Research Article

JOURNAL OF HEPATOLOGY

Ursodeoxycholic acid inhibits hepatic cystogenesis in experimental models of polycystic liver disease

Patricia Munoz-Garrido[1,2], José J.G. Marin[2,3], María J. Perugorria[1,2,4], Aura D. Urribarri[5], Oihane Erice[1], Elena Sáez[5], Miriam Úriz[5], Sarai Sarvide[5], Ainhoa Portu[2,5], Axel R. Concepcion[5], Marta R. Romero[2,3], María J. Monte[2,3], Álvaro Santos-Laso[1], Elizabeth Hijona[1,2], Raúl Jimenez-Agüero[1], Marco Marzioni[6], Ulrich Beuers[7], Tatyana V. Masyuk[8], Nicholas F. LaRusso[8], Jesús Prieto[2,5], Luis Bujanda[1,2,†], Joost P.H. Drenth[9,†], Jesús M. Banales[1,2,4,5,*]

[1]*Department of Liver and Gastrointestinal Diseases, Biodonostia Health Research Institute – Donostia University Hospital, University of the Basque Country (UPV/EHU), San Sebastián, Spain;* [2]*National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd, Instituto de Salud Carlos III), Spain;* [3]*Experimental Hepatology and Drug Targeting (HEVEFARM), Biomedical Research Institute of Salamanca (IBSAL), University of Salamanca, Salamanca, Spain;* [4]*IKERBASQUE, Basque Foundation for Science, Spain;* [5]*Division of Gene Therapy and Hepatology, CIMA of the University of Navarra, Pamplona, Spain;* [6]*Department of Gastroenterology, "Università Politecnica delle Marche", Ancona, Italy;* [7]*Department of Gastroenterology and Hepatology, Tytgat Institute for Liver and Intestinal Research, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands;* [8]*Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA;* [9]*Department of Gastroenterology & Hepatology, Radboud University Nijmegen Medical Center, The Netherlands*

Background & Aims: Polycystic liver diseases (PLDs) are genetic disorders characterized by progressive biliary cystogenesis. Current therapies show short-term and/or modest beneficial effects. Cystic cholangiocytes hyperproliferate as a consequence of diminished intracellular calcium levels ($[Ca^{2+}]_i$). Here, the therapeutic value of ursodeoxycholic acid (UDCA) was investigated.

Methods: Effect of UDCA was examined *in vitro* and in polycystic (PCK) rats. Hepatic cystogenesis and fibrosis, and the bile acid (BA) content were evaluated from the liver, bile, serum, and kidneys by HPLC-MS/MS.

Results: Chronic treatment of PCK rats with UDCA inhibits hepatic cystogenesis and fibrosis, and improves their motor behaviour. As compared to wild-type animals, PCK rats show increased BA concentration ([BA]) in liver, similar hepatic *Cyp7a1* mRNA levels, and diminished [BA] in bile. Likewise, [BA] is increased in cystic fluid of PLD patients compared to their matched serum levels. In PCK rats, UDCA decreases the intrahepatic accumulation of cytotoxic BA, normalizes their diminished [BA] in bile, increases the BA secretion in bile and diminishes the increased [BA] in kidneys. *In vitro*, UDCA inhibits the hyperproliferation of polycystic human cholangiocytes via a PI3K/AKT/MEK/ERK1/2-dependent mechanism without affecting apoptosis. Finally, the presence of glycodeoxycholic acid promotes the proliferation of polycystic human cholangiocytes, which is inhibited by both UDCA and tauro-UDCA.

Conclusions: UDCA was able to halt the liver disease of a rat model of PLD through inhibiting cystic cholangiocyte hyperproliferation and decreasing the levels of cytotoxic BA species in the liver, which suggests the use of UDCA as a potential therapeutic tool for PLD patients.

© 2015 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.

Keywords: Polycystic liver diseases (PLDs); Ursodeoxycholic acid (UDCA); Cystogenesis; Cholangiocyte; Intracellular calcium; Therapy.

Received 1 December 2014; received in revised form 26 May 2015; accepted 27 May 2015; available online 1 June 2015

* Corresponding author. Address: Department of Liver and Gastrointestinal Diseases, Biodonostia Health Research Institute – Donostia University Hospital, Paseo del Dr. Begiristain s/n, E-20014 San Sebastian, Spain. Tel.: +34 943006067. *E-mail address:* jesus.banales@biodonostia.org (J.M. Banales).

† Both authors contributed equally to this work.

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ADPLD, autosomal dominant polycystic liver disease; AKT, v-akt murine thymoma viral oncogene homolog 1; ALP, alkaline phosphatase; α-Sma, alpha-smooth muscle actin; ALT, alanine aminotransferase; ARPKD, autosomal recessive polycystic kidney disease; AST, aspartate aminotransferase; BA, bile acid; BDL, bile-duct ligation; $[Ca^{2+}]_i$, intracellular calcium concentration; CA, cholic acid; cAMP, 3′-5′-cyclic adenosine monophosphate; CDCA, chenodeoxycholic acid; CK19, cytokeratin 19; Col1a1, collagen type 1 alpha 1; Ctgf, connective tissue growth factor; Cxcl1, C-X-C motif ligand 1 [interleukin 8 (IL8) homolog]; Cyp7a1, cytochrome P450 7A1; DCA, deoxycholic acid; DMEM/F-12, Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12; ERK1/2, extracellular signal-regulated kinases 1/2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; IL6, interleukin 6; LCA, lithocholic acid; MAP kinases, mitogen-activated protein kinases; MEK, mitogen-activated protein kinase kinase; mRNA, messenger RNA; pAKT, phosphorylated AKT; PCK rat, polycystic kidney rat; PLDs, polycystic liver diseases; PCNA, proliferating cell nuclear antigen; pERK, phosphorylated ERK; PKA, protein kinase A; PKDs, polycystic kidney diseases; PKHD1, polycystic kidney and hepatic disease 1; qPCR, quantitative polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; Tgfβ1, transforming growth factor beta 1; TUDCA, tauroursodeoxycholic acid; UDCA, ursodeoxycholic acid.

Introduction

Polycystic liver diseases (PLDs) are a group of genetic disorders characterized by bile-duct dilation and/or development of biliary cysts, which progressively grow and are the main cause of morbidity [1]. The large volume of hepatic cysts may cause different symptoms and complications such as severe hepatomegaly, abdominal distension with local pressure, back pain, hypertension, gastro-esophageal reflux, dyspnea, as well as bleeding, infection and/or rupture of the cysts [1]. PLDs are inherited in dominant or recessive form and developed alone or in association with polycystic kidney diseases (PKDs). Autosomal dominant polycystic liver disease (ADPLD) develops cysts preferentially in the liver, whereas the kidney is also markedly affected in autosomal dominant (ADPKD) and recessive (ARPKD) forms of PKDs [1]. Local surgery of symptomatic cysts is commonly employed and partially prevents and/or ameliorates the disease progression. However, this therapy show short-term and/or modest beneficial effects, liver transplantation being the only curative option [1].

Current research is focused on elucidating the molecular mechanisms involved in the pathogenesis of PLDs with the aim of identifying new potential targets for pharmacological therapy [2]. Novel evidence suggests that PLDs share several pathological mechanisms that may provide a key for treatment [2]. In this regard, cAMP-mediated hyperproliferation of cystic cholangiocytes is considered a central event in PLDs [2]. Different experimental and clinical studies have previously demonstrated the potential therapeutic value of somatostatin analogues in partially reducing the increased intracellular cAMP concentration $[cAMP]_i$ and the concomitant cAMP-mediated hyperproliferation in cystic cholangiocytes [3–10], indicating that drugs specifically directed to inhibit this pathway could have key therapeutic value. In this regard, we have previously reported that cholangiocytes from PCK rats, an animal model of ARPKD, are characterized by increased $[cAMP]_i$ and diminished intracellular calcium concentration $[Ca^{2+}]_i$ [11]. Restoration of $[Ca^{2+}]_i$ with a calcium ionophore inhibited both basal and cAMP/PKA/MEK/ERK1/2-stimulated proliferation of PCK rat cholangiocytes via activation of the PI3K/AKT pathway [11]. These data indicate that normalization of $[Ca^{2+}]_i$ in cystic cholangiocytes may have a translational impact. Different molecules and mechanisms are able to upregulate the $[Ca^{2+}]_i$ in cholangiocytes. In particular, ursodeoxycholic acid (UDCA; 3α, 7β-dihydroxy-5β-cholanoic acid) is a minor endogenous hydrophilic bile acid (BA) used for the treatment of several cholestatic disorders [12,13]. Experimental evidence suggests that UDCA stimulates the hepatobiliary secretion of bicarbonate and protects cholangiocytes against the cytotoxicity of hydrophobic BAs, and that these effects are mediated in part by increasing the $[Ca^{2+}]_i$ [12,13]. In an experimental model of obstructive cholestasis, such as bile-duct ligation (BDL) in rats, cholangiocytes hyperproliferate as a consequence of increased $[cAMP]_i$ and decreased $[Ca^{2+}]_i$ resulting in bile-duct proliferation. Interestingly, treatment of this animal model with UDCA for 1 week inhibits the aforementioned cholangiocyte hyperproliferation by normalizing the $[Ca^{2+}]_i$ [14,15].

In the present study, we explored the potential therapeutic value of UDCA in inhibiting the hepatic cystogenesis of experimental models of PLD and the molecular mechanisms of action.

Materials and methods

Treatment of PCK rats with UDCA

The PCK rat (Charles River Laboratory) is a well characterized animal model of ARPKD that presents a spontaneous mutation in the *PKHD1* orthologous gene [16,17]. Hepatic cystogenesis and fibrosis, as well as serum biochemical markers were analysed in non-treated wild-type (n = 12) and PCK (n = 10) rats, as well as in PCK rats (n = 10) orally-treated with UDCA (25 mg/kg/day) for 5 months. The details are described in Supplementary data.

Determination of messenger RNA expression

Total RNA was obtained from rat liver tissue with TriReagent (Sigma). Detection and quantification of messenger RNAs (mRNAs) were performed via reverse transcription polymerase chain reaction (RT-PCR) and real-time quantification (qPCR) as we previously described [18] (cf. Supplementary Table 1 for specific primers). The *Gapdh* gene expression was employed as a normalizing control.

Western blot analysis

Changes in protein expression and/or phosphorylation were detected through immunoblotting using cell extracts or PCK rat liver tissue as detailed in Supplementary data.

Bile acid measurement

BAs were analysed in total liver, bile collected before the sacrifice, peripheral blood and total kidney of rats by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) after C18-based extraction as we previously reported [19,20]. Similarly, BA concentration ([BA]) was determined in cystic fluid of patients with PLD and compared with their paired samples of peripheral blood. All human procedures were approved by the Medical Ethical Committee of the Radboudumc, Nijmegen, The Netherlands.

Animal motor behaviour

The degree of motor activity in PCK rats was analysed using the *Open field* test [21]. One day prior to sacrifice, both untreated and UDCA-treated PCK rats were individually placed in the centre of a quadrangular box (511 cm × 511 cm) and the free trajectory was monitored for 5 min using an infrared X-ray camera. The distance and the average speed were measured. Each animal performed one trial.

Isolation and culture of normal and polycystic human cholangiocytes

Normal and polycystic human cholangiocytes were isolated and characterized as we previously described [18].

Measurement of intracellular Ca^{2+} level in normal and polycystic human cholangiocytes in culture

Intracellular Ca^{2+} level was determined as we previously described [11]. Briefly, normal and polycystic human cholangiocyte cultures were loaded with 4 μM Fura-2/AM (Biotium) for 30 min in HCO_3^- -Ringer's solution (with 2 mM $CaCl_2$) at 37 °C and washed twice. Then, the fluorescence intensities (dual excitation at 340/380 nm and emission at 510 nm) were measured overtime in the presence or absence of 100 μM UDCA using an inverted Leica DMIRB fluorescent microscope equipped with a software to acquire and analyse the images.

Proliferation assays in normal and polycystic human cholangiocytes in culture

Proliferation of both normal and polycystic human cholangiocytes was assessed in the presence or absence of different doses of UDCA, TUDCA (tauroursodeoxycholic acid) or GDCA (glycodeoxycholic acid) (i.e. 100, 200 and/or 500 μM) and/or inhibitors (MEK inhibitor U0126: 15 μM; Calbiochem) in quiescent medium (DMEM-Ham's-F12 with 3% fetal bovine serum and 1% penicillin/streptomycin) for 48 h at 37 °C using the *CellTrace™ CFSE Cell Proliferation Kit* (Invitrogen) for flow cytometry (*FC 500 MCL System*, Beckman Coulter).

Download English Version:

https://daneshyari.com/en/article/6102832

Download Persian Version:

https://daneshyari.com/article/6102832

Daneshyari.com