Pathogenesis of gallstones in Crohn's disease
Brink, M.A.

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Chapter 8

SUMMARY
In this thesis a mechanism is proposed that may explain increased levels of pigments in bile of patients with ileal Crohn's disease. In addition the thesis focuses on the role of bile pigments and biliary proteins in the pathogenesis of gallstones in these patients. **Chapter 2**, reviews the literature on gallstone prevalence in Crohn's disease (which is 30-40% compared to 10-15% in the general population). In the literature there is controversy as to whether Crohn's disease patients are at risk for cholesterol or black pigment gallstones, and possibly these patients might form either cholesterol or black pigment gallstones or both types of stones simultaneously. The increased gallstone prevalence is usually thought to be caused by bile salt malabsorption due to ileal dysfunction. Bile salt malabsorption may deplete the bile salt pool and this in turn may lead to supersaturation of bile with cholesterol. It is generally assumed that cholesterol supersaturation is the primary factor in cholesterol gallstone formation. Nevertheless, gallbladder biles of the majority of patients with Crohn's disease are unsaturated or marginally saturated with cholesterol, probably because de novo bile salt synthesis by the liver compensates for ileal bile salt loss unless the latter is excessive. In contrast, increased concentrations of both unconjugated and conjugated bilirubin were found in bile samples from patients with ileal Crohn's disease, predisposing these patients to black pigment gallstone formation. We hypothesize that higher bile pigment concentrations are the result from enhanced colonic absorption of unconjugated bilirubin, formed by bacterial deconjugation and solubilization by the bile salts that escape reabsorption in the terminal ileum because of inflammation or resection of the ileum. Intestinal reabsorption of unconjugated bilirubin has been documented before, but the intestinal fate of bilirubin in diseases other than neonatal and hereditary hyperbilirubinemias, e.g. Crigler Najjar disease, has not been established.

In **chapter 3**, the hypothesis of enterohepatic cycling of bilirubin as a consequence of bile salt malabsorption was tested in the ileectomized rat. This chapter shows that in the ileectomized rat UCB was solubilized in the large intestine presumably by excess of bile salts spilled into the colon. Bilirubin secretion rates into bile were increased 3-5 days after ileal resection, but bilirubin secretion rates remained normal in animals subjected to jejunal resection. The bilirubin hypersecretory state was proven to be reversible in the ileectomized rat. By 8-11 days after surgery, intestinal adaptation normalized bile salt reabsorption, and
hypersecretion of bilirubin disappeared. Because overt hemolysis was not detected, bilirubin hypersecretion was considered to be caused by enhanced absorption of unconjugated bilirubin from the colon.

Bile salt malabsorption from any cause might lead to enterohepatic cycling of bilirubin leading either to hyperbilirubinemia or to increased secretion of biliary pigments or both, depending on the capacity of the liver with respect to uptake, conjugation and secretion of unconjugated bilirubin. Ursodeoxycholic acid (UDCA), a hydrophilic bile salt and dietary cholesterol may cause bile salt malabsorption in rodents, the former by competition for and the latter by down-regulation of ileal bile acid transporters. Chenodeoxycholic acid (CDCA), a hydrophobic bile salt, competes with endogenous bile salts for ileal bile salt transporters to a mild degree. In chapter 4 we describe that UDCA and cholesterol administration in rodents doubled bilirubin secretion rates into bile, whereas CDCA caused a moderate elevation of bilirubin secretion into bile. In UDCA-fed mice, gallbladder biles contained increased levels of bilirubin conjugates and unconjugated bilirubin and precipitates of amorphous calcium bilirubinate were observed. Dietary cholesterol and bile acids, particularly UDCA, increased cecal bile salt levels, unconjugated bilirubin and urobilinogen concentrations, and decreased fecal bilirubin outputs, providing indirect evidence for enhanced absorption of unconjugated bilirubin from the colon.

Whether enterohepatic cycling of bilirubin may occur in patients with ileal Crohn's disease as well was studied in chapter 5. Gallbladder bile was obtained from a large cohort of Crohn's disease patients during elective bowel surgery and control bile samples were obtained from patients with ulcerative colitis. A striking increase in bile pigments (both conjugates and UCB) and calcium was observed in biles of Crohn's disease patients with a compromised ileum. Bilirubin levels in bile matched to the anatomical length and duration of ileal disease or resection. In patients with Crohn's colitis, bilirubin levels in gallbladder bile were comparable to patients with ulcerative colitis. Hemolysis, increased tissue heme turnover or vitamin B$_{12}$ deficiency were unlikely causes for increased pigment levels in bile. Therefore, enterohepatic cycling of bilirubin was believed to be the dominant mechanism leading to enhanced secretion of biliary pigments in gallbladder biles in patients with ileal Crohn's disease or after ileectomy. Total calcium levels in gallbladder bile were also increased with ileal dysfunction, but the cause for this phenomenon remained unknown. Perhaps increased calcium
concentrations in bile may be due to increased levels of BMG and BDG, causing diffusion of calcium ions from the blood into bile by Gibbs-Donnan forces.

Both black pigment gallstones and cholesterol gallstones were found in low numbers in patients with Crohn's disease. In a significant number of patients with Crohn's disease cholesterol monohydrate crystals were observed. For cholesterol crystallization, supersaturation of bile with cholesterol is required. Gallbladder biles of the Crohn's disease patients studied in this thesis were unsaturated, marginally saturated or supersaturated with cholesterol. In gallbladder biles of cholesterol gallstone patients, proteins have been characterized that promote cholesterol crystallization. In chapter 6 it was investigated whether these proteins also play a role in cholesterol crystallization in patients with Crohn's disease. Biles of Crohn's disease patients had an increased propensity for cholesterol crystal formation compared to patients with ulcerative colitis, but no differences in biliary lipid composition were observed. In gallbladder biles of Crohn's disease patients, crystallization promoting activity was found, but this activity was absent in biles obtained from patients with ulcerative colitis. In a number of bile samples (particularly in patients with ulcerative colitis) crystallization inhibition was noticed. Levels of Immunoglobulin A (IgA), the only characterized glycoprotein which inhibits cholesterol crystallization, appeared to be significantly higher in Crohn's disease compared to ulcerative colitis. This indicates that differences in crystallization behaviour could not be explained by differences in IgA levels.

Thus, as shown in chapters 5 and 6, pigment and cholesterol crystallization promoting factors were both increased in biles of Crohn's disease patients explaining their potential risk for both types of stones. Whether presence of these factors indeed induces stone formation is difficult to address. So far, studies have not been reported of formation of stones in vitro. We therefore investigated whether incubation of preexisting cholesterol gallstones in gallbladder biles from patients with Crohn's disease or patients with ulcerative colitis would reveal differences in stone growth. In chapter 7, growth characteristics are presented showing similar increases in the mass of cholesterol gallstones in all groups. Unconjugated bilirubin levels in gallbladder bile correlated with gallstone growth in biles of Crohn's disease patients, but not in ulcerative colitis patients. Microscopical analyses revealed cholesterol crystals at the surface of the incubated stones, and more intense pigmentation. These observations seem to indicate that in this system stones accrete cholesterol foremost, whereas bile pigments were
deposited on stones to a lesser extent. Because cholesterol gallstones are porous structures, some pigment deposition in the interior of the stone could not be ruled out. The fact that both patients with Crohn's disease and with ulcerative colitis carry the same potential for growth of gallstones and that the gallstone prevalence rate is much higher in Crohn's disease than in ulcerative colitis, suggests that a certain initiating event, i.e. the nidation of a stone, occurs more often in Crohn's disease patients. Since the increase of bile pigments is the only clear difference between biles from both groups of patients, we postulate that in patients with functional impairment or exclusion of the ileum, increased bilirubin levels in bile predispose to bilirubin precipitation and nidation of stones. This process may be mediated by stasis due to gallbladder hypomotility, or mucous glycoproteins that may function as scaffolding agents. We propose that the stone nidus may grow into a pigment or cholesterol gallstone depending on whether bile is supersaturated with bile pigments or with cholesterol, respectively. The growing stones may probably irritate the gallbladder wall leading to increased mucin formation as well as secretion of immunoglobulins and acute-phase proteins. The stone on the cover of this thesis was obtained from a Crohn's disease patient and is a pure example of the proposed pathogenic mechanism of gallstone formation in Crohn's disease. The cholesterol monohydrate crystals are transparent thus allowing us to see the pigmented nucleus.