) EndoCAb growth was observed only after 5th day of disease. In 4 (4.6%) cases only IgM increased, while IgG level remained low. NO levels rose reliably (p < 0.05) in all observed patients with EDS (42.96 ± 2.75 mmol/l vs. 34.61 ± 3.07 mmol/l in healthy subjects). Strong negative correlation (r = -0.79, p < 0.05) between EndoCAb and NO levels was found only in negative course cases.

Discussion/Conclusion: Excessive growth of conditionally pathogenic Enterobacteriacea and endotoxin release is associated with insufficient production of antinuclear anti-endotoxin antibodies (EndoCAb). NO aggravate HEDS gravity by means of decreased motility and increased gut permeability. This "intestinal leakage" plays role as pathophysiologic vicious circle.
Elevated serum cholesterol: A new marker for hepatic disorder and abdominal sepsis?

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Bukovinian State Medical University, Chernivtsi. Ukraine

**Introduction:** Few publications predict increased cholesterol (CL) as a marker of acute septic complications associated with abdominal surgery and enteral dysfunction syndrome (S. Leardi et al., 2000). We hypothesized increased cholesterol as a marker of abdominal sepsis (AS) but this requires clinical validation. The aim is to reveal changes of systemic CL under AS and establish its diagnostic value.

**Methods:** CL concentration assessed dynamically using automatic system in 364 AS patients (mean age 43.91 ± 2.87 yrs). Systemic inflammatory response syndrome (SIRS) was a major criterion for AS diagnosis. All patients were divided accordingly into SIRS-2 (2 SIRS symptoms) 1st group, SIRS-3 (3 SIRS symptoms) 2nd, and SIRS-4 (4 SIRS symptoms, heavy sepsis) 3rd group. 26 patients who underwent surgery without abdominal pathology formed control group.

**Results:** CL changes were time dependent. In control group we found the permanent reduction of the CL from the day of the surgery (5.38 ± 0.19 mmol/l) till the 10th day. In contrast, reliable elevation of CL level on the first day after surgery was observed in all AS groups, with the next decrease till the 3rd day in the 1st group (from 4.68 ± 0.19 to 4.23 ± 0.25 mmol/l*), 5th day in the 2nd group (from 6.27 ± 0.29 to 5.64 ± 0.18 mmol/l*), and the 7th day in the 3rd group (from 6.79 ± 0.27 to 5.27 ± 0.32 mmol/l*). Maximum growth of CL in 1st group – 5.08 ± 0.22 mmol/l*, in 2nd group – 6.19 ± 0.73 mmol/l* on the 7th day, and 5.41 ± 0.41 mmol/l* in 3rd group on the 10th day. Correlation coefficients -0.189, 0.355, and 0.859 characterized interrelations between different research groups and control.

**Discussion/Conclusion:** Higher CL values with slower normalizing tendency generally characterize serious course of AS and poor outcome. CL significance as AS severity marker is determined by its role in cyclopentan-perhydrophenantren associated metabolism of hormones and regulatory messengers emphasizing regulatory disorders and hepatic dysfunction.
Association analyses between variants in genes conferring diet-induced gallbladder contraction and bile salt-induced gallbladder relaxation and gallstone susceptibility in two distinct human populations

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¹Department of Gastroenterology and Hepatology, University of Leipzig, Germany, ²Department of Endocrinology, University of Leipzig, Germany, ³Department of Internal Medicine II, Saarland University Hospital, Homburg, Germany, ⁴Interdisciplinary Centre for Clinical Research, University of Leipzig, Leipzig, Germany

Introduction: Formation of cholesterol gallstones requires the supersaturation of bile with cholesterol, a mucin gel in the gallbladder as nucleation matrix for monohydrate crystals and gallbladder hypomotility leading to stasis of bile. Gallbladder motility is regulated by postprandial release of cholecystokinin A (CCKA) from specialized cells in the intestine and bile salt induced release of FGF19 from the distal ileum. Following binding to the CCKAR and FGFR3 receptors on gallbladder smooth muscle cells these intestinal hormones mediate gallbladder contraction and gallbladder filling, respectively. In isolated cases of cholelithiasis, mutations in the CCKAR gene were identified.

Methods: To systematically explore the hypothesis that genetic variants in genes controlling gallbladder motility affect gallstone susceptibility we employed a HapMap-based approach and identified single nucleotide polymorphisms (SNPs) that captured the genetic variation of FGF19, FGFR3, CCKA and CCKAR (r² cut-off 0.8; minor allele frequency > 0.05). 13 SNPs were genotyped in a total of 373 gallstone carriers and 1017 control subjects from two distinct populations employing the TaqMan method. Data were analyzed in the additive model using logistic regression including age, sex and body mass index as covariates.

Results: Neither the analyses in individual populations nor the analysis in subjects from both populations combined identified an association of cholelithiasis with any of the SNPs in the four genes under investigation (all p > 0.1).

Discussion/Conclusion: In conclusion, CCKAR mutations resulting in gallstone formation appear to be exceptionally rare. Furthermore, our systematic analysis does not support a major role of genetic variation of genes controlling gallbladder motility and cholelithiasis.
TIPS procedure does not change endotoxin levels in blood of patients with alcoholic liver cirrhosis

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Background: Endotoxins represent one factor leading to portal hypertension. It has been shown in experimental and human cirrhosis that portal hypertension promotes bacterial translocation with increase of serum endotoxin. Thus we investigated whether the decrease of portal pressure following TIPS leads to a drop of endotoxin levels.

Methods: 15 patients with alcoholic liver cirrhosis receiving TIPS for refractory ascites were investigated. During the TIPS procedure and at invasive control two weeks later, portal and central venous blood was taken and endotoxin levels were measured via chromogenic limulus-Assay.

Results: We found no significant difference between endotoxin levels in portal (0.35 ± 0.3 ng/ml) and central venous blood (0.30 ± 0.2 ng/ml). Portal pressure decreased by 21% (19.9 ± 3.1 and 15.7 ± 4.8 mmHg). This decrease in portal pressure was not associated with significant changes of endotoxin levels. The portal endotoxin levels correlated directly with MELD-Score (p = 0.02) and age (p = 0.03), as well as inversely with systolic arterial pressure (p = 0.009) and portal vein flow velocity (p = 0.016) before TIPS.

Discussion: This study shows that a fall in portal hypertension did not lead to reduction of endotoxin levels in both compartments, which may well be explained by the shunt counterbalancing gut leakiness. The endotoxin levels correlated with the severity of liver dysfunction and hyperdynamic circulation in alcoholic cirrhosis.
Increased eNOS- and decreased Rho-kinase activity are associated with portal-hypertensive gastropathy in human cirrhosis

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Department of Internal Medicine I, University of Bonn, Germany

Background: Portal-hypertensive gastropathy (PHG) is characterized by an increased blood flow in gastric mucosa and submucosa. The underlying mechanism is still not known. Others and we showed that eNOS and Rho-kinase signaling take part in the regulation of the splanchnic vascular tone. Here, we investigated the activity of both enzymes in gastric mucosa of 26 cirrhotic patients (Child A 12; B 9 and C 5) and 10 controls.

Methods: Gastric mucosa biopsies were collected from patients with liver cirrhosis and PHG (14 mild, 12 severe) as well as from 10 controls. Activity of eNOS was assessed as its phosphorylation at serin-1177, and activity of Rho-kinase as phosphorylation of its substrate, moesin, at threonine-558, using Western blot with phospho- and site-specific antibodies.

Results: In the mucosa of cirrhotic patients we found significantly increased eNOS phosphorylation and decreased Rho-kinase activity as compared to non-cirrhotic controls. The activity of eNOS and Rho-kinase were depended on the grade of PHG.

Discussion: Our findings suggest that changes of the gastric mucosa in portal hypertension are driven by an altered eNOS- and Rho-kinase signaling.
Soluble TNFα-receptor p55 as prognostic marker in liver cirrhosis with portal hypertension and TIPS

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Department of Internal Medicine I, University of Bonn, Germany

Background: TNFα levels are increased in liver cirrhosis even without obvious infection signs probably by continuous endotoxin influx in portal blood. Soluble TNFα receptors (p55, p75) are useful tools to investigate TNFα release, which itself has a short half-life. p75 levels correlate with mortality in liver cirrhosis (Clin Gastroenterol Hepatol. 2008; 6: 1255–1262). Here, we investigated p55- and p75-levels in patients receiving TIPS.

Methods: 41 patients with liver cirrhosis and portal hypertension (12 viral, 29 alcoholic) received TIPS. Portal and central venous blood was withdrawn in these patients during the TIPS-procedure and in the invasive control two weeks later. In these samples levels of p55 and p75 were measured via ELISA.

Results: We found no significant difference of p55- and p75-levels in portal and central venous blood. Both p55- and p75-levels correlated directly with the MELD-score (p = 0.001) and creatinine (p = 0.0001), as well as inversely with albumin (p = 0.02) and cholinesterase (p = 0.01). The portal pressure measured during TIPS-procedure did not correlate with p55- and p75-levels. In contrast, portal pressure during the invasive control two weeks after TIPS correlated directly and systolic arterial pressure inversely with central venous p55-levels. Survival correlated inversely with central venous p55-levels (p = 0.007).

Discussion: This study shows that central venous p55 levels correlate with severity of hyperdynamic circulation as well as with increased mortality in patients receiving TIPS. This parameter could represent a prognostic marker for patients receiving TIPS.
Does the chronic intermittent hypoxia link between obstructive sleep apnea and fatty liver disease?

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*Fatih University Medical School Department of Internal Medicine, Division of Gastroenterology, Ankara, Turkey
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Introduction: Currently the common pathogenetic mechanisms in Nonalcoholic fatty liver disease (NAFLD) and Obstructive sleep apnoea (OSA) are targeted growing attention. The aim of this study is to find out the influence of CIH and OSA related parameters to severity of NAFLD with liver functions tests.

Methods: We examined the liver functions tests and ultrasonographic data of liver as well as markers of OSA severity (AHI, oxygen desaturation index, nadir oxygen saturation, percentage of time spent with SpO2 < 90% [%T < 90]) of 106 subjects.

Results: Fatty liver disease was diagnosed in 71 subjects (group-1) and remaining 35 subjects were taken as controls (group-2). As NAFLD severity increased from mild to severe form, mean AHI and ODI values also increased significantly. Our multivariate analysis showed that AHI, ODI, lowest desaturation values and percentage of sleep duration with SpO2 < 90 were independent predictors of NAFLD after adjustment for BMI, weight and insulin resistance. Furthermore, the most correlated parameter for the severity of NAFLD was found as the duration of hypoxia.

Conclusion: We postulate that sleep fragmentation, or disruption because of frequent hypopnoeic and apnoeic episodes in sleep apnoea patients, may result in elevated levels of pro-inflammatory cytokines that may promote oxidative stress.
Treatment with the non-peptide chemokine receptor 1 (CCR1) antagonist BX471 has no effect on fibrogenesis in a toxic model of liver fibrosis in mice

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¹Department of Medicine II, Saarland University Hospital, Saarland University
²Bayer Healthcare, Berkeley, CA, USA

Introduction: Induction, progression and resolution of liver fibrosis are influenced by multiple chemokines. Recently, recruitment of peripheral blood monocytes via CCR2 stimulation has been shown to induce hepatic fibrosis in a toxic mouse model (Karlmark et al. 2008). In humans, monocyte recruitment is also influenced by CCR1 signalling (Heydtman and Adams, 2009). Inhibition of CCR1 signalling by a specific non-peptide inhibitor (BX471) reduces kidney fibrosis after unilateral ureteral obstruction by suppression of leukocyte recruitment. However, currently it remains unclear whether CCR1 inhibition may also have an influence on liver fibrogenesis. We therefore aimed to study the effect of CCR1 inhibition on liver fibrosis in a toxic mouse model.

Methods: We induced fibrosis in susceptible female BALB/c mice (n = 16) by intraperitoneal injection of 0.7 ml/kg CCl₄ over 6 weeks. The verum group of 8 mice were treated with subcutaneous injections of 50 mg/kg BX471 bid, while the controls received vehicle only. Collagen contents in the liver were determined by enzymatic hydroxyproline (HYP) assays and liver histopathology was assessed.

Results: BX471 injections were tolerated well by all mice, and all mice developed portoseptal liver fibrosis. No significant differences were observed in ALT levels after 6 weeks of treatment between the two groups. Interestingly, in mice treated with BX471 there was a trend for higher hepatic collagen contents compared to control mice; however, this trend was not significant (338.4 ± 78.4 g Hyp/g vs. 279.0 ± 41.0 g Hyp/g; p = 0.078).

Discussion/Conclusion: Despite evidence of an involvement of CCR1 induced recruitment of pro-inflammatory cells in the liver during liver fibrogenesis and antifibrotic effects of CCR1 inhibition in fibrosis models in lung and kidney we could not detect a beneficial effect of CCR1 inhibition in BALB/c inbred mice challenged with a hepatotoxin. In contrast, there was a trend for augmented fibrosis in the verum group, indicating strain-specific and/or potential antifibrotic effects of CCR1 during liver fibrogenesis.
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The Gut and the Liver

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