Non-conjugated chenodeoxycholate induces severe mitochondrial damage and inhibits bicarbonate transport in pancreatic duct cells

We read the manuscripts by Lee and Muallem1 and Venglovecz et al2 recently published in Gut with great interest. In both articles the authors highlighted the role of pancreatic ducts in maintaining the integrity of the pancreas. Venglovecz et al showed that a high concentration of the non-conjugated chenodeoxycholate (CDC) inhibits pancreatic ductal bicarbonate secretion; however, the mechanisms of the inhibition were not clarified. This is a follow-up study in which we show that this reduction of ductal bicarbonate secretion by CDC is evoked by inhibition of glycolytic and oxidative (caused by severe mitochondrial damage) metabolism with a consequent depletion of intracellular ATP levels.

Physiologically, pancreatic ductal fluid and HCO₃⁻ secretion are necessary to wash out the digestive enzymes from the acinar cells into the duodenum. Under pathophysiological conditions toxic factors (such as bile acids and ethanol) involved in the pathogenesis of acute pancreatitis have dual effects on ductal HCO₃⁻ secretion.1 Low doses of CDC and ethanol were found to stimulate fluid and HCO₃⁻ secretion. However, these toxic agents in high concentrations inhibit the secretion. These data suggest that an elevation in pancreatic ductal fluid and HCO₃⁻ secretion may have protective roles. However, since under physiological conditions the pressure in the main pancreatic duct is higher than in the bile ducts, it is still controversial as to whether bile acids enter the pancreatic ductal tree.

We have recently shown that a high concentration (1 mM) of the non-conjugated bile acid CDC has strong inhibitory effects on the activities of acid/base transporters (Na⁺/H⁺ exchanger (NHE), Na⁺/HCO₃⁻ cotransporter (NBC) and Cl⁻/HCO₃⁻ exchanger (CBE)) in pancreatic ductal epithelial cells (PDECs). Although the fluid and HCO₃⁻ secretion. However, these toxic agents in high concentrations inhibit the secretion. These data suggest that an elevation in pancreatic ductal fluid and HCO₃⁻ secretion may have protective roles. However, since under physiological conditions the pressure in the main pancreatic duct is higher than in the bile ducts, it is still controversial as to whether bile acids enter the pancreatic ductal tree.

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conjugated bile acid glycochenodeoxycholate (GCDC) also enters the cell, it has no effect on the ion transporters.

In this study intra/interlobular pancreatic ducts were isolated from the pancreas of guinea pigs. CDC or GCDC (0.1–1 mM) was administered basolaterally for 1–10 min. The intracellular pH (pHi) of PDECs was measured by microfluorometry using BCECF (2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein). The intracellular ATP level (ATPₐ) was determined using Mg-Green which has been shown indirectly to reflect ATP. It is well described that an elevation in fluorescence intensity caused by the increase in free intracellular Mg²⁺ concentration suggests a reduction of ATPₐ. The ATPₐ measurement was performed in standard HEPES-buffered solution whereas the pHi measurements were performed in 25 mM HCO₃⁻/CO₂-buffered solution. Morphological changes of PDECs were evaluated by transmission electron microscopy.

Administration of a low concentration (0.1 mM) of CDC or GCDC for 1–10 min had no effects on the intracellular organelles (data not shown). In addition, a high concentration (1 mM) of the conjugated GCDC did not induce morphological changes. Importantly, exposure to 1 mM CDC for 10 min greatly damaged all of the mitochondria (figure 1A). The mitochondria swelled up and their inner membranes were disrupted. Other intracellular organelles such as nuclei or Golgi apparatus seemed to be unaltered. In agreement with this, Benedetti et al. have shown that non-conjugated bile salts induce mitochondrial damage in bile epithelial cells.

For positive control experiments we used the mitochondrial toxin carbonyl cyanide m-chlorophenyl hydrazone (CCCP, 100 µM) which mimicked the effect of CDC on mitochondria. Next we set out to investigate whether ATPₐ is affected due to the mitochondrial damage. Administration of a low concentration of CDC or GCDC for 10 min had no effect on the ATPₐ; however, a high concentration of CDC and/or CCCP markedly decreased the ATPₐ (figure 1B, C). Although 1 mM GCDC also decreased ATPₐ, this depletion was reversible and significantly less than the depletion caused by CDC or CCCP. The fact that CDC caused a greater depletion of ATPₐ suggests that bile acids have additional effects which further decrease ATPₐ. Therefore, we used the deoxy-glucose (DOG)/iodoacetamide (IAA) model which inhibits intracellular glycolytic metabolism. Administration of 10 mM DOG and 5 mM IAA decreased ATPₐ (figure 1B, C). Importantly, CCCP or DOG/IAA administered after high concentrations of CDC resulted in further ATPₐ depletion; however, their effects were significantly smaller after CDC than when administered alone. Exposure of pancreatic ducts to CCCP and DOG/IAA totally mimicked the effect of CDC. These data indicate that CDC inhibits both the oxidative and glycolytic metabolism of PDECs.

Finally, we provided evidence that ATPₐ depletion is crucial in the toxic inhibitory effect of CDC on the ion transporters. To characterise the effects of ATPₐ depletion on the activities of NHE, NBC and CBE, we used the NH₄Cl pulse technique in HCO₃⁻/CO₂-buffered solution. CCCP strongly inhibited NBC, NHE (recovery from acid load) and CBE (recovery from alkaline load) (figure 2A–C). Administration of 10 mM DOG and 5 mM IAA also inhibited the ion transporters (figure 2A–C). Significantly higher inhibition was evoked by parallel administration of CCCP and DOG/IAA. These observations suggest that depletion of ATPₐ is the key factor which inhibits NBC, NHE and CBE. Our findings are in accordance with the results of other authors who showed that depletion of ATPₐ inhibits many of the ion transporters such as the NHEs in different cell types.³

In conclusion, our results clearly show that (1) a high concentration of the non-conjugated bile acid CDC, but not the conjugated bile acid GCDC, causes mitochondrial damage followed by ATPₐ depletion; (2) CDC inhibits the glycolytic metabolism of

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**Figure 2** Effect of bile acids on the rate of intracellular pH (pHi) recovery from an alkali and an acid load. (A) Representative pH traces showing the effects of 1 mM glychenodeoxycholate (GCDC), 1 mM chenodeoxycholate (CDC), 100 µM carbonyl cyanide m-chlorophenyl hydrazone (CCCP) or 10 mM deoxyglucose (DOG) with 5 mM iodoacetamide (IAA) administered from the basolateral membrane of pancreatic ductal epithelial cells (PDECs) in the presence of 25 mM HCO₃⁻/CO₂. CDC, the mitochondrial toxin CCCP and DOG/IAA markedly inhibited both recoveries. (B) Summary data for the initial rate of recovery from alkaline load. CDC, CCCP and DOG/IAA decreased the recovery from alkaline load. (C) Summary data for the initial rate of recovery from acid load. CDC, CCCP and DOG/IAA decreased the recovery from acid load. Data are shown as the means ± SEM from 25–35 regions of interests (ROIs) in 5–7 ducts. a, p<0.01 vs control, b, p<0.01 vs CDC.
FDECs, and (5) ATP, depletion by itself can be responsible for the impaired fluid and HCO$_3^-$ secretion. The relationship between mitochondrial function and HCO$_3^-$ secretion and the differences between the effects of conjugated and non-conjugated bile acids needs further investigation.

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J Maléth, V Venglovecz, Zs Rázga, L Tiszlavicz, Z Rakonczay Jr, P Hegyi

1First Department of Medicine; 2Department of Pharmacology and Pharmacotherapy; 3Department of Pathology, University of Szeged, Szeged, Hungary

Correspondence to Dr Péter Hegyi, First Department of Medicine, University of Szeged, PO Box 427, H-6701 Szeged, Hungary; hep@in1st.szote.u-szeged.hu

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How accurate are the Swansea criteria to diagnose acute fatty liver of pregnancy in predicting hepatic microvesicular steatosis?

We noted that in two recent reports in Gut, none of 62 patients diagnosed by the Swansea criteria to have acute fatty liver of pregnancy (AFLP) underwent liver biopsy.1 2

We retrospectively assessed the accuracy of the Swansea criteria to predict hepatic microvesicular steatosis in 34 patients with suspected pregnancy-related liver disease who underwent liver biopsy at our centre between 1998 and 2006. These patients tested negative for other causes of acute liver dysfunction such as hepatitis viruses (hepatitis B virus surface antigen (HBsAg), hepatitis C virus (HCV) antibody, immuno-}

globulin (Ig) M hepatitis A virus (HAV) antibody, IgM hepatitis E virus (HEV) antibody), malarial parasite and sepsis (blood culture). No patient gave a history of ingestion of a potentially hepatotoxic drug. We excluded 10 patients (details in figure 1).

The remaining 24 patients included in this study were at 56 (21–40) weeks gestation (median (range)), 25 (17–29) years old and 71% were primigravida. The interval from the first symptom to presentation to our centre was 5 (1–14) days.

Abnormal variables in Swansea criteria for AFLP at presentation in the 24 study patients were: vomiting (11/21 patients), abdominal pain (3/14), polydipsia/polyuria (1/1), encephalopathy (9/24), hyperbilirubinaemia (24/24), hypoglycaemia (8/24), hyperuricaemia (8/10), leucocytosis (20/25), ascites/bright liver on ultrasound (16/22), elevated transaminases (23/24), hyperammonaemia (2/2), renal impairment (17/24), coagulopathy (23/24) and hepatic microvesicular steatosis (17/24). Some variables were not recorded/not tested in all 24 patients (eg, vomiting recorded in only 21; uric acid tested in only 10). Baseline laboratory results in study patients were: serum total bilirubin, 12.7±6.2 mg/dl (mean±SD); alanine aminotransferase (ALT), 119±62 IU/l; prothrombin time, 39±50 s; serum creatinine, 1.7±0.9 mg/dl; and MELD (Model for End-Stage Liver Disease) score, 30 (range 13–46).

Liver biopsy was done either immediately postmortem (5/24) or postnataally via the transjugular route (19/24). Biopsies were fixed in formalin and routinely stained for H&E and

Figure 1 Flowchart of patients with suspected pregnancy-related liver disease who underwent liver biopsy. *Refers to diffuse/perivenular hepatic microvesicular steatosis.
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