REVIEW ARTICLE

Review of experimental animal models of biliary acute pancreatitis and recent advances in basic research

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Abstract

Acute pancreatitis (AP) is a formidable disease, which, in severe forms, causes significant mortality. Biliary AP, or gallstone obstruction-associated AP, accounts for 30–50% of all clinical cases of AP. In biliary AP, pancreatic acinar cell (PAC) death (the initiating event in the disease) is believed to occur as acinar cells make contact with bile salts when bile refluxes into the pancreatic duct. Recent advances have unveiled an important receptor responsible for the major function of bile acids on acinar cells, namely, the cell surface G-protein-coupled bile acid receptor-1 (Gpbar1), located in the apical pole of the PAC. High concentrations of bile acids induce cytosolic Ca^{2+} overload and inhibit mitochondrial adenosine triphosphate (ATP) production, resulting in cell injury to both PACs and pancreatic ductal epithelial cells. Various bile salts are employed to induce experimental AP, most commonly sodium taurocholate. Recent characterization of taurolithocholic acid 3-sulphate on PACs has led researchers to focus on this bile salt because of its potency in causing acinar cell injury at relatively low, sub-detergent concentrations, which strongly implicates action via the receptor Gpbar1. Improved surgical techniques have enabled the infusion of bile salts into the pancreatic duct to induce experimental biliary AP in mice, which allows the use of these transgenic animals as powerful tools. This review summarizes recent findings using transgenic mice in experimental biliary AP.

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Introduction

Biliary acute pancreatitis (BAP) refers to acute pancreatitis (AP) caused by biliary calculous diseases. It is the most common cause of AP and is associated with significant morbidity and mortality.[1] Obstruction of the common biliopancreatic duct (CBPD) by gallstones blocks the efflux of pancreatic zymogens, creates elevated pressure in the pancreas and leads to bile reflux into the pancreatic duct. A number of experimental models have been designed to recreate this condition. The purely surgical models of BAP, such as

closed duodenal loop-induced pancreatitis and CBPD or pancreatic duct ligation-induced pancreatitis have been reviewed in detail previously[2,3] and thus are beyond the scope of the current review. Cannulation of the pancreatic duct has enabled researchers to apply the bile components into the pancreas of experimental animals in a more controlled way. One of the most significant recent advances involves the adaptation of this technique to a mouse model, which opens the door to transgenic studies of BAP. Another significant development refers to observations of a variety of bile salts that induce pathological responses in single acinar cells *in vitro*, elucidating some molecular mechanisms of the detrimental action of pancreatic bile. In addition, some

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important knowledge on the role of pancreatic duct epithelial cells (PDECs) in the pathogenesis of BAP has recently emerged. Finally, this review summarizes recent advances in basic research and highlights further research prospects in the pursuit of effective clinical interventions in BAP.

Bile acids and their targets

Bile acids, in addition to their known functions in dietary fat absorption and cholesterol metabolism through nuclear receptors,[4,5] also induce cellular signalling through the recently described cell surface G-protein-coupled bile acid receptor-1 (Gpbar1), found in brown adipose tissue, intestine and gallbladder in mammals.[5–7] When Gpbar1 receptor is activated by bile acids, it can modulate energy homeostasis, lipid homeostasis and glucose homeostasis,[8,9] and stimulate gallbladder filling.[6]

In hepatocytes, choleretic and cholestatic bile acids activate and inhibit store-operated Ca^{2+} channels, respectively, through mechanisms which involve reversible redistribution of stromal interaction molecule 1.[10,11] In pancreatic acinar cells (PACs), bile acid transporters, namely, the Na^+-dependent Na^+ taurocholate co-transporting polypeptide (NTCP), located at the apex of the cell, and HCO_3^--dependent organic anion transporting polypeptide-1 (OATP1), located on the basolateral portion of the cell membrane, have recently been identified.[12] The bile receptor Gpbar1 is also located in the apical region of the cell and is positioned to respond to bile in the lumen of the duct.[13] The bile acid transporters and receptor constitute the molecular machinery responsible for the pathological action of refluxed or circulated bile acids on PACs.[14,15] Detailed bile acid uptake and targets in PACs have been reviewed recently by Lerch and Aghdassi.[14]

Effects of bile acids on acinar cells

In 2002, Voronina *et al.*[16] demonstrated the effect of taurolithocholic acid 3-sulphate (TLC-S) on isolated murine PACs. TLC-S induced Ca^{2+} oscillations at concentrations as low as 25 µM and triggered responses in almost all cells at 200 µM. At higher concentrations of 300–500 µM, TLC-S caused longlasting Ca^{2+} rises comprised of initial release from intracellular Ca^{2+} stores and followed by the influx of extracellular Ca^{2+}. The study found that other bile salts, taurodeoxycholate (TDC) and taurocholate (TC), also triggered local and global Ca^{2+} oscillations, although at much higher concentrations (1 mM and 5 mM, respectively).[16]

These data highlight the role of TLC-S not only as the most potent Ca^{2+} releaser among bile acids tested on PACs so far, but also as the most effective bile acid in inducing Ca^{2+}-independent current, even at 10 µM. This concentration is close to the concentrations of sulphated lithocholic acid conjugates detected in serum in different pathological conditions.[17] Indeed, in patients with severe extrahepatic duct obstruction, concentrations of bile acids of ~200 µM have been detected in peripheral circulation.[18]

Subsequently, Voronina *et al.* and other groups revealed that bile acids mediated intracellular Ca^{2+} release from both endoplas-

mic reticulum and acidic intracellular Ca^{2+} stores through the activation of inositol trisphosphate receptors (IP_3R) and ryanodine receptors,[19–21] the inhibition of sarco/endoplasmic reticulum Ca^{2+}-ATPase pumps and the activation of store-operated Ca^{2+} entry,[12] and also reduced mitochondrial membrane potential[22] and depleted both cytosolic and mitochondrial adenosine triphosphate (ATP),[23] leading to cellular injury.

Effects of bile acids on pancreatic duct cells

Although PACs have been extensively characterized, it is surprising that few studies have addressed issues of pancreatic duct cells under stressful conditions such as bile acid stimulation. Encouragingly, the work carried out by Venglovecz *et al.*[24] investigated the effects of bile acids on cells of the pancreatic duct. This study has shed some light on the role of pathological agents in the function of the ductal system.

Pancreatic duct epithelial cells and PACs have mutual communication and share similar responses to bile acids.[25] Pancreatic duct epithelial cells play a fundamental role in secreting fluid rich in HCO_3^- to wash out harmful digestive enzymes secreted by PACs and in neutralizing acid chyme in the duodenum. Therefore, PDECs represent the first line of defence against bile acid reflux.[25] Venglovecz *et al.* found that a low concentration (100 µM) of unconjugated bile acid chenodeoxycholate (CDC) stimulated HCO_3^- secretion via phospholipase C- and IP_3-mediated Ca^{2+} signalling in PDECs.[24] By contrast, high-concentration (1 mM) CDC inhibited HCO_3^- secretion, suppressed the glycolytic metabolism of PDECs and depleted mitochondrial ATP, causing mitochondrial damage.[26] However, conjugated bile salt glycochenodeoxycholate (GCDC)-elevated intracellular Ca^{2+} signals failed to stimulate or inhibit HCO_3^- secretion at various concentrations and caused no morphological change in mitochondria.[26] Further work in guinea pigs identified Ca^{2+}-activated large conductance K^+ channels expressed at the apical membrane of PDECs, which play a crucial role in regulating bile acid-stimulated or -inhibited HCO_3^- secretion.[27]

Induction of AP by bile salts

The first experimental BAP model was established in 1856 by Bernard,[28] who developed a method of retrograde injection of bile and olive oil into a canine pancreas through the ampulla of Vater. Since then, various bile salts such as sodium CDC (Na-CDC), sodium TC (Na-TC), sodium glycodeoxycholic acid (Na-GDC), TDC (Na-TDC) and TLC-S have been reported to induce AP in different species.

Model of pancreatitis induced by Na-CDC

Very few studies have employed the non-conjugated bile salt, Na-CDC, to study the pathogenesis of AP or the effects of treatment regimens. This procedure sometimes requires simultaneous