

Gallstone-related pathogenesis of acute pancreatitis

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Abstract

Acute pancreatitis is now the most common reason for hospital admission among all gastrointestinal disorders. In most countries the presence of gallbladder stones represents the most frequent and significant risk factors for developing acute pancreatitis and underlying gallstone disease accounts for between 30 and 50% of cases with pancreatitis. The question via what mechanism gallstones trigger pancreatitis on their passage through the biliary tract is not purely academic. Considering what is known today about the onset mechanisms of the disease restoring impaired pancreatic secretion, rather than preventing the reflux of bile into the pancreatic duct, should become the primary therapeutic or preventive strategy.

1. Etiology and pathogenesis of pancreatitis

Acute Pancreatitis is an inflammatory disorder of the exocrine pancreas caused, in most cases, by immoderate alcohol consumption or the passage of gallstones. Population-based studies indicate that the incidence of acute pancreatitis is rising from 14.8 in 100,000 (1990-1994) to 31.2 in 100,000 (2010-2013) among British males (14). Acute pancreatitis is currently the most frequent reason for hospital admission among all non-malignant gastrointestinal diseases (38). It also remains a lethal disease with an overall mortality of 4.3% within 90 days and a one year mortality of 7.9% (14). Not only heavy alcohol consumption

but also gallstone disease is becoming more common. Population-based studies indicate that the prevalence of gallstones in some western countries surpasses 20% of the adult population (45). While genetic predispositions clearly play an important part in gallstone formation (5, 46) they cannot explain the continuous rise in gallstone prevalence which is much more likely to be due to nutritional and life style factors. Once a patient has developed pancreatitis due to gallstones the disease is likely to recur if the source of migrating bile duct stones is not removed or their impaction at the duodenal papilla is not prevented. In a study involving some 5,000 patients admitted for a first episode of acute gallstone-associated pancreatitis, endoscopic sphincterotomy reduced the recurrence rate from approximately 30% during the first weeks to 6.7 %, an elective interval cholecystectomy reduced it to 4.4 %, and performing endoscopic sphincterotomy during the same hospital admission combined with elective cholecystectomy reduced it further to 1.2 % (32). Another way to address the problem is the transient insertion of small plastic stent into the pancreatic duct. After manipulation of the papilla, e.g. to remove a gallstone or to perform a sphincterotomy, the consequent swelling can obstruct the pancreatic duct, an event as we shall later see, that triggers pancreatitis in some patients. The inserted plastic stent prevents the prolonged impairment of pancreatic secretion and has been shown to significantly reduce the incidence of ERCP-induced pancreatitis (9). Taken together these clinical and population-based observations indicate that a) carrying

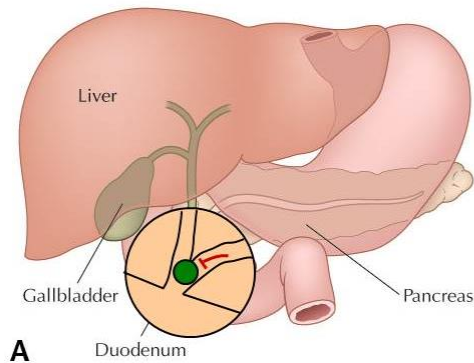
gallstones increases the risk of developing acute pancreatitis; b) only gallstones that are small enough to pass through the biliary tract, rather than the ones that remain asymptotically in the gallbladder, confer a pancreatitis risk; c) strategies intended to remove the source of migrating gallstone or that prevent their impaction near the duodenal papilla reduce the risk of developing pancreatitis in the first place and the risk of a recurrence of pancreatitis; and d) preserving the flow from the pancreatic duct is an effective way of preventing ERCP-induced pancreatitis, a clinical entity considered to be caused by obstruction of the pancreatic duct. The next paragraph will review the century old discussion about the underlying mechanism how a wandering gallstone initiates pancreatitis.

2. Possible mechanisms of gallstone-induced pancreatitis

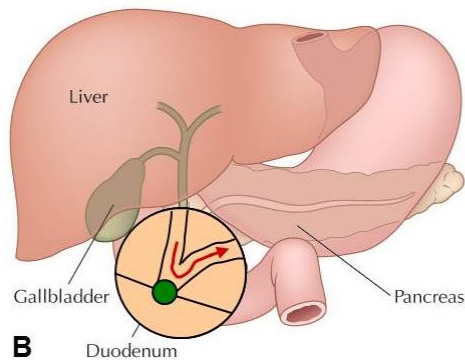
A connection between gallbladder stones and pancreatitis has been suspected since at least the 17th century (12) but how gallstones confer that risk has been the matter of much debate. Claude Bernard discovered in 1856 (4) that bile is an agent that, when injected into the pancreatic duct of laboratory animals, can cause pancreatitis. Since that time many studies have been performed to elucidate the underlying mechanisms. It is firmly established today that the initiation of pancreatitis requires the passage of a gallstone from the gallbladder through the biliary tract (1) and gallstones, that remain in the gallbladder will not cause pancreatitis. However, the various hypotheses that were proposed to explain this association have sometimes been contradictory. In 1901 Eugene Opie postulated that impairment of the pancreatic outflow due to obstruction of the pancreatic duct causes pancreatitis (36). This initial 'duct obstruction hypothesis' was somewhat forgotten when Opie

published his second 'common channel' hypothesis during the same year (35). This later hypothesis predicted that an impacted gallstone at the papilla of Vater creates a communication between the pancreatic and the bile duct (the so called 'common channel') through which bile flows into the pancreatic duct and thus causes pancreatitis (**Figure 1**).

Although Opie's 'common channel' hypothesis seems rational from a mechanistic point of view and has become one of the most popular theories in the field, considerable experimental and clinical evidence is incompatible with its assumptions (23, 33). Anatomical studies have shown that the communication between the pancreatic duct and the common bile duct is much too short (< 6 mm) to permit biliary reflux into the pancreatic duct (8) and an impacted gallstone would most likely obstruct both, the common bile duct and the pancreatic duct (27). Even in the event of an existing anatomical communication pancreatic secretory pressure would still exceed biliary pressure and pancreatic juice would flow into the bile duct rather than bile into the pancreatic duct (6, 29). Late in the course of pancreatitis when necrosis is firmly established a biliopancreatic reflux due to a loss of barrier function in the damaged pancreatic duct may well explain the observation of a bile-stained necrotic pancreas at the time of surgery. This, however, should not be regarded as evidence for the assumption that reflux of bile into the pancreas is a triggering event for the disease onset. Experiments performed on the American opossum, an animal model that is anatomically well suited to test the common channel hypothesis, have revealed that neither a common channel, nor a biliopancreatic reflux is required for the development of acute necrotizing pancreatitis but obstruction of the pancreatic duct is required (23).



Opie's duct obstruction
– impaired secretion hypothesis



Opie's common channel
- bile reflux hypothesis

Figure 1: The two “Opie hypotheses” for the pathogenesis of gallstone induced pancreatitis, both reported in 1901. A: A gallstone on its passage through the biliary tract obstructs the pancreatic duct. The impaired flow from the exocrine pancreas triggers acinar cell or duct cell damage. Whether or not the common bile duct is also obstructed is immaterial to the triggering mechanism of pancreatitis in this scenario. B: A gallstone, impacted at the duodenal papilla, creates a communication between the pancreatic duct and the common bile duct. Behind it, bile can flow through this “common channel” into the pancreatic duct and would trigger the onset of acute pancreatitis. Modified from reference (19).

In order to overcome the inconsistencies of the ‘common channel’ hypothesis it was proposed that the passage of a gallstone could damage the duodenal sphincter in a manner that sphincter insufficiency results. This, in turn, could permit duodenal content, including bile and activated pancreatic juice, to flow through the incompetent sphincter and into the pancreatic duct (28) thus inducing pancreatitis. While this hypothesis would, indeed, avoid most of the inconsistencies of Opie’s ‘common channel’ hypothesis it was shown not to be applicable to the human situation in which sphincter stenosis, rather than sphincter insufficiency, results from the passage of a gallstone through the papilla and flow of pancreatic juice into the bile duct, rather than flow of duodenal content into the pancreas, is the

consequence (15). A final argument against the ‘common channel’ hypothesis is that the perfusion of bile through the pancreatic duct has been shown to be completely harmless (41) and only a potential influx of infected bile, which might occur after prolonged obstruction at the papilla when the pressure gradient between the pancreatic duct (higher) and the bile duct (lower) is reversed (2, 7), may represent an aggravating factor, as opposed to an initiating event, for the course of pancreatitis.

Taken together these data suggest that the initial pathophysiological events during the course of gallstone-induced pancreatitis affect acinar cells (21) and are triggered, in accordance with Opie’s initial hypothesis, by obstruction or impairment of

flow from the pancreatic duct (25). A reflux of bile into the pancreatic duct – either through a common channel created by an impacted gallstone or through an incompetent sphincter caused by the passage of a gallstone – is neither required nor likely to occur during the initial course of acute pancreatitis (40).

3. Cellular events during pancreatic duct obstruction

To investigate the cellular events involved in gallstone-induced pancreatitis an animal model based on pancreatic duct obstruction in rodents has been employed (30). In addition to a morphological and biochemical characterization of this experimental disease variety the intracellular calcium release in response to hormonal stimuli was investigated. Under physiological resting conditions most cell types, including the acinar cells of the exocrine pancreas, maintain a Ca^{2+} -gradient across the plasma membrane with low intracellular (nanomolar range) facing high extracellular (millimolar range) Ca^{2+} -concentrations. A rapid Ca^{2+} -release from intracellular stores in response to external and internal stimuli is used by many of these cells as a signaling mechanism that regulates such diverse biological events as growth and proliferation, locomotion and contraction, and the regulated secretion of exportable proteins. An impaired cellular capacity to maintain the Ca^{2+} -gradient across the plasma membrane has previously been identified as a common pathophysiological characteristic of vascular hypertension, malignant tumor growth, and cell damage in response to toxins. It has also been observed in a secretagogue-induced model of acute pancreatitis (31, 42) where a rapid and sustained rise of intracellular Ca^{2+} caused by release from apical stores and a rapid entry of extracellular Ca^{2+} has been shown to be involved in the pathogenesis of experimental pancreatitis. Up to 6 hours of pancreatic duct ligation in rats and mice, a condition that mimics the situation in human gallstone-induced pancreatitis, induced leukocytosis, hyperamylasemia, pancreatic edema and granulocyte immigration into the

lungs, all of which were not observed in bile duct-ligated controls (30). It also led to significant intracellular activation of pancreatic proteases such as trypsin, an event we will discuss in the next paragraph in more detail. While the resting $[\text{Ca}^{2+}]_i$ in isolated acini rose by 45% to 205 ± 7 nM, the acetylcholine- and cholecystokinin-stimulated calcium peaks as well as amylase secretion declined. However, neither the $[\text{Ca}^{2+}]_i$ signaling pattern nor the amylase output in response to the Ca^{2+} -ATPase inhibitor thapsigargin, nor the secretin-stimulated amylase release, were impaired by pancreatic duct ligation. On the single cell level pancreatic duct ligation reduced the percentage of cells in which a physiological secretagogue stimulation was followed by a physiological response (i.e. Ca^{2+} -oscillations) and increased the percentage of cells with a pathological response (i.e. peak-plateau or absent Ca^{2+} -signal). Moreover, it reduced the frequency and amplitude of Ca^{2+} -oscillation as well as the capacitative Ca^{2+} -influx in response to secretagogue stimulation.

To test whether these prominent changes in intracellular calcium signaling not only parallel pancreatic duct obstruction but are directly involved in the initiation of pancreatitis, animals were systemically treated with the intracellular calcium chelator BAPTA-AM. As a consequence, both the parameters of pancreatitis as well as the intrapancreatic trypsinogen activation induced by duct ligation, were found to be significantly reduced. These experiments suggest that pancreatic duct obstruction, the critical event involved in gallstone-induced pancreatitis, rapidly changes the physiological response of the exocrine pancreas to a pathological Ca^{2+} -signaling pattern. This pathological Ca^{2+} -signaling is associated with premature digestive enzyme activation and the onset of pancreatitis – both of which can be prevented by administration of an intracellular calcium chelator. A number of preclinical and ongoing clinical trials have employed the dependence of pancreatitis on intracellular calcium signaling and have identified Mg^{2+} as a suitable calcium antagonist in this context (31, 42) in which a calcium chelator would

be too toxic and have proceeded to test its efficiency in patients (11).

Whether or not premature intra-acinar cell protease activation provides a sufficient explanation for triggering pancreatitis has recently come under discussion and covered elsewhere in this volume as well as in recent reviews (20).

4. Cellular signaling and sorting mechanisms

Subsequent investigations have focused on the cellular signaling and sorting mechanisms involved in the disease onset. Essential events that have been identified are colocalization and transactivation of lysosomal cathepsins with zymogens and the above-mentioned pathological Ca^{2+} -release from intracellular stores. Both processes were found to be of critical importance not only in supramaximal stimulation-induced models of pancreatitis (13, 18) but also in clinically more relevant duct obstruction-induced pancreatitis (16, 24).

Two inconsistencies of the duct-ligation models of pancreatitis have led to renewed interest in the role of bile in the disease onset. The first was that duct-ligation alone, with the notable exception of the opossum, induces mostly mild pancreatitis, rather than fully developed necrosis, particularly in the rat (30). The second inconsistency was that some studies employing the opossum model, while still refuting the common-channel-hypothesis, reported that bile duct-ligation, when added to pancreatic duct-ligation, increased the severity of the disease (43). This suggests that elevated bile acids in the systemic circulation could aggravate the disease process and the subsequent line of arguments has previously been summarized (19).

A first confirmation for this assumption came from studies reporting that bile acids have a direct effect on pancreatic acinar cells and elicit an oscillatory release of Ca^{2+} from intracellular stores (47). This bile acid effect on $[\text{Ca}^{2+}]_i$ is either mediated via bile acid inhibition of the sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) pump with consecutive depletion of ER Ca^{2+} -stores and activation of significant capacitative Ca^{2+} -entry into the cytosol (10, 17) or, alternatively, by potentiation of Ca^{2+} -release from the ER and apical (vesicular) Ca^{2+} -stores (3, 47, 48). Most studies agree that monohydroxy-bile-acids, such as tauro lithocholic-acid-3-sulfate (TLC-S) have a more potent effect on acinar cells than dihydroxy-bile-acids (i.e. TCDC) or trihydroxy-bile-acids and can cause damage independently of their properties as detergents or ionophores. Most importantly, TLC-S can induce pathological Ca^{2+} -signals and lead to trypsinogen activation at concentrations that correspond to those found in the serum of patients with gallstone-induced biliary obstruction (48, 49). The disease-aggravating effect of common bile duct obstruction in pancreatitis would therefore not require bile reflux into the pancreatic duct but be elicited readily by bile acids in the serum or interstitial space of jaundiced patients.

The question remains as to how bile acids enter the acinar cell and whether this entry occurs from the basolateral or the luminal surface. An elegant study by Kim (17) identified two potential mechanisms (**Figure 2**): The first involves a Na^+ -dependent-co-transporter (Na^+ -taurocholate co-transporting-polypeptide, NTCP), that accounts for approximately 25% of the bile acid uptake and is predominantly operative at the luminal membrane.

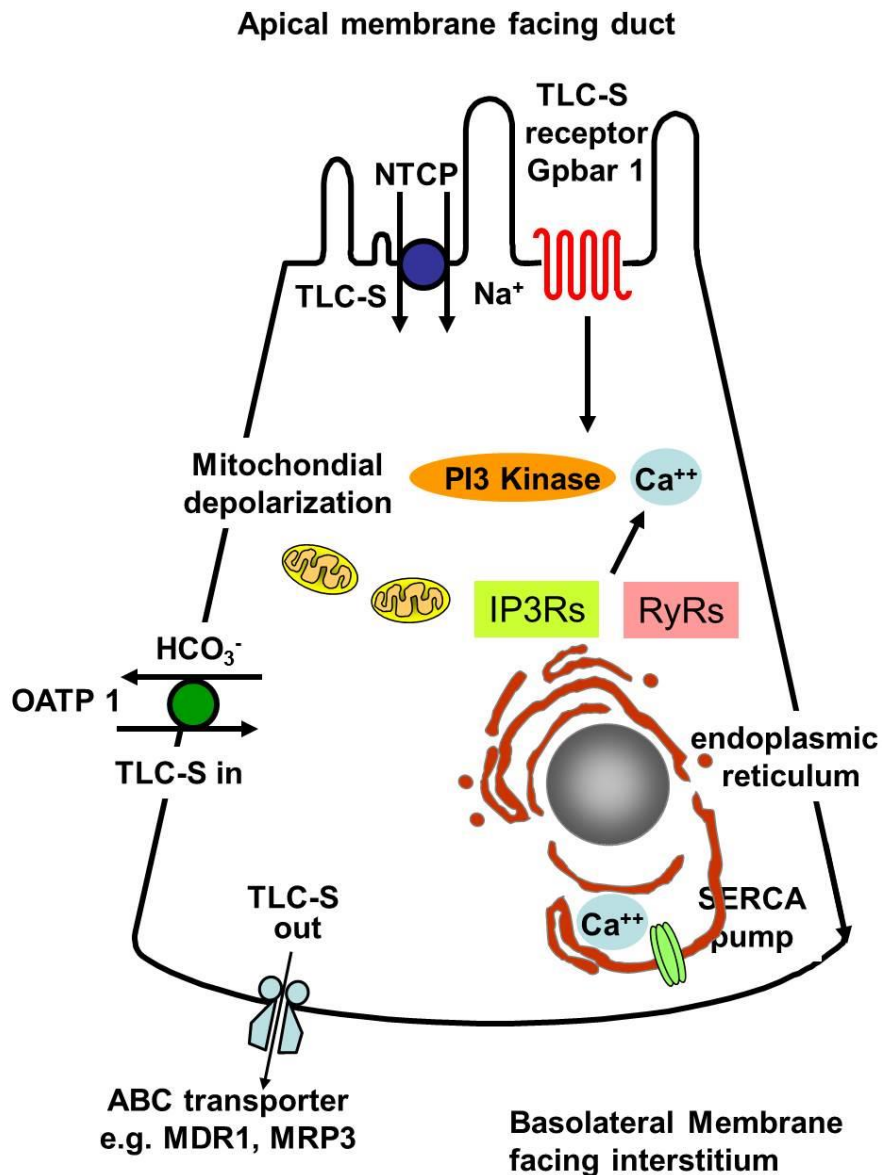


Figure 2: Different cellular mechanisms that can all mediate the uptake of bile acids into either duct or acinar cells from either the luminal side of the cell or from the interstitial/vascular surface. Modified from reference (19).

Bile acid uptake via this transporter would thus require bile reflux to reach the pancreatic acinar cell via the duct. The other uptake mechanism involves an HCO₃⁻-dependent exchanger (organic-anion-transporting polypeptide, OATP3) which operates from the basolateral acinar cell surface and could thus be supplied with serum or interstitial bile acids.

Perides and co-workers (39) have recently identified an additional mechanism for the effects of bile acid on pancreatic acinar cells which 1)

seems to require action only at the luminal cell surface; 2) is independent of bile acid uptake mechanisms into the cell; and 3) involves G-protein-receptor-coupled signalling events elicited by TLC-S, which suggests biliary pancreatitis to be a surface-receptor-mediated disease. The authors used a mouse strain deleted for the G protein-coupled bile acid receptor-1 (Gpbar1) (44) and induced pancreatitis by injecting bile acids (50µl 3mM TLC-S or Na⁺-taurocholate) into the pancreatic duct which results in mild acute pancreatitis involving only the head of the

pancreas and was not burdened with the high mortality of the established Na⁺-taurocholate-induced models of pancreatitis (50). Interestingly, only TLC-S injection resulted in pancreatitis in this setting whereas Na⁺-taurocholate did not. Gpbar1^{-/-} mice were completely protected against TLC-S-induced pancreatitis. These studies have led to renewed interest in the events that take place inside the pancreatic duct during the initiating phase of gallstone induced pancreatitis. Some appear to involve impairment of pancreatic fluid secretion (26), others the intraductal action of prematurely activated trypsin (37), the action of

intraductal lysosomal enzymes (16, 22), signal transduction events within ductal cells (39) or changes in the intraductal pH (34). While Eugene Opie challenged preconceived theories about the mechanisms that trigger gallstone-induced pancreatitis and has set us on path towards actionable results (such as restoring the flow of pancreatic juice or preventing its blockage) clinically relevant information is still accumulating at a rapid pace from laboratories the world over that hopefully results in better treatment and prevention strategies for this still deadly disease.

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