mice express higher levels of several key molecules in the Akt signaling pathway, including phosphorylated PTEN (upstream negative regulator of Akt), phosphorylated Akt, total Akt, and two Akt downstream molecules (Mcl-1, BcL-XL), when compared to the wild type mice; the levels of these molecules are lowest in the COX-2 KO mice. More cleaved PARP was observed in the liver tissues from the COX-2 KO mice when compared to the wild type mice; no PARP cleavage was found in the liver tissues from the COX-2 Tg mice after Jo2 treatment. Conclusion: Hepatocyte COX-2 protected against Fas-induced liver injury by activation of Akt pathway.

Disclosures:

The following people have nothing to disclose: Guiying Li, Chang Han, Tong Wu

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MUSCARINIC RECEPTOR BLOCKADE IN MICE EXACERBATES AZOXYMETHANE (AOM)-INDUCED CHRONIC LIVER INJURY

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Recently, we reported that repeated administration of AOM, a potent carcinogen, leads to chronic liver injury that is more severe in mice with genetic ablation of muscarinic receptor type-3 ($M_3R^{-/-}$ mice) (Gastroenterology 2008;134:A820). The aim of the present study was to determine if pharmacological blockade of muscarinic receptors mimics genetic ablation of M_3R. Male wild type mice (genetic background, 129S6/SvEv-Tac x CF1; 50%/50%; N=26) were treated with intraperitoneal AOM (10 mg/kg/wk for 6 wks). In addition, mice received either subcutaneous scopolamine (SCOP), a muscarinic receptor antagonist, (3 mg/kg/day in 2 divided doses, N=13) or PBS (N=13) for 20 weeks. An additional 4 control mice were treated with intraperitoneal PBS and subcutaneous SCOP. Mice were euthanized at 20 wks. Body weights were measured weekly. Liver weights were measured at euthanasia. Liver injury was assessed by gross inspection (0-normal; 1-mild nodularity; 2-moderate nodularity; 3-severe nodularity plus ascites), histology, immunohistochemistry for markers of proliferation (BrdU) and apoptosis (activated caspase-3), and by determination of fibrosis by Sirius red staining. Fibrotic area was calculated using Image Pro Plus 5.0. Statistical analysis was performed using Student's t-test for normally distributed data and the Mann-Whitney U-test for non-parametric data. Control mice did not develop gross or microscopic liver injury. At week 20 in AOM-treated mice, there was no difference in body weight (32.6±0.7 vs. 33.8±1 gm, PBS vs. SCOP, P>0.05) or liver weight (5.2±0.3 vs. 5.0±0.1% body wt, P>0.05). On gross examination, 21 of 25 AOM-treated mice (84%) had liver nodularity. H&E staining revealed enlarged hepatocytes, periportal and bridging liver fibrosis and bile ductular proliferation. Compared to PBS-treated mice, SCOP-treated mice had a higher liver injury score (0.8±0.1 vs. 1.3±0.2, P<0.05), increased liver fibrosis (3.4±0.8, vs. 10.3±1.0% Sirius red-stained area, SCOP vs. PBS, P<0.001), increased apoptosis (7.5±2.5 vs. 27.8±7.2 activated caspase-3-stained cells/1000 hepatocytes, P<0.05), and decreased proliferation (10.7±1.6 vs. 5.7±1.3 BrdU-positive cells/1000 hepatocytes, P=0.02). In mice, scopolamine treatment exacerbates AOM-induced chronic liver injury. These findings indicate that in AOM-induced murine liver injury, pharmacological inhibition of muscarinic receptor activation mimics genetic ablation of M_3R.

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PUTATIVE EFFECT OF CYCLOSPORINE ON HEPATOCELLULAR APOPTOSIS: ROLE OF CREMOPHOR EL AS THE VEHICLE

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INTRODUCTION: The impact of cyclosporine on hepatocyte apoptosis is controversial. In vitro, cyclosporine is known to block the mitochondrial permeability transition observed during the apoptotic process. Cyclosporine, which is very lipophilic, requires adequate solubilization with agents such as Cremophor® EL (CrEL). However, CrEL has been recognized to be associated with side effects and is also known for its capacity to inhibit the multidrug resistance p-glycoprotein (as cyclosporine does). We have previously reported that an iv-formulation of CsA protected against Fas-induced apoptosis in the liver. The following studies were performed to assess the effects of CrEL, the vehicle used in the cyclosporine formulation, on Fas-induced liver injury in vivo. METHODS: BALB/C mice were injected intraperitoneally (ip) with 0.45 μg/g anti-Fas antibody, a dose that induces acute liver injury after 5-6 hours without animal demise. The formulation-vehicle, (CrEL/Ethanol(EtOH)) was injected ip 45 min prior to anti-Fas. Animals were sacrificed at six time points up to 7.5hrs following anti-Fas. Standard enzymatic assays were used for serum-ALT/AST and caspase-3 determinations. tBID/BID was evaluated by Western blot and GSH/GSSG by HPLC.RESULTS: Induction of hepatocellular injury in vivo by anti-Fas led to a characteristic sequence of biochemical events. The appearance of tBID (35.3±4.9% BID) and induction of Caspase-3 activity (26.8±5.8 U/L) were first observed 3h post-injection. Pre-treatment with CrEL/EtOH abolished the increases in tBID (6.8±2.0%; P<0.001 vs. Fas-only) and Caspase-3 activity (3.8±2.2 U/L; P<0.001 vs. Fas-only). Five hours post-anti-Fas, serum-ALT/AST levels rose to 7584±2419/5369±1158 U/L (Fas-only) and intracellular glutathione (GSH) were decreased (34.6±7.5% control, P<0.001 vs. control). This was associated with the appearance of massive liver injury as shown on histology. CrEL/EtOH significantly attenuated the increase in serum-ALT/AST (895±474/1230±448 U/L; P<0.05 vs. Fas-only) as well as the decrease in GSH levels (86.6±3.9% control; P<0.001 vs Fas-only). Morphological analysis also supported the effect of CrEL/EtOH on Fas-induced apoptosis. CONCLUSION: Our results demonstrate a protective effect of the vehicle used in the intravenous formulation of cyclosporine on all levels of the injury process studied in Fas-induced hepatocyte apoptosis. Experiments are underway to determine the mechanisms responsible for this observation. These results might explain the discrepancy observed in some of the previous experiments performed with cyclosporine. They also underline the importance of the choice of vehicle in drug development.

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