0.338 ±0.021 in the severe cases. No mild cases had a ratio exceeding 0.1. 1H-MRS ratio had no significant relationship with body mass index (BMI), HOMA-IR or low-density-lipoprotein (LDL) cholesterol. The analysis of biochemical parameters and 1H-MRS ratio showed significant correlations of 1H-MRS ratio with γ-GTP, TG, AST, and ALT (P<0.01). An examination was performed on the 1H MRS ratio and 3D-US H/S histogram ratio. There tended to be a significant correlation between them with a correlation coefficient of r = 0.679 (Y = 1.157 + 0.951 * X; R^2 = 0.461) (P=0.015). There was a significant correlation between 1H-MRS ratio and H/K histogram ratio with a correlation coefficient of r = 0.635 (Y = 1.411 + 2.021 * X; R^2 = 0.404) (P=0.026). Conclusion: 1H-MRS can be used to quantitatively measure hepatic triglyceride content. 3D-US is useful to detect hepatic steatosis and to examine hepatic steatosis. These two methods can be easily applied to non-invasively monitor steatosis during repeated follow up measurements in clinical settings.

Tu2047

Acetaldehyde-Derived Advanced Glycation End-Products Promote Alcoholic Liver Disease

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Abstract Background: Chronic ingestion of ethanol increases acetaldehyde and leads to the production of acetaldehyde-derived advanced glycation end-products (AA-AGE). We evaluated the cytotoxicity of AA-AGE on hepatocytes and examined the role of AA-AGE in the pathogenesis of alcoholic liver disease (ALD). Methods: Rat hepatocyte cultures were treated with N-ethyllysine (NEL) or AA-AGE and the cell viability was evaluated using MTT assay. Male Wistar rats were fed with liquid diet containing 5% ethanol for 8 weeks followed with normal diet for 12 weeks. A group of animals were sacrificed at 4, 6, and 8th week and the remaining animals at 12, 14, 16, 18 and 20th week. The liver sections were stained for AA-AGE and 4-hydroxynonenal (4-HNE). Liver biopsy obtained from ALD patients was also stained for AA-AGE and 4-HNE. Results: The hepatocyte viability was significantly reduced in cultures treated with AA-AGE compared to control cultures or with NEL. Severe fatty degeneration was observed during the chronic administration of ethanol increasing from 4-8 weeks. The staining of AA-AGE and 4-HNE was strongly correlated with the degree of ALD in both rat and human. In rats, hepatic fatty degeneration was completely disappeared and the staining for both AA-AGE and 4-HNE returned to normal at 12th week of abstinence. Staining for AA-AGE and 4-HNE was completely absent in normal human liver. Conclusions: The data demonstrated that AA-AGE is toxic to hepatocytes, but not NEL. Chronic ethanol ingestion produces AA-AGE and reactive oxygen species (ROS) contributing to the pathogenesis of ALD. Abstinence of alcohol results in complete disappearance of both AA-AGE and 4-HNE suggesting that AA-AGE may play a significant role in the pathogenesis of ALD. * Presenting author

Tu2048

Histological Features Using Quantitative Morphometric Digital Image Analysis Can Predict Hpvg and Help in Sub-Classification of Cirrhosis

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Background: The definitive diagnosis of cirrhosis is histological however, it is the degree of portal hypertension, as determined by the hepatic venous pressure gradient (HVPG), that is an important determinant of the severity of cirrhosis. An HVPG > 10 mmHg, termed clinically significant portal hypertension (CSPH) is predictive of the development of complications of cirrhosis. We have earlier shown that, extent of fibrosis, nodule size and septal thickness are independent predictors of CSPH. This study aimed to confirm these findings using quantitative analysis of whole slide digital images (WSI). Methods: Fifty seven slides from 40 patients with biopsy-proven cirrhosis who had HVPG measured within six months of the biopsy were included in the study. WSI of trichrome-stained slides were created using a Zeiss Mirax Midi with a 40x objective. Various parameters were determined by software algorithms developed specifically for this project. Fibrosis area (FA) was estimated using color discrimination on trichrome stains. The software used the staining pattern to detect and outline the cirrhotic nodules and estimated the nodule size and number of nodules per mm2 of the biopsy. The following parameters were measured without knowledge of HVPG results: FA, nodule area (NA) expressed as the areas occupied by the nodules as a percentage of the total biopsy area, and number of nodules per mm2 (NN) of the liver biopsy. Using non-parametric statistics, HVPG and CSPH were correlated to each parameter, along with the appropriate use of multivariable models Result: HVPG correlated significantly with FA (pearson's correlation = 0.73, CI 0.56-0.84) and NN (correlation = 0.68, CI 0.48-0.80). On stepwise linear regression, only FA and NN independently correlated with HVPG (correlation = 0.77, p=0.005). On univariate logistic analysis, biopsies of patients with CSPH had a significantly greater FA and higher NN compared to those without CSPH (p=0.014). On binary backward logistic regression, FA was the only histological parameter predictive of the presence of CSPH (p=0.018, correlation = 0.55). Conclusion: In this study, using an objective, quantitative methodology, which can be automated, we confirm that there is a significant relationship between HVPG and a combination of increased number of nodules per mm2 (small nodularity) and the extent of fibrosis in liver biopsies. This confirmatory study validates the proposed pathologic sub-classification of cirrhosis into different severity stages based mostly on fibrosis area.

Tu2049

Hydrogen Sulfide (H2S) Regulates Liver Development and Protects From Acetaminophen (APAP)-Induced Liver Injury in Zebrafish

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APAP toxicity is the leading cause of acute liver failure in the United States, despite the success of the clinically approved antidote N-acetylcysteine. Consequently, there is an unmet need to develop new therapeutic strategies to treat APAP-induced liver injury. We have recently established a model of APAP-induced liver injury in zebrafish that closely reflects human physiology. Using a chemical screen in zebrafish, we identified modulators of H2S as potential therapies to treat APAP injury. H2S is a recently appreciated gasotransmitter, which plays an important role in regulating human physiology. H2S is generated metabolically in small quantities by the enzyme cystathionase (cth). The aim of this study was to determine to role of H2S signaling both in liver development and in recovery from APAP-induced liver injury. Exposure of zebrafish embryos to H2S donors (sodium hydrogen sulfide, NaHS, 10 µM; sodium sulfide nonahydrate, Na2S 10 µM; or diallyltrisulfide, DATS, 1 µM) from 24 h post fertilization (hpf) to 72 hpf increased liver size, as determined by in situ hybridization for the hepatocyte-specific gene liver fatty acid binding protein (lfabp). Whereas, exposure to inhibitors of cth (β-cyanoalanine, BCA, 50 µM; or DL-propargylglycine, PAG, 50 µM) dramatically decreased liver size, as determined by lfabp expression. To confirm these findings, we used morpholino antisense oligonucleotides to knock down the expression of cth, which inhibited liver development at 72 hpf. The effect of H2S on survival following APAP-induced liver injury in larval and adult zebrafish was assessed in zebrafish larvae exposed to a lethal dose of APAP (10 mM) from 72 hpf onwards in the presence or absence of chemical modulators of H2S signaling. NaHS significantly improved larval survival from 5% to 80% at 144 hpf, whereas DATS, which generates H2S at a more gradual rate, enhanced survival even more dramatically to 95%. Conversely, exposure to the cth inhibitors BCA or PAG exacerbated APAP toxicity, as evidence by a complete loss of survival at 96 hpf. Consistent with these findings, cth morphants were sensitized to APAP-induced toxicity. Finally, we examined whether the hepatoprotective effects of H2S were conserved in adult zebrafish exposed to APAP. NaHS improved survival in adult zebrafish from 8 % to 40% at 54 h post exposure (hpe), and DATS was even more effective, enhancing survival to 65% at 54 hpe. Together, these studies demonstrate an important role for H2S during liver development and suggest that H2S donors could be clinically useful therapeutic agents in the treatment of APAP-induced acute liver failure.

Tu2050

Dose-Dependent Effects of Udenafil - A New Phosphodiesterase-5 (PDE-5) Inhibitor - On Portal Hypertension: An Open-Label Dose-Finding Trial

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BACKGROUND: Non-selective beta-blockers (NSBB) are used as the standard treatment for primary or secondary prevention of esophageal variceal bleeding in liver cirrhosis. Effective long-term treatment should result in a decrease of HVPG by ≥20% from baseline or to ≤12 mmHg. HVPG decrease by ≥10% or to ≤12 mmHg after acute administration of NSBB predicts long-term clinical response, as well. However, the response rate to NSBB is only about 50% and a considerable part of patients cannot tolerate the required doses due to cardiovascular side effects. There still is an unmet clinical need for new treatment options. Several studies have shown that PDE-5 inhibitors increase portal venous blood flow and lower portal pressure both in rats with normal liver and in individuals with normal or cirrhotic liver by counteracting the sinusoidal constriction being typical for liver cirrhosis. AIMS: The effect of udenafil, a new PDE-5 inhibitor with a half-life of 12h, on HVPG in patients with portal hypertension was investigated. Here we present the final evaluation of the study. Preliminary results of lower dosing groups have been published (Gastroenterology. 2011;140(5 Suppl 1):S-598). METHODS: Patients with liver cirrhosis, HVPG ≥12 mmHg, and low risk of bleeding were eligible for this open-label, multicenter phase II trial. Patients were treated with ascending doses of oral udenafil (12.5 mg/day, 25 mg/day, 50 mg/day, 75 mg/day [5 patients for each dose], and 100 mg/day [10 patients]) once daily (OD) for one week. Routine laboratory tests and systemic hemodynamic parameters were checked regularly. On day 0 and 6 HVPG was measured prior to and one hour after drug administration. We investigated whether udenafil lowers HVPG by ≥10% in the acute setting (day 0 or/and 6) or by ≥20% or to ≤12 mmHg in the one week OD setting (day 6 post-dose compared to day 0 pre-dose). RESULTS: 30 patients qualified for per protocol analysis. Data for the one week OD setting are shown in Table 1. The doses of 12.5 to 50 mg/day had only minor effects. In 4 of 5 patients treated with 75 mg/day and in 7 of 10 patients treated with 100 mg/day HVPG was lowered by ≥10% on day 0 or/and day 6. Fig. 1 shows the mean decrease of HVPG on day 0 and on day 6 for the different dose levels. In the 10 patients who were treated with 100 mg/day the mean decrease was significant on day 0 (p= 0.012) and on day 6 (p=0,045). On day 6 the response to udenafil was consistently lower than on day 0. There were no cardiovascular side effects due to the drug in any of the groups. Standard liver biochemical tests did not change during the study. CONCLUSION: The new PDE-5 inhibitor udenafil lowers HVPG in the acute setting in a dose-dependent manner without relevant cardiovascular side effects. 75 mg or 100 mg/day seem to be suitable doses and will be investigated in further clinical studies.

Change of HVPG in one week OD setting

	12.5 mg n=5	25 mg n=5	50 mg n=5	75 mg n=5	100 mg n=10
Responders	3/5	2/5	2/5	1/5	4/10
Day 0 pre-dose 1 h post-dose	14.3 ± 4.0 13.9 ± 4.7	20.9 ± 4.4 20.0 ± 5.4	22.9 ± 4.5 21.2 ± 6.0	19.1 ± 4.8 15.1 ± 7.4	15.5 ± 2.7 12.9 ± 4.4
Day 6 pre-dose 1 h post-dose	13.6 ± 6.6 12.3 ± 4.4	22.6 ± 8.9 22.3 ± 9.4	20.9 ± 4.1 19.5 ± 4.3	18.3 ± 4.2 16.3 ± 4.1	15.2 ± 6.2 13.0 ± 5.7
% difference (day 6 post-dose vs. day 0 pre-dose)	-14.4 ± 14.9	3.1 ± 24.9	-14.0 ± 16.5	-13.5 ± 16.2	-16.8 ± 30.9

"Response" is defined as: HVPG post-dose at day 6 ≥ 20% lower than HVPG pre-dose at day 0 or ≤ 12 mm Hg