Ursodeoxycholic Acid Inhibits Hepatic Cystogenesis in Experimental Models of Polycystic Liver Disease

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Disclosures: None

BACKGROUND

➢ Polycystic Liver Diseases (PCLDs) are genetic disorders characterized by bile duct dilatation, and/or biliary cyst development which progressively grow and are the main cause of morbidity. Current therapies show short-term and/or modest benefits, being liver transplantation the only curative option.

➢ Novel evidence suggests that PCLDs share several pathological mechanisms that may provide a key for treatment, considering as a central event the cAMP-mediated hyperproliferation of cystic cholangiocytes (*Perugorria et al. Nature reviews Gastroenterology and Hepatology 2014*).

➢We have recently reported that cholangiocytes from PCK rats (model of ARPKD) have increased levels of cAMP and decreased intracellular calcium levels. Restoration of $[Ca^{2+}]i$ levels with a calcium ionophore inhibits the cAMP-dependent cholangiocyte hyper-proliferation via activation of the PI3K/AKT pathway *(Banales et al. Hepatology 2009;49:160-74)*.

HYPOTHESIS

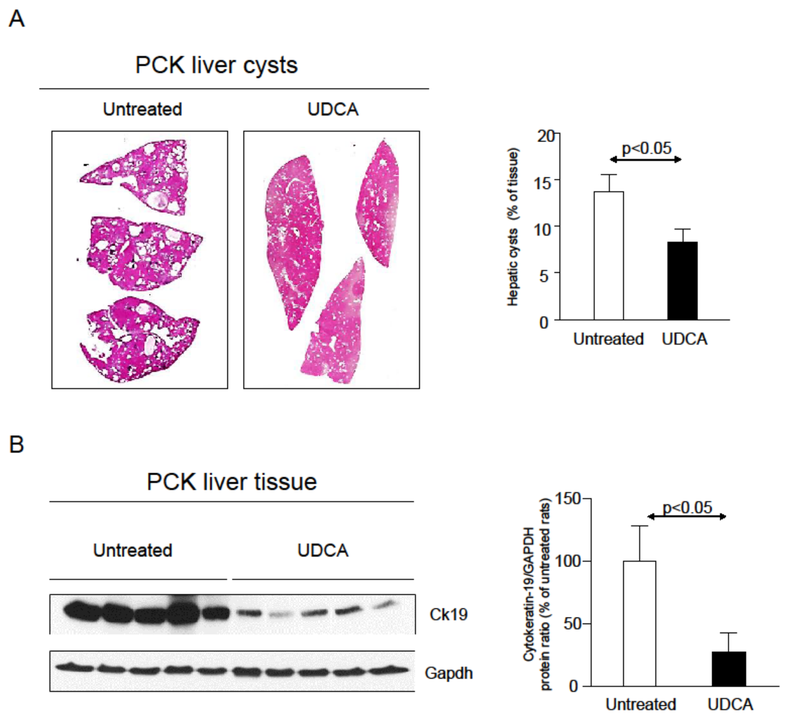
Therapies specifically directed to inhibit the cAMP-dependent hyperproliferation in PCLDs by normalization of [Ca2+]i levels could have a key therapeutic value.

➢ Ursodeoxycholic acid (UDCA) is an endogenous hydrophilic bile acid used for the treatment of several cholestatic disorders.

➢Experimental evidence suggests that UDCA stimulates the hepatobiliary secretion of bicarbonate and protects cholangiocytes against the cytotoxicity of hydrophobic bile acids, and that these effects are mediated in part by increasing the [Ca2+]i .

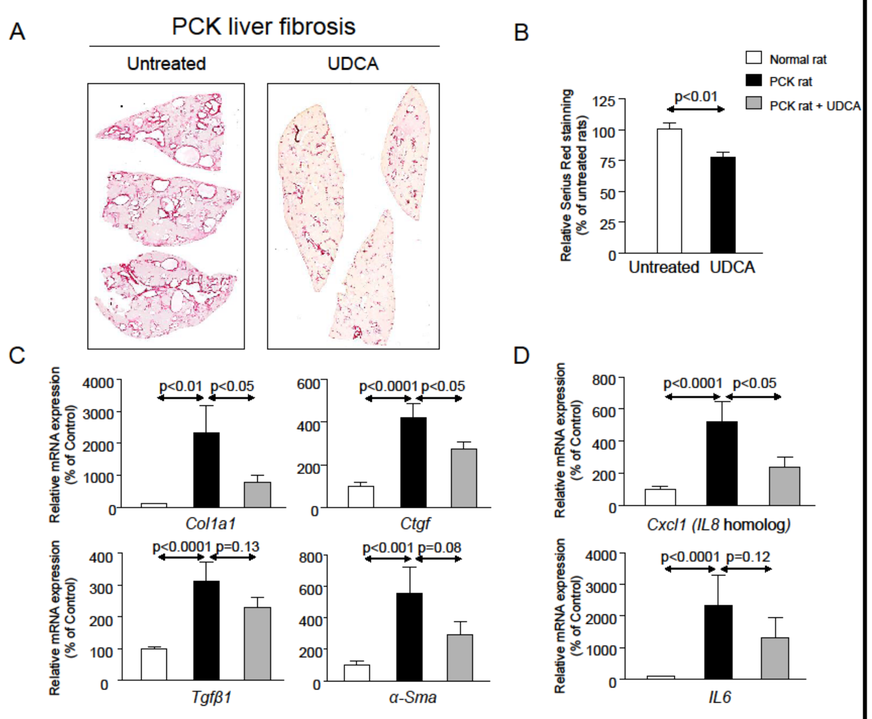
RESULTS

Figure 1
Chronic treatment of PCK rats with UDCA halts hepatic cystogenesis.



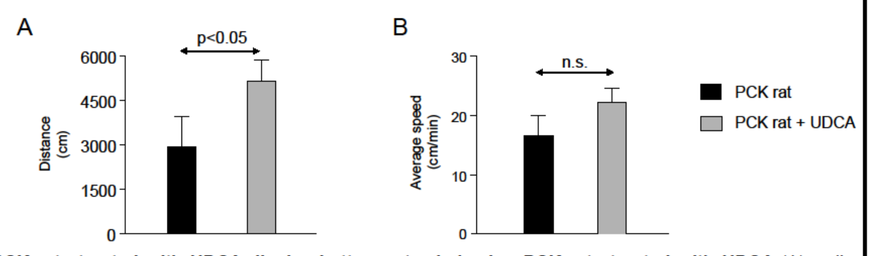
Treatment of PCK Rats with UDCA Halts Hepatic Cystogenesis and Fibrosis. (A) Representative images and bar graph showing that hepatic cystic areas are decreased in UDCA-treated PCK rats compared to PCK matched controls. Livers were stained with hematoxylin-eosin. (B) This event was associated with hepatic downregulation of the cholangiocyte-marker Ck19 at protein level. The western blot shows Ck19 protein expression of 3 representative PCK rats treated with UDCA or placebo. Bar graph shows the Ck19 quantification (n=10 and n=9 in PCK and PCK+UDCA groups, respectively).

Figure 2
Chronic treatment of PCK rats with UDCA halts hepatic cystogenesis and inflammation



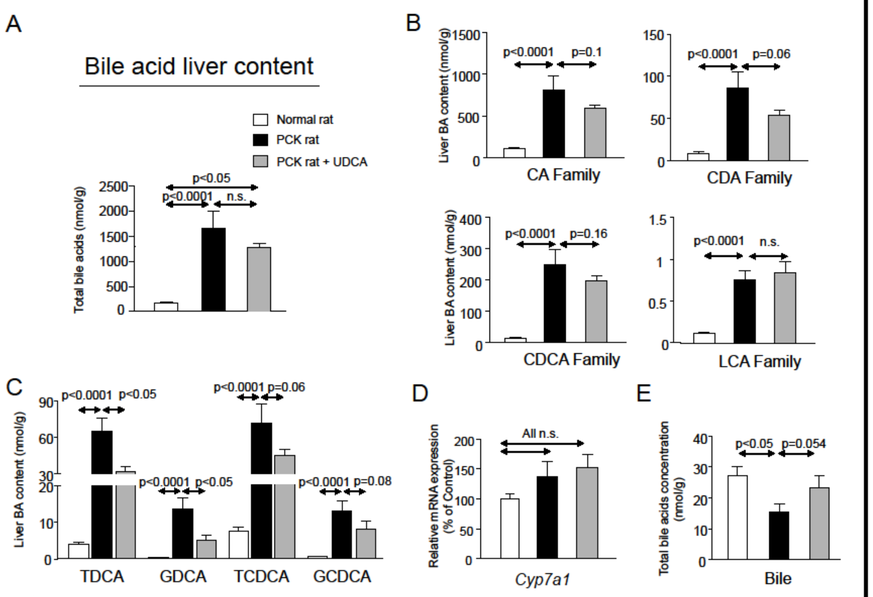
(A) Representative images of sirius-red staining and (B) bar graph showing that the hepatic areas of collagen deposition are decreased in UDCA-treated PCK rats compared to PCK matched controls. In PCK rat livers, the increased mRNA expression levels of (C) pro-fibrotic genes *collagen-1a1* and *Ctgf* and (D) pro-inflammatory gene *IL6* were found downregulated after UDCA-treatment; in addition, the increased mRNA expression levels of pro-fibrotic genes *Tgfβ1* and *α-Sma*, as well as pro-inflammatory gene *IL8*-homolog *Cxcl1*, showed an almost statistical significant downregulation after UDCA-treatment.
N=12 in normal (untreated) and n=10 in PCK (untreated and UDCA-treated) groups unless specified.

Figure 3
UDCA administration to PCK rats improves their motor behaviour



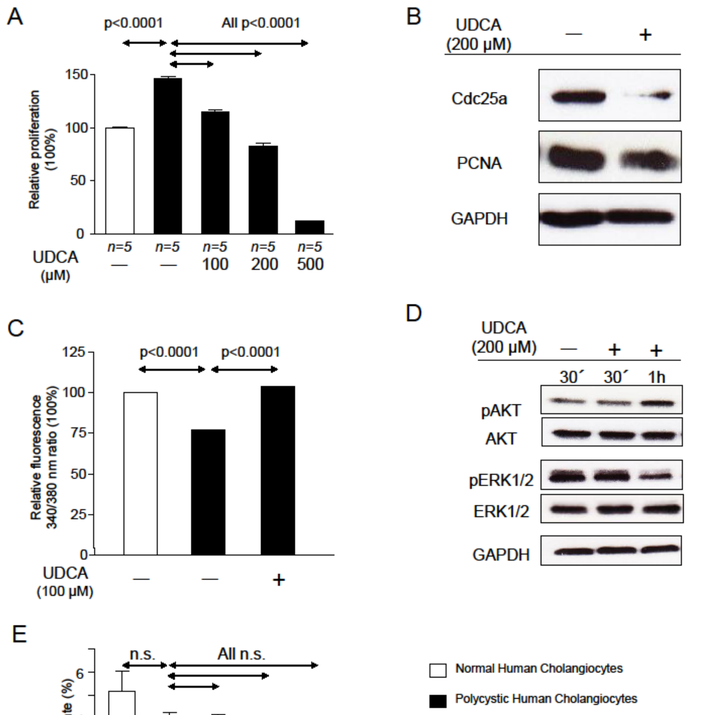
PCK rats treated with UDCA display better motor behavior. PCK rats treated with UDCA (A) walk more distance than untreated PCK rats in 5 min and (B) do not alter their average speed. N=10 in both PCK groups (untreated and UDCA-treated).

Figure 4
PCK rats show increased intrahepatic concentration of bile acids compared to normal control rats, and UDCA decreases accumulation of cytotoxic bile acids



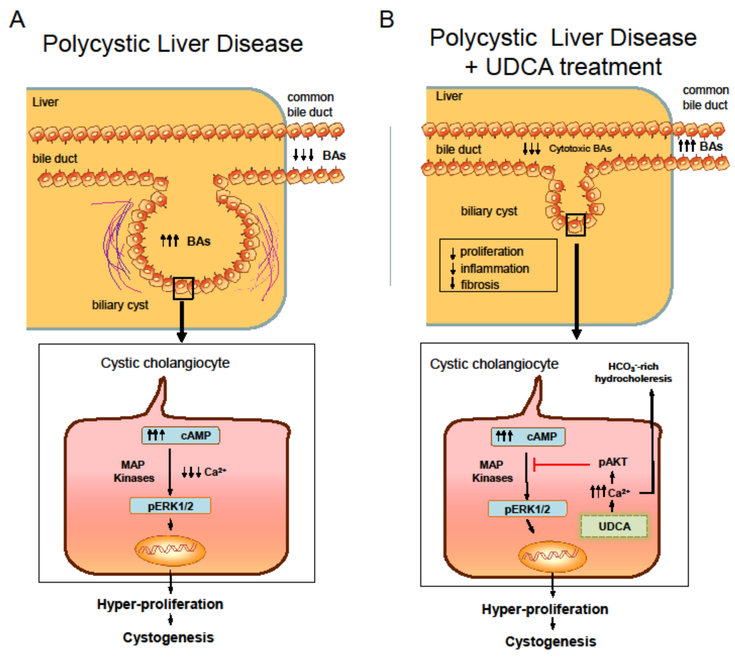
(A) The total bile acids (BAs) concentration in PCK rats is increased compared to normal control animals, and UDCA-treatment does not alter this increased BAs concentration. (B) The levels of both primary −CA (cholic) and CDCA (chenodeoxycholic)− and secondary −DCA (deoxycholic) and LCA (lithocolic)− major species of bile acids were higher in the liver of PCK than normal rats; UDCA-treatment showed an almost statistical significant reduction of CA, CDA and CDCA families in PCK rats compared to non-treated PCK animals. (C) The intrahepatic levels of the toxic dihydroxylated bile acids −TDCA (taurodeoxycholic),TCDCA (taurochenodeoxycholic), GDCA (glycodeoxycholic) and GCDCA (glycochenodeoxycholic)− were markedly higher in PCK than in normal rats. UDCA decreased the hepatic concentration of TDC and GDC and almost the concentration of TCDC and GCDC in PCK rats. (D) The mRNA expression levels of Cyp7a1 were similar in the liver of the normal (untreated) and PCK rats (untreated and UDCA-treated). (E) The total bile acids concentration in the bile of PCK rats is decreased compared to control normal rats; this reduction was almost completely prevented by treatment with UDCA. N=12 in normal (untreated) and n=10 in PCK (untreated and UDCA-treated) groups.

Figure 5
UDCA inhibits hyperproliferation of polycystic human cholangiocytes



UDCA inhibits the proliferation of polycystic human cholangiocytes by raising the intracellular calcium levels that increases AKT phosphorylation and diminishes ERK1/2 phosphorylation. (A) Polycystic human cholangiocytes hyperproliferate compared to normal human cholangiocytes in culture. This hyperproliferation was inhibited by UDCA in a dose-dependent manner. N=5 in all experimental groups. (B) UDCA dowregulates the protein expression of the pro-mitotic markers Cdc25a and PCNA. (C) Polycystic human cholangiocytes show decreased intracellular free calcium levels compared to normal human cholangiocytes in culture, which are restored by UDCA. (D) UDCA upregulates the phosphorilation of AKT and downregulates the phosphorylation of ERK1/2. (E) The basal apoptotic rates do not differ between normal and polycystic human cholangiocytes in the presence or absence of UDCA; n=5 in all experimental groups.

Figure 6
Working model of the steps by which UDCA may modulate hepatic cystogenesis in PCLDs



(A) Hepatic cystogenesis in PCLDs is characterized by cAMP/PKA/MEK/ERK1/2-dependent cholangiocyte hyperproliferation associated to decreased intracellular free calcium. Cystic fluid contains increased bile acids (primary, secondary and toxic) concentration. (B) UDCA restores the diminished intracellular calcium levels in polycystic cholangiocytes that induce the phosphorylation of AKT and the subsequent inhibition of the cAMP/MAP kinases-dependent proliferation. This results in decreased hepatic cystogenesis, fibrosis and inflammation. UDCA, through its choleretic features, may also flow the increased concentration of cytotoxic bile acids in the cystic fluid, preventing their biliary pathogenic effects.

CONCLUSION

UDCA represents a promising therapeutic tool for the treatment of patients with PCLDs and deserves clinical testing