1353
THE C-REL SUBUNIT OF NF-κB IS A REGULATOR OF HEPATOCYTES PROLIFERATION DURING RECOVERY FROM CHRONIC LIVER INJURY AND REGENERATION IN MICE

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Background and aims: NF-κB is an important regulator of cell proliferation and survival. NF-κB consists of five subunits (p50, p52, p65 (RelA), RelB and c-Rel) and increasing evidence suggests that they have individual functions in normal and diseased livers. The aim of this study was to investigate the role of the c-Rel subunit of NF-κB in liver disease. We found evidence that c-Rel is critically involved in hepatocyte proliferation. Methods: Mice hepatocytes were isolated with a pronase perfusion of the livers and a low-speed centrifugation. [3H]Thymidine incorporation was measured 72 hr after hepatocytes were isolated. In addition, FACS cell cycle analysis by propidium iodide staining was performed on freshly isolated hepatocytes. The role of c-Rel in proliferation in vivo was studied during recovery from chronic carbon tetrachloride (CCl4)-induced liver injury and partial hepatectomy and livers were stained for different markers of proliferation (BrdU, PCNA and mitotic bodies). The expression of growth regulating genes was studied with TaqMan Low Density Array (TLDA) and real-time PCR. Results: Incorporation of [3H]thymidine was 70% higher in wildtype than c-Rel knockout mice indicating a deficiency in basal hepatocyte proliferation. Flow cytometry of propidium iodide-stained nuclei demonstrated a significant difference in DNA content between wildtype and c-Rel knockout indicative of a cell cycle arrest in G1. During recovery from chronic CCl4-induced liver injury, staining for PCNA and mitotic bodies revealed significant lower levels of hepatocyte proliferation in c-Rel knockout mice. These mice also showed lower hepatocyte proliferation levels at 72 hrs after partial hepatectomy in BrdU and PCNA-stained sections (14- and 4-fold lower, successively). Transcript levels of FoxM1b associated with hepatocyte regeneration were significantly lower in c-Rel knockout mice. Conclusion: Our finding reveals that c-Rel is an important factor in control of hepatocyte proliferation in the liver following injury and regeneration. This novel function of c-Rel makes it a potential therapeutic target to stimulate hepatocyte regeneration in patients with liver fibrosis. Increasing c-Rel in livers of hepatectomy patients could enhance the liver regeneration in the remnant liver.

Disclosures:

The following people have nothing to disclose: Roben G. Gieling, Ahmed M. Elsharkawy, David Cowie, Matthew Wright, Fiona Oakley, Derek A. Mann

1354
PAI-1 DEFICIENT MICE ARE PROTECTED FROM ANGIOTENSIN II-INDUCED HEPATIC FIBROSIS

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Background. Plasminogen activator inhibitor-1 (PAI-1) is an acute phase protein known to correlate with hepatic fibrosis. It has been hypothesized that PAI-1 may directly contribute to fibrosis by inhibiting matrix resolution. Indeed, recent work by this group has also shown that PAI-1 plays a causal role in experimental hepatic fibrosis caused by bile duct ligation; however whether or not PAI-1 contributes to experimental fibrosis in other models is unclear, and is critical to extrapolation to human disease. **Methods.** Wild-type or PAI-1$^{-/-}$ mice were administered AngII (500 ng/kg/min) subcutaneously for 4 wks via an osmotic pump. Plasma and histologic indices of liver damage were determined, as well as expression of key genes involved in hepatic fibrosis. Accumulation of extracellular matrix was evaluated by Sirius red, and reticulin staining; fibrin accumulation was determined immunofluorometrically. **Results.** Chronic infusion of AngII under these conditions caused no detectable hepatocellular damage, as determined by histologic assessment and transaminases release. In contrast, AngII infusion caused significant hepatic fibrosis, as determined by Sirius red staining. The pattern of the fibrotic changes was 'chickenwire,' and differed from lobular fibrosis found after bile duct ligation. PAI-1$^{-/-}$ mice were protected from fibrosis, indicating a causal role of PAI-1 in this model of fibrosis. Protection correlated with a blunting of the increase in αSMA expression, an index of stellate cell activation. Furthermore, this protective effect correlated with an increase in the activity of MMP-9, a collagenase known to be involved in matrix resolution. The increase in the deposition of sinusoidal fibrin matrix caused by AngII under these conditions was also blunted in the PAI-1$^{-/-}$ mice. **Conclusions.** These data suggest that PAI-1 plays a causal role in mediating fibrosis caused by AngII. The protection in this model in which hepatocellular damage is minimal, indicates that PAI-1 may be profibrotic at the level of the stellate cell activation and matrix resolution. (supported, in part, by NIAAA)

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1355
INTERFERING OF CONNECTIVE TISSUE GROWTH FACTOR MRNA PROTECTS N-NITROSODIMETHYLAMINE INDUCED TOXIC LIVER INJURY IN RATS

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Connective tissue growth factor (CTGF) is a profibrogenic molecule and plays a crucial role in the pathogenesis of hepatic fibrosis. CTGF is dramatically upregulated in toxic liver injury including alcoholic fibrosis. The aim of our present investigation was to examine whether interference of CTGF at the mRNA level could prevent the progression of NDMA-induced hepatic fibrosis in rats. Liver injury was induced by intraperitoneal injections of N-nitrosodimethylamine (NDMA) in adult male albino rats in doses of 10 mg/kg body weight daily for seven consecutive days. The animals were left for an additional 7 days without any treatment. Another set of animals received intraperitoneal injections of a mammalian expression vector carrying CTGF siRNA cDNA in doses of 1 mg DNA/kg body weight, daily 2 h prior to the administration of NDMA and afterwards every day until the sacrifice of the animals on day 14. Serial administrations of NDMA resulted in activation of hepatic stellate cells, upregulation of CTGF and TGF-β1 both at mRNA and protein levels and well developed fibrosis in the liver. CTGF siRNA treated animals showed marked decrease of hepatic stellate cell activation, downregulation of CTGF and TGF—β1 both at mRNA and protein levels, remarkable reduction in fibrosis and deposition of collagen fibers in the liver and significant decrease of serum hyaluronic acid and TGF-β1. Our study

demonstrated that knockdown of CTGF mRNA has potential therapeutic application to prevent hepatic fibrogenesis.

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1356
PKCε PLAYS A CRITICAL ROLE IN CCl_4-INDUCED HEPATIC FIBROSIS IN MICE

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Background. Protein kinases C (PKC) are serine/threonine kinases that play an important role in mediating many signal transduction pathways. Previous studies have indicated that PKCs are activated in models of hepatic fibrosis, and that broad-spectrum inhibition of PKCs blunts fibrogenesis. Recent studies have also identified that the isoform, PKCε, contributes to hepatic steatosis in models of both ALD and NAFLD. However, whether or not this specific PKC isoform also contributes to later (fibrotic) stages of the disease was unclear. The purpose of the current study was to therefore test the hypothesis that knockdown of PKCε with antisense oligonucleotides (ASO) would prevent experimental hepatic fibrosis in mice caused by carbon tetrachloride (CCl_4). **Methods**. C57Bl/6J mice were injected with CCl_4 (1 ml/kg 2×/wk) for 4 wks. Mice were also administered PKCε ASO (25 mg/kg 2×/wk) or vehicle over the course of the study. This dose regimen decreased PKCε steady-state mRNA to less than 20% of saline injected animals, as determined by real-time rtPCR. Mice were sacrificed 24 h after the last injection of CCl_4. **Results**. Chronic CCl_4 administration caused robust necroinflammatory liver damage and elevated plasma transaminases (e.g., AST) values ~20-fold. Administration of the PKCε ASO did not detectably affect this variable. In contrast to indices of hepatic inflammation and damage, the accumulation of extracellular matrix (as determined by Sirius red stain) was attenuated by PKCε ASO coadministration. Whereas hepatic fibrosis was attenuated by the PKCε ASO, increases in mRNA indices of stellate cell activation and matrix synthesis caused by CCl_4 were not significantly attenuated. Instead, protection against fibrosis by administration of the PKCε ASO correlated with a blunting of the increase in expression of plasminogen activator inhibitor-1 (PAI-1), a protein that contributes to hepatic fibrosis by blocking matrix resolution. **Conclusions**. Taken together, these data suggest that PKCε plays a causal role in CCl_4-induced fibrosis. This protective effect of the PKCε ASO is not mediated at the level of fibrogenesis per se, but rather at the level of matrix resolution by blocking the induction of PAI-1. (Supported, in part, by NIAAA).

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1357
CHANGES OF LIVER-SPECIFIC MICRORNA EXPRESSION DURING CCl_4 INDUCED CHRONIC LIVER INJURY IN RECOMBINANT INBRED MICE

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Background: Hepatic fibrosis represents a complex response to chronic liver injury. Genetic determinants of liver fibrosis can be identified by quantitative trait loci (QTL) analysis in experimental crosses of inbred mouse lines (Hillebrandt et al. Nat Genet 2005). Micro (mi) RNA 122 displays liver-specific expression (Landgraf et al. Cell 2007) and is differentially expressed in hepatocellular carcinoma. Our specific aim now was to determine liver-specific miRNAs during liver fibrogenesis in a panel of genetically distinct recombinant inbred mouse lines. Methods: As genetic reference population, we availed of 30 BXD recombinant inbred lines. Each line shows a unique mosaic gene set of the two founder strains (C57BL/6J and DBA/2J). We induced liver fibrosis by challenging the mice twice weekly with CCl_4 (12 injections i.p.; 0.7 mg/kg). For phenotypic characterization, we determined histological stages of liver fibrosis and measured hepatic collagen contents. Small RNAs were isolated using the mirVana miRNA isolation kit, and quantitative RT-PCR assays were employed for miRNA expression profiling. Results: The BXD lines display marked differences in susceptibility to fibrosis. After CCl_4 challenge, histological fibrosis stages vary from F0 to F3 and strain means of collagen contents range from 181 to 506 μg/g liver. The analysis of miRNA expression shows marked induction during fibrogenesis and strain-specific differences for miRNA 122 and miRNA 192. miRNA 192 expression levels range from 3.8 to 12.6-fold induction compared to untreated F2 controls; miRNA 122 levels range from 6.3 to 10.4 times baseline in CCl_4 treated BXD lines. Conclusions: Expression of liver-specific miRNA is altered profoundly during experimental fibrogenesis. Strain-specific differences indicate that miRNA expression is under genetic control. Our findings are consistent with a network of environmental and host factors affecting miRNA expression during chronic liver injury.

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1358
KUPFFER CELL ACTIVATION BY CIRCULATING AIR PARTICULATE MATTER EXACERBATES NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN A TLR4 DEPENDENT MANNER

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Background: Fine air particulate matter (<2.5um diameter; PM2.5) exacerbates pulmonary diseases in a TLR4 dependent manner. Inhaled PM2.5 that crosses into the circulation is believed to exacerbate atherosclerosis. Our pilot study indicated that PM2.5 inhalation also significantly increased hepatic inflammation and fibrosis in a mouse model of NAFLD. The mechanism by which this occurred is unclear. We hypothesize that circulating PM2.5 may be taken up by liver cells and induce inflammation and fibrosis. Aim: To assess circulating PM2.5 uptake by liver cells and its effect on pro-inflammatory cytokine and collagen production. Methods: PM2.5 (SRM1649a) was obtained from the National Institute of Standards and Technology. Wild-type (wt) and TLR4 knockout (TLR4-/-) C57BL/6 mice were sacrificed 24h after intravenous injection of PM2.5 (500ug). Formalin-fixed liver sections were prepared and stained with H&E. Isolated primary cells and cell lines were exposed in culture to media containing PM2.5. After exposure, mRNA and protein expression levels were assayed by qRT-PCR, and ELISA or western blotting, respectively. Statistical comparisons were performed using Student's t-