

(39.2% compared to 18.4% in control). **Conclusion:** *jnk2* KO hepatocytes are resistant to TNF α -induced apoptosis. In addition to the absence JNK2, compensatory activated JNK1 contributes to the anti-apoptotic phenotype of *jnk2* KO hepatocytes through phosphorylation and stabilization of Mcl-1.

467

Aspirin Blocks Acetaminophen Induced Hepatotoxicity and Mortality in Mice - Dependent On the ASC/Caspase-1 Inflammasome

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Acetaminophen (APAP) hepatotoxicity is dependent on the immune system, but the responsible molecular pathways have not been identified. Recently it was reported that IL-1 receptor signaling is required for APAP induced hepatotoxicity (Nature Med. July 2007, vol. 13,7. p851). The protein complex of pattern recognition molecules, the adaptor protein ASC and caspase-1 constitutes the inflammasome. Activation of the inflammasome results in production of IL-1 beta, resulting in IL-1 receptor signaling. Aims: i) Determine the role of the inflammasome in APAP hepatotoxicity. ii) Test if aspirin down-modulates the inflammasome pathway. iii) Test if aspirin blocks APAP hepatotoxicity. Methods: APAP hepatotoxicity (500 mg/kg ip single injection) was induced in wild-type C57BL/6 mice, and mice deficient in inflammasome components ASC, or caspase-1. An *In-Vivo* assay of inflammation dependent on the ASC/Caspase-1 pathway was used to test if aspirin can reduce inflammation mediated by this pathway. This consisted of intraperitoneal (ip) injection of monosodium urate (MSU) (3mg/mouse), with quantification of peritoneal neutrophils at 3 hrs. The ability of aspirin (approx 6mg/kg in drinking water starting 3 days prior to APAP), the platelet inhibitor clopidogrel (30 mg/kg by gavage every 24 hrs starting 2 days prior to APAP) and a cox-1 inhibitor (SC-560, 5mg/kg by gavage every 12 hrs starting 60 hrs before APAP) to protect from APAP induced injury was tested. Results: Mice deficient in the adaptor protein ASC or caspase-1 had reduced mortality from hepatotoxic doses of APAP (P<0.027 and P<0.013 respectively). In wild-type mice aspirin reduced inflammation mediated by the ASC/Caspase-1 inflammasome as demonstrated by reduced ip neutrophil accumulation (P<0.015). In wild-type mice at 12 hrs after APAP injection aspirin improved histology and transaminitis (control group mean ALT 2365 iu/ml SD 1801 vs aspirin group mean ALT 944 iu/ml SD 373 p<0.04). In wild-type mice aspirin also reduced mortality from APAP hepatotoxicity (at 72 hrs after APAP mortality was 10/13 in control group and 3/17 in group receiving aspirin P<0.015). Clopidogrel and the cox-1 inhibitor SC-560 did not reduce mortality from APAP hepatotoxicity, suggesting that the mechanism of action of aspirin was not via inhibition of platelet degranulation or cox-1. Conclusions: The ASC/Caspase-1 inflammasome is required for APAP induced hepatotoxicity, and can be suppressed by aspirin. Aspirin reduces APAP induced mortality. Co-formulation of aspirin with APAP may reduce APAP induced liver failure.

468

CTS-1027, a Broad-Spectrum Matrix Metalloprotease Inhibitor, Attenuates Liver Injury and Fibrosis in the Bile Duct-Ligated Mouse

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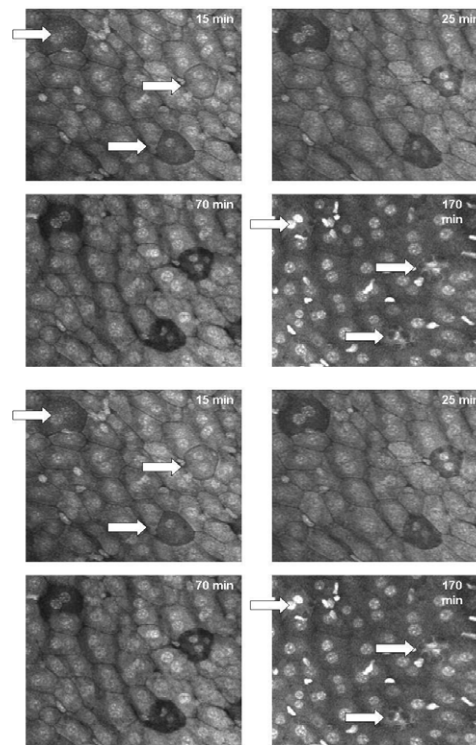
Excessive or inappropriate matrix metalloproteinases (MMP) activity has been implicated in the pathogenesis of acute liver injury. However, there are no data examining MMP inhibition in cholestatic liver injury. CTS-1027 is a broad-spectrum MMP inhibitor that inhibits MMP 2, 3, 8, 9, 12, 13 and 14, which has previously been studied in humans. Thus, our AIM was to ascertain if CTS-1027 is hepatoprotective during cholestatic liver injury. METHODS: C57/BL6 wild-type (wt) mice (6 to 8 weeks of age, n = 10 per experimental group) were used for these studies. Mice were subjected to bile duct ligation (BDL) for 14 days. Sham-operated wt mice were used as controls. Either CTS-1027 or the vector CMC (carboxymethyl-cellulose) were administered by gavage in a dose of 10 mg/kg body weight once a day. Hepatocyte apoptosis was quantified by the TUNEL assay and immunofluorescence for activated caspases 3/7. Liver injury was assessed by histopathology, and quantification of bile infarcts. Hepatic fibrosis was assessed by Sirius red staining and quantitative morphometry. Real-time polymerase chain reaction (PCR) was used to measure mRNA transcripts for collagen 1alpha (I) and alpha-smooth muscle actin. RESULTS: Following 14 days of BDL, wt mice treated with CTS-1027 demonstrated a 3-fold decrease in TUNEL and a 5-fold decrease in caspase 3/7-positive hepatocytes (p <0.01) as compared to animals treated with the vehicle. Consistent with the apoptosis data, histologic examination of livers from BDL wt animals treated with CTS-1027 also demonstrated a >70% reduction in the number of bile infarcts as compared to vehicle treated BDL mice. These differences could not be ascribed to differences in cholestasis as serum total bilirubin concentrations were nearly identical in BDL wt mice treated with CTS-1027 or the vehicle (18-22 mg/dl). Hepatic transcripts for alpha-smooth muscle actin, a marker for stellate cell activation, and collagen I were increased 6- and 8-fold in 14-day BDL mice as compared to sham-operated controls. The mRNA for these transcripts were reduced by >60% in CTS-1027 vs. vehicle-treated BDL animals. Sirius red staining of hepatic collagen was also reduced 3-fold in BDL wt mice treated with CTS-1027. Finally, overall animal survival following 14 days of BDL was also significantly enhanced in the group receiving active drug (p<0.05). In CONCLUSION, the present study demonstrates that in the BDL mouse, liver injury and hepatic fibrosis are attenuated upon treatment with the MMP inhibitor CTS-1027. Given its strong safety profile in prior human studies, CTS-1027 appears to be an attractive hepatoprotective drug for the treatment of human liver diseases.

469

In Vivo Confocal Imaging of Apoptosis of the Liver

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Introduction: Apoptosis is a dynamic process of programmed cell death. However, the sequence of events involved in this cellular suicide are still not clearly understood, partly because single cells cannot be visualized continuously over the time course of apoptosis. Aim of the present study was therefore to monitor single hepatocytes, nuclei and vessel leakiness in apoptosis of the liver with confocal laser endomicroscopy in live mice. Methods: Apoptosis was induced by injection of Jo-2 in n=16 BL/6 mice. Cellular events were followed in anaesthetized mice with a miniaturized confocal probe (FIVE1, Optiscan), providing a high resolution of 0.7 μ m and an adjustable imaging depth of 0-250 μ m, which was brought in direct contact with the tissue. Acriflavine was applied for intravital nuclear staining and FITC-labeled dextrans for intravascular contrast. *In Vivo* findings were correlated to histopathology and TUNEL staining. Results: Continuous microscopy of individual hepatocytes was performed over a time course of up to 240 min in living mice. Apoptosis started in a patchy pattern. Cytoplasmic vesicle formation was seen as early as 15 min after induction, and nuclear condensation, blebbing and disintegration were visualized continuously. Mononuclear cells were rarely seen to infiltrate areas of apoptotic hepatocytes. Enhanced vessel leakiness for high molecular weight dextrans was not observed. Discussion: This is the first study to continuously follow the physiologic processes of hepatocyte apoptosis at high resolution in live animals, monitoring early cytoplasmic disintegration followed by nuclear blebbing. Confocal endomicroscopy is a novel promising tool to visualize such dynamic processes of cells in their natural environment over a long time course and, once combined with multi-channel molecular imaging, may contribute significantly to our understanding of apoptosis.



574

Down-Regulation of Sulf1 By Hypermethylation Mediates Resistance of Hepatocellular Carcinoma Cells to Chemotherapy-Induced Apoptosis

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Background: Hepatocellular carcinoma (HCC) is the fifth most frequent cancer worldwide and the third most common cause of cancer death. Down-regulation of tumor suppressor genes in cancer frequently occurs through epigenetic mechanisms. Sulfatase 1 (SULF1) is a heparin-degrading endosulfatase which functions as a tumor suppressor in HCC. Aim: 1) To test the hypothesis that expression of SULF1 is down-regulated in HCC by gene methylation. 2) To examine the effect of treatment of HCC cell lines with the DNA methylation inhibitor, 5-aza-2' deoxycytidine, on sulfatase activity and susceptibility to chemotherapy-induced apoptosis. Methods: First, SULF1 expression level was determined in 11 HCC cell lines and 8 primary HCC tumors by real-time PCR. Second, fluorescence in situ hybridization (FISH) using a bacterial artificial chromosome (BAC) containing the SULF1 gene locus was performed on 2 HCC cell lines with low SULF1 expression and 2 with high SULF1 to determine SULF1 gene copy number. Third, the methylation status of 9 HCC cell lines and 6 primary tumors with low SULF1 expression was examined using bisulfite genomic sequencing (BGS). Fourth, the effect of treatment with 5-aza-2' deoxycytidine on sulfatase activity and drug-induced apoptosis was studied in 2 HCC cell lines with endogenous methylation of the SULF1 gene. Results: SULF1 was down-regulated in 9 out of 11 HCC cell lines and 6 out of 8 primary HCC tumors. Cell lines with low SULF1 mRNA expression had intact SULF1 genes, suggesting that they are down-regulated by epigenetic means. 10