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The effects of bile acids on pancreatic ductal cells

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Bile acids (BAs) are natural end products of cholesterol metabolism (10). The physiological functions of BAs are the emulsification of lipid aggregates and solubilization of lipids in an aqueous environment. The major BAs in humans are chenodeoxycholic acid (CDC) and cholic acid (CA), which are known as primary BAs since they are synthesized in the liver (36). Before secretion by hepatocytes, primary BAs are conjugated with either taurine or glycine, which increases their polarity and water solubility. Secondary bile acids such as deoxycholic acid (DCA) and lithocholic acid (LCA) are produced in the colon by bacterial dehydroxylation of the primary bile acids. Under physiological conditions, BAs are temporarily stored in the gallbladder and are released to the intestine. Most of the BAs are then efficiently reabsorbed from the ileum and transported back to the liver via the portal vein (enterohepatic circulation). Under normal, physiological conditions, BAs cannot get into the pancreas. However, under pathophysiological conditions, such as obstruction of the ampulla of Vater by an impacted gallstone, bile can diffuse into the pancreatic ducts and trigger pancreatitis (25). Unfortunately, we do not know the concentration of bile acids that can reach the pancreatic ductal cells under pathological conditions. It probably varies among patients and mainly depends on the duration of ampullary gallstone obstruction. However, previous studies have shown that relatively low concentrations of BAs (25-200 µM) are able to cause intracellular Ca^{2+} signaling and cell death in acinar cells (19, 39).

The close relationship between the passage of a gallstone and the development of acute pancreatitis (AP) has been known for more than a hundred years (25) and has been confirmed in a number of studies (22, 26, 35). However the pathogenesis underlying the development of biliary AP is not well understood.

Most of the research investigating the pathomechanism of AP has been done on pancreatic acinar cells. These studies have demonstrated that the central intra-acinar events in pancreatitis are elevation of intra-acinar Ca^{2+} concentration, premature activation of trypsinogen and the activation of the proinflammatory transcription factor, nuclear factor-κB which leads to pancreatic injury (6, 14-16, 28, 34). It has been shown that one of the most toxic BAs to acinar cells is the secondary BA, taurolithocholic acid (TLC) that forms from LCA after re-absorption from the intestine. The sulfated form of TLC causes Ca^{2+} signaling in pancreatic acinar cells via an inositol 1,4,5-trisphosphate (IP_3)-dependent mobilization of intracellular Ca^{2+} (39). The elevated intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) can lead to enzyme activation (28) and/or cell death (19) and result in severe acute necrotizing pancreatitis.

In contrast, the role of pancreatic ductal epithelial cells (PDECs) in the pathogenesis of biliary AP has received much less attention, despite being the first pancreatic cell type exposed to the refluxed bile.

Pancreatic ducts can be divided into three main types on the basis of their size and location. These are the main duct, the inter- and intralobular ducts. The main duct mostly collects and drains the juice secreted by other branches of the ductal tree, whereas intra/interlobular ducts are the main sites of HCO_3^- secretion. Although, several studies have shown that ductal fluid and HCO_3^- secretion are crucially important to maintain the integrity of the pancreas (4, 8, 9, 17), the role of PDECs in the development of AP has only been highlighted recently. *In vivo* studies have shown that pancreatic hypersecretion (with hypoproteinaemia) occurs in the early phase of AP, which develops into hyposecretion during the onset of pancreatitis (8, 9, 17). This hypersecretion may represent a defence mechanism by washing out the toxic factors from the pancreas. The beneficial effect of fluid hypersecretion is further supported by studies in which secretin, one of the major secretagogues of ductal fluid secretion, was shown to reduce the severity of caerulein-induced AP (32, 33). In addition, impaired or insufficient ductal fluid secretion, such as observed in cystic fibrosis, increases the risk of AP (11, 12). Taken together, these data strongly suggest that PDECs represent an important and essential protective mechanism in the exocrine pancreas.

1. Effect of Bile Acids on the Main Pancreatic Duct

In the 1980s, it was postulated that the breakdown of the pancreatic duct permeability barrier is a risk factor for the development of AP. Therefore, researchers extensively investigated the effect of BAs on the morphology and permeability of the main pancreatic duct. In these *in vivo* studies various BAs were perfused through the cannulated main duct and the permeability of the pancreatic duct mucosal barrier was measured using different techniques (5, 13, 23, 29-31). It has been shown that BAs in high concentrations (2-15 mM), made the ducts permeable to molecules as large as 20,000-daltons, whereas they are normally impermeable to molecules over 3,000-daltons (5, 13). BAs in millimolar concentrations also increase the permeability of the main duct to Cl^- and HCO_3^- (23, 29-31). The effect of dihydroxy BAs was significantly greater than the effect of trihydroxy BAs on the permeability of these anions, most probably because trihydroxy BAs are less lipid soluble and therefore less toxic to the cells. The changes in ductal permeability were in accord with the changes in the morphology of the ductal epithelia. Perfusion with higher concentrations of BAs (15 mM) caused disruption of cell integrity, flattening of the ductal epithelium and cell loss (5). Due to their detergent properties, this harmful effect of BAs is not surprising.

It was also highlighted that infected bile is more harmful to the duct cells than sterile bile (23, 29, 30). The higher toxicity of infected bile is probably due to bacterial deconjugation, which produces more toxic unconjugated BAs. The toxicity of BAs mainly depends on their solubility and the degree of ionisation. At neutral pH, unconjugated BAs exist in an unionized (7), electrically neutral form and therefore can pass easily through the cell membrane. In contrast, glycine- and taurine-conjugated BAs, which have a lower pK_a (around 4 and 2 respectively), are ionized at neutral pH (7) and are therefore less lipid soluble.

Although, *in vivo* animal studies have a very important significance, their relevance to human disease is doubtful. One of the major problems with these studies is that BAs were used in relatively high concentrations; probably higher than would be present in the pancreatic duct if reflux occurs. Moreover, at these extremely high concentrations, BAs caused excessive and uncontrolled destruction of both acini and ducts.
In vitro studies have allowed the investigation of more pathophysiologically relevant effects of BAs on the ductal epithelium. Okolo et al. studied the

effects of BAs (100 μM – 2 mM) on the ion conductances and monolayer resistance of cultured PDECs isolated from the accessory pancreatic duct of dog (24). They found that taurodeoxycholate (TDC) and taurochenodeoxycholate caused a concentration-dependent increase in both Cl^- and K^+ conductances, whereas the trihydroxy BA, taurocholate, was completely ineffective. The increases in Cl^- and K^+ conductances were mediated via elevation of $[Ca^{2+}]_i$ and blocked by 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) and charybdotoxin, respectively. Using Ussing chambers, they could localize the Cl^- conductance to the apical, and the K^+ conductance to the basolateral membrane of PDECs. In addition, they showed that only higher concentrations of BAs decreased the monolayer transepithelial resistance. Similar results have been found in bovine PDECs, where TDC markedly increased transepithelial ion transport and decreased the electrical resistance of the tissue (1). On the other hand, TDC caused dose-dependent mucosal damage (2) and at higher concentrations extensive loss of the epithelial cell lining (1).

2. Effect of Bile Acids on the Intra/Interlobular Pancreatic Ducts

Although, the earlier studies described above characterized the effects of BAs on the permeability and morphology of the main pancreatic duct, no information was available about their effects on the smaller ducts. However, the development of microdissection techniques for the isolation of small intra/interlobular ducts (3), led to a break-through in our understanding of the cellular physiology of the ductal cells.

The major physiological function of the intra/interlobular pancreatic ductal cells is to secrete a HCO_3^--rich alkaline fluid that washes digestive enzymes out of the gland and neutralizes acid chyme in the duodenum (3). The effects of BAs on HCO_3^-- secretion have been intensively investigated in the last few years (20, 37, 38), and these studies suggest that the role of ductal cells in the pathomechanism of biliary AP is complex.

Our research group has shown that both basolateral and luminal administration of either non-conjugated or glycine-conjugated forms of CDC causes a dose-dependent intracellular acidification in guinea pig PDECs (38). Interestingly, basolateral administration of 1 mM CDC for 6–8 min damaged the membrane integrity, and the duct cells lost the fluorescent dye very quickly. The same concentration of CDC had no toxic effects on the luminal membrane. Okolo et al. also found differences between the effects of BAs on the luminal and basolateral membranes (24). In addition, both CDC and glycochenodeoxycholate (GCDC) induced a dose-dependent increase in $[Ca^{2+}]_i$ via phospholipase C- and IP_3 receptor-mediated mechanisms. GCDC had a smaller effect on intracellular pH (pH_i) and $[Ca^{2+}]_i$ than CDC, most probably because conjugated BAs are ionised at neutral pH and therefore require active transport mechanisms for cellular uptake.

We also found that the effect of CDC on ductal HCO_3^- efflux depends on its concentration (38). At low concentrations (0.1 mM), CDC significantly stimulated HCO_3^- efflux by a DIDS–sensitive Cl^-/HCO_3^- exchange mechanism. The stimulatory effect of CDC was observed only when CDC was added to the lumen of the ducts and was dependent on Ca^{2+} mobilization. In contrast, high concentrations of CDC (1 mM) caused pathological Ca^{2+} signaling and strongly inhibited HCO_3^- efflux. This inhibitory effect of high concentrations of CDC was independent of changes in $[Ca^{2+}]_i$ and was observed when CDC was applied to either the luminal or the basolateral membrane of the ducts (38). The effect of the conjugated GCDC on pH_i and $[Ca^{2+}]_i$ suggest that although GCDC can enter the cells,

most probably by a transporter-mediated mechanism, it had no effect on HCO_3^- efflux at both high and low concentrations.

The differences in the effects of low and high concentrations of the CDC suggest that non-conjugated BAs have a specific mode of action on PDECs which strongly depends on their concentration. The key question is the identification of the cellular mechanisms by which BAs exert these opposite effects. Perides et al. have recently identified the presence of a G-protein-coupled bile acid receptor-1 (*Gpbar1*) on the apical membrane of acinar cells (27). They showed that *Gpbar1* knock out mice were completely protected against TLC 3-sulfate-induced pancreatitis and suggested that this receptor has a central role in the BA-induced acinar cell injury in mice. In contrast, guinea pig pancreatic ductal cells do not express *Gpbar1* (37) suggesting that this receptor is not involved in the effect of CDC on PDECs.

3. Stimulatory Effect of Low Concentrations of Bile Acids

Since CDC only increased HCO_3^- efflux when applied to the luminal membrane, it is likely that the stimulatory effect of CDC is due to activation of one, or more, Ca^{2+}-dependent apical transporters. Several ion transporters have been identified in the apical membrane of PDECs. The cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel is a Ca^{2+}-independent transporter, making it unlikely that this transporter is involved in the stimulatory mechanism of CDC. In fact, we have recently shown that CDC does not influence the activity of CFTR in PDECs (18). In contrast, the SLC26 anion transporters (21, 40) and the Ca^{2+}-activated Cl^- channel (CaCC) are known to be activated by an increase in $[Ca^{2+}]_i$ suggesting that these transporters could be the target for the CDC-induced increase in Ca^{2+}.

Using the whole cell configuration of the patch clamp technique, CDC failed to activate CaCC, but induced a robust and reversible increase in K^+ currents (37). The activated currents could be blocked by the specific large-conductance Ca^{2+}-activated K^+ channel (BK) inhibitor, iberiotoxin. In contrast, the small- and intermediate Ca^{2+}-activated K^+ channel inhibitors, UCL1684 and TRAM34 had no effect on the CDC-activated currents. Luminal administration of iberiotoxin completely blocked the stimulatory effect of CDC on HCO_3^- efflux in microperfused ducts. In contrast, basolateral iberiotoxin had no effect on the luminal CDC-stimulated HCO_3^- efflux. These data strongly indicate that BK channels play a central role in the stimulatory effect of CDC on HCO_3^- efflux and that they are localized to the luminal membrane of the ductal cells. This latter hypothesis has been confirmed by immunohistochemistry which showed strong expression of BK channels at the apical membrane of guinea pig intra/interlobular ducts (37). Moreover, activation of BK channels by luminal administration of a pharmacological compound, NS11021, increased HCO_3^- efflux in a similar manner to CDC. Our hypothesis is that activation of apical BK channels leads to the hyperpolarisation of the apical plasma membrane, which in turn increases the electrochemical driving force for anion efflux through the CFTR Cl^- channels and also by electrogenic SLC26 anion exchangers **(Fig. 1).** The stimulatory effect of low concentrations of CDC highlights the importance of ductal fluid secretion in the protection of the pancreas.

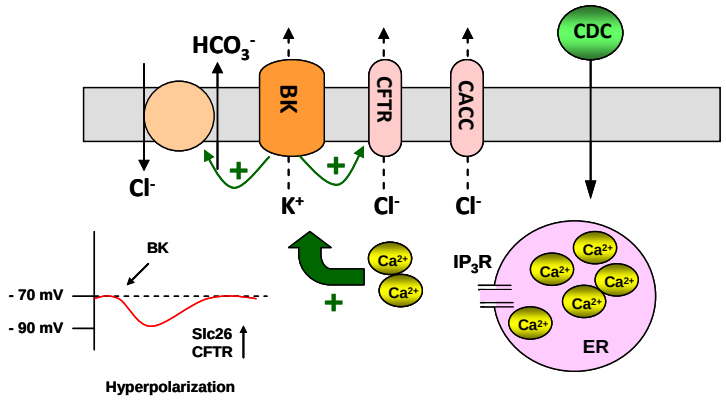

Figure. 1. Cellular mechanism of the stimulatory effect of chenodeoxycholate on pancreatic ductal HCO_3^- efflux. Low concentrations of CDC induce an elevation of intracellular calcium concentration $[Ca^{2+}]_i$ via phospholipase C and inositol 1,4,5-triphosphate receptor - mediated mechanisms. The increase in $[Ca^{2+}]_i$ will activate large-conductance Ca^{2+}-activated K^+ channel (BK) which leads to the hyperpolarisation of the plasma membrane which in turn increases the electrochemical driving force for anion secretion through cystic fibrosis transmembrane conductance regulator Cl^- channel (CFTR) and SLC26 anion Cl^-/HCO_3^- exchangers. CDC: chenodeoxycholate, CACC: Ca^{2+}-activated Cl^- channel, ER: endoplasmic reticulum, IP_3R: inositol 1,4,5-trisphosphate receptor, +: stimulation.

The increased volume of fluid can be beneficial in several ways:

i. High concentrations of HCO_3^- in the secreted fluid promote the deprotonation of BAs to less toxic bile salts.
ii. The increased volume of fluid decreases the concentration of BAs in the ducts.
iii. The greater ductal flow may push stones through the papilla of Vater to clear the obstruction.
iv. Increased fluid secretion may wash out the toxic BAs from the ductal tree in order to avoid pancreatic injury.

4. Inhibitory Effect of High Concentrations of Bile Acids

If the stimulated secretion is not able to wash out BAs from the ductal tree, the luminal concentrations of BAs will increase further. In this situation, high concentrations of CDC cause pathologic Ca^{2+} signaling and inhibition of the acid/base transporters of PDECs (38). We have recently provided evidence that mitochondrial damage and depletion of intracellular ATP concentration ($[ATP]_i$) are the most crucial factors in the toxic inhibitory effect of CDC on pancreatic ductal secretion (20). Administration of 1 mM CDC to PDEC for 10 minutes caused swelling of all of the mitochondria and disruption of their inner membranes. Damage of the mitochondria markedly and irreversibly reduced $[ATP]_i$. Exposure of pancreatic ducts to carbonyl cyanide m-chlorophenyl hydrazone and deoxyglucose/iodoacetamide (inhibitors of oxidative phosphorylation and the glycolytic pathway respectively) totally mimicked the effect of 1 mM CDC. These data indicate that CDC inhibits both the oxidative and glycolytic pathways in PDECs. In addition, it has been shown that $[ATP]_i$ depletion is crucial in the inhibitory effect of CDC on ductal ion transport mechanisms. In the absence of intracellular ATP the acid/base transporters do not work properly, which leads to impaired fluid secretion and finally cell death. In this case, BAs in the duct could reach the acinar cells, either by diffusion up the ductal tree or by leakage into the gland interstitium, where they will switch on pathologic Ca^{2+} signaling and trigger AP **(Fig. 2).**

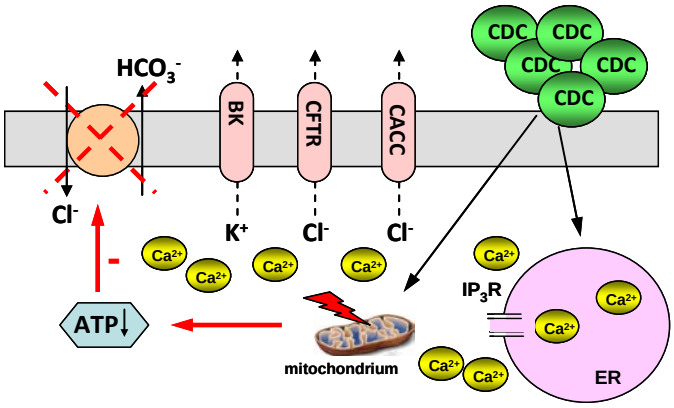

Figure. 2. Cellular mechanism of the inhibitory effect of CDC on pancreatic ductal HCO_3^- efflux. High concentrations of CDC induce toxic inracellular Ca^{2+} signaling and depletion of $[ATP]_i$ which will inhibit all the acid-base transporters in the PDEC, including the Cl^-/HCO_3^- exchangers. CDC: chenodeoxycholate BK: large conductance Ca^{2+}-activated K^+ channel, CACC: Ca^{2+}-activated Cl^- channel, CFTR: cystic fibrosis transmembrane conductance regulator Cl^- channel, ER: endoplasmic reticulum, IP_3R: inositol 1,4,5-trisphosphate receptor, -: inhibition.

Taken together, both *in vivo* and *in vitro* studies indicate that once BAs reach the ductal epithelium, depending on their concentration, they either stimulate or inhibit pancreatic ductal bicarbonate efflux. This biphasic effect of BAs on ductal secretion may be a significant factor in the pathomechanism of biliary AP.

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