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Targets for Current Pharmacological Therapy in Cholesterol Gallstone Disease

Agostino Di Ciaula, MD [Internist][a], **David Q.-H. Wang, MD, PhD [Assistant Professor of Medicine]**[b], **Helen H. Wang, BS, MS [Research Associate]**[c], **Leonilde Bonfrate, MD [Research Fellow]**[d], and **Piero Portincasa, MD, PhD [Associate Professor of Medicine]**[e]

Agostino Di Ciaula: agostinodiciaula@tiscali.it; David Q.-H. Wang: dqwang@caregroup.harvard.edu; Helen H. Wang: hjiang@caregroup.harvard.edu; Leonilde Bonfrate: microscopia@semeiotica.uniba.it

[a]Division of Internal Medicine, Hospital of Bisceglie, via Bovio 279 - 70052 - Bisceglie (Bari), Italy, +39-80-3363271, +39-80-3363232 (fax)

[b]Liver Center and Gastroenterology Division, Beth Israel Deaconess Medical Center, Department of Medicine, Harvard Medical School and Harvard Digestive Diseases Center, 330 Brookline Avenue, DA 601, Boston, MA 02215, (617) 667-0561, (617) 975-5071 (fax)

[c]Department of Medicine, Liver Center and Gastroenterology Division, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, DA 601, Boston, MA 02215, (617) 667-5156, (617) 975-5071 (fax)

[d]Clinica Medica “A. Murri”, Department of Internal and Public Medicine, University of Bari Medical School, Piazza Giulio Cesare 11, Policlinico, 70124 Bari, Italy. +39-80-5478227, +39-80-5478232 (fax)

[e]Clinica Medica “A. Murri”, Department of Internal Medicine and Public Medicine, University Medical School, Bari, Italy

Summary

Gallstone disease is a frequent condition throughout the world and cholesterol stones are the most frequent form in western countries. Current standard treatment of symptomatic gallstone subjects remains laparoscopic cholecystectomy. The selection of patients amenable for non-surgical, medical therapy is of key importance: a careful analysis should consider the natural history of the disease and the overall costs of therapy. Only patients with mild symptoms and small, uncalcified cholesterol gallstones in a functioning gallbladder with a patent cystic duct will be considered for oral litholysis by the hydrophilic ursodeoxycholic acid (UDCA) hopefully leading to cholesterol desaturation of bile and progressive stone dissolution. Recent studies have raised the possibility that cholesterol-lowering agents which inhibit hepatic cholesterol synthesis (statins) or intestinal cholesterol absorption (ezetimibe), or drugs acting on specific nuclear receptors involved in cholesterol and bile acid homeostasis may offer, alone or in combination, additional medical therapeutic tools for treating cholesterol gallstones. Recent perspectives on medical treatment of cholesterol gallstone disease will be discussed in this chapter.

[e]Corresponding author for proof and reprints: Piero Portincasa, MD, PhD, Clinica Medica “A. Murri”, Department of Internal and Public Medicine, University Medical School, Piazza Giulio Cesare 11, Policlinico, 70124 Bari, Italy. +39-80-5478227, +39-80-5478232 (fax), p.portincasa@semeiotica.uniba.it.

Keywords

bile salts; cholesterol absorption; ezetimibe; gallbladder; nuclear receptors; statins

Introduction

Gallstone disease is one of the most frequent and costly digestive diseases in western countries, as its prevalence in adults ranges from 10% to 15% (1-4). Although frequent, many patients with gallstones remain “silent”, symptoms and/or complications occur in approximately a third of patients. In the United States, medical expenses for the treatment of gallstones exceeded $6 billion in the year 2000. Furthermore, the prevalence of gallstones seems to be rising and approximately one million new cases are discovered each year (5). About 75% of the gallstones in the United States and westernized countries, including Italy are cholesterol gallstones (6-8). The remaining gallstones are pigment stones that contain less than 30% cholesterol by weight, which can be subclassed into two groups: black pigment stones (about 20% of all gallstones, found in the gallbladder and/or bile duct, containing mainly insoluble bilirubin pigment polymer mixed with calcium phosphate and carbonate, and cholesterol) and brown pigment stones (about 5% of all gallstones, found mainly in bile ducts, containing calcium bilirubinate, calcium palmitate, and stearate and cholesterol)(9).

Cholesterol gallstones are associated with well known risk factors, such as obesity, type 2 diabetes, dyslipidaemia, and hyperinsulinaemia (1), which are often components of the metabolic syndrome epidemic (10-14), which prevalence is greater than 35% in the adult pupulation and continues to raise in westernized countries (15;16). Epidemiological surveys have observed that cholesterol cholelithiasis is prevalent in populations consuming a “Western” diet (i.e. enriched in saturated fatty acids, cholesterol, and rapidly absorbed refined carbohydrates), rather than a more “prudent” diet (i.e. enriched in mono-polyunsaturated fats, fruit, vegetables and low in refined carbohydrates) associated with physical activity (17-25). Thus, the prevalence of cholesterol gallstone disease is significantly higher in North and South American as well as European populations than that in Asian and African populations (6). In China, the prevalence of cholesterol gallstones appears to increase with the “westernization” of the traditional Chinese diet (26-28). Even in Japan, the adoption of Western-type dietary habits has resulted in a marked increase of the prevalence of cholesterol cholelithiasis over the past 40 years (29;30). As discussed later, high efficiency of intestinal cholesterol absorption and high dietary cholesterol appear to be two key and independent risk factors for the formation of cholesterol gallstones. The complex pathogenesis of cholesterol gallstones depends on the concurrent existence of hepatic hypersecretion of cholesterol into bile leading to bile supersaturation with cholesterol, accelerated nucleation/crystallization of cholesterol in gallbladder bile, impaired gallbladder motility leading to gallbladder stasis, and increased cholesterol availability from the small intestine, as well as *LITH* genes and genetic factors (1;31;32). A complex genetic basis plays a key role in determining individual predisposition to develop cholesterol gallstones in response to environmental factors (33-37). Some “gallstone genes” might also play a potential role, including some genes governing the nuclear bile acid receptors such as farnesoid X receptor (FXR). For example, *FXR* variants seem to affect gallbladder motor-function and intestinal microflora in Mexicans (38), while functional variants in FXR might account for intrahepatic cholestasis of pregnancy in Caucasians, as well as be associated with other cholestatic and dyslipidemic disorders (39)).

From a therapeutic point of view, although gallstone disease is frequent in the general population and the costs of therapeutic interventions are high, the natural history of the

disease suggests to restrict the medical treatment of gallstones to a subgroup of symptomatic patients (1;36;40). The selection of patients eligible for medical or surgical therapy, therefore, is of key importance. The onset of biliary pain is the only suggestive marker of "symptomatic" gallstone disease (41;42), although it can be difficult to distinguish between symptomatic and asymptomatic subjects in a random population of gallstone patients (43). The diagnosis can be misleading if patients inadequately describe "typical" symptoms or suffer from highly atypical symptoms (44). Of note, previously symptomatic patients who are symptom-free for five consecutive years should be included in the group of asymptomatic subjects again. After this time, the risk of pain attacks gradually decreases towards values similar to those of patients with asymptomatic gallstones (45). Classical drug therapy for cholesterol gallstones (*i.e.* oral litholysis by the bile acid ursodeoxycholic acid, UDCA) plays at the moment a limited role, but novel interesting therapeutic options might arise in the near future, when looking at the molecular mechanisms responsible for the formation of cholesterol gallstones (1). Such novel therapeutic approaches might involve subgroups of patients permanently or temporarily at risk for gallstone formation. In this respect, recent studies in both animal models and humans have found that blocking the intestinal absorption of cholesterol with ezetimibe (EZT), the potent inhibitor of the Niemann-Pick C1-like 1 (NPC1L1) protein (46), may provide a novel powerful strategy for the medical treatment of cholesterol gallstones (47). Modification of the expression levels of specific nuclear receptors in the liver might also provide a clue for novel therapeutic approaches for cholesterol gallstones via manipulation of cholesterol and bile acid homeostasis. Current views and perspectives on medical treatment of cholesterol gallstone disease will be discussed in the present paper.

Guidelines for Management of Gallstone Disease

Gallbladder stones are frequently found in asymptomatic subjects during routine abdominal ultrasonography, since in the majority of the cases (60-80%) gallstones do not generate symptoms (43;48;49). Previous observations have shown that average risk of developing symptomatic gallstones is as low as 2.0-2.6% per year (45;50). By contrast, the presence of microstones and sludge in the gallbladder is a major risk factor for the development of biliary pain and complicated gallstone disease, and also plays a main role in the etiology of acute otherwise idiopathic pancreatitis (51-53). Nevertheless, the yearly incidence of complications is very low (0.3%), and the annual risk for gallbladder cancer is as low as 0.02% (54;55). Treatment of "asymptomatic" gallstone patients, therefore, is not routinely recommended, as the overall risk of biliary colic, complications and gallbladder cancer are very low (56-58). Rather, the expectant management is currently considered the appropriate choice in the vast majority of asymptomatic gallstone patients (**Grade A**). The decision is different in symptomatic gallstone patients and should follow the algorithm depicted in Figure 1, where surgery (namely laparoscopic cholecystectomy) represents the gold standard for treatment while oral litholysis with hydrophilic bile salts plays a limited role (1;36). Other non-surgical (non pharmachological) therapies include direct contact dissolutions of gallstones using the potent cholesterol solvent methyl tert-butyl ether (MTBE)(59), and extracorporeal shock wave lithotripsy (60). Both options, however, have lost their interest because of potential side effects (MTBE) and/or high post-dissolution recurrence rate (1). Available medical treatments for gallstones are discussed in the following paragraphs and include the treatment of biliary colic (all types of stones), oral litholysis by hydrophilic bile acids and novel approaches with statins, EZT, and agonists/antagonists of nuclear receptors (NR) (all for cholesterol gallstones).

Medical Treatments of Gallstone Disease

Treatment of the biliary colic

The presence of gallstone of any type and size may put the patient at risk of biliary pain. As the intensity of pain is usually high (mean visual analogue scale of 9 cm on a 0-10 cm scale), patients require immediate medical attention and analgesia. The pain is not exclusively postprandial, and is typically intermittent. The most frequent localization is the right upper quadrant of the abdomen and/or the epigastrium (representative dermatomes T8/9), and the duration is generally longer than 15-30 minutes. The pain is radiating to the angle of the right scapula and/or shoulder in about 60% of cases. In less than 10% of cases the pain is radiating to the retrosternal area. About two-third of patients experience an urge to walk (44), and often are nauseated or vomit (41;44;49). In biliary colic, the pain is visceral and is caused by the impaction of the stone in cystic duct or sphincter of Oddi. Distension of the gallbladder and/or biliary tract with activation of visceral sensory neurons may follow (41). The pain can last for several hours and be associated with non-specific symptoms of indigestion. The pain could be relieved if the stone returns into the gallbladder lumen, passes through the sphincter into the duodenum or migrates back to the common bile duct (40). The biliary pain is rapidly responsive to narcotic analgesics (meperidine (61)) or non-steroidal anti-inflammatory drugs (NSAIDs) (such as i.m. or i.v. ketorolac or ibuprofen p.o.) which could also reduce the risk of evolution towards acute cholecystitis (62-65). A second-line therapy includes the use of antispasmodic (anticholinergic) agents like hyoscine (scopolamine) which are known to be less effective than NSAIDs (62) (**Grade A**). The patient with biliary colic should remain fasting to avoid release of endogenous cholecystokinin and further gallbladder contraction. If a complicated biliary pain is suspected (association of leukocytosis, nausea, jaundice, vomiting, and fever), the patient should be quickly admitted to hospital and treated accordingly. Typical complications of gallstone disease are acute pancreatitis, acute cholecystitis, biliary obstruction and cholangitis, gallbladder perforation, abscess formation, mucocele of the gallbladder which may require additional medical therapy with antibiotics or invasive procedures with or without surgery. In case of mild and moderate acute cholecystitis, early laparoscopic cholecystectomy is recommended between 2 and 4 days (66) (**Grade A**). The risk of biliary pain in asymptomatic carriers are estimated to be approximately 1-2% annually (67;68). Early not randomized or placebo-controlled studies found that UDCA, beside its litholytic effect (see below), might also reduce the risk of biliary colic (69;70). In a non-randomized study, Tomida *et al.* treated patients referred for symptomatic or asymptomatic gallstones with 600 mg UDCA per day (71) and used those who refused as a control group. The incidence of biliary pain was apparently reduced by UDCA in asymptomatic patients, although a bias might include a misclassification of symptoms. However, in a large randomized, double-blind, and placebo-controlled trial on the effects of UDCA in highly symptomatic gallstone patients scheduled for cholecystectomy, UDCA did not exert a beneficial effect on biliary colic. The likelihood of remaining colic-free was comparable in patients with strong or weak baseline gallbladder contraction as determined by ultrasonography after a standard mixed meal (72).

Dissolution of cholesterol gallstones by oral bile acids

About two-thirds of the gallstones in western countries are composed mainly of cholesterol. However, dissolution therapy by oral administration of the hydrophilic bile acid UDCA, the 7β-epimer of chenodeoxycholate is suitable only for a small subgroup (about 15%) of symptomatic patients (1;40). Similar results are reached with the taurine-conjugated UDCA, i.e. tauroursodeoxycholic acid (TUDCA). Chances of dissolution are much higher if gallstones are small (less than 0.5 cm in size), not calcified (radiolucent on X-ray, including CT scan), cholesterol-enriched (i.e. more than 80%) and contained within a functioning

gallbladder with a patent cystic duct (73). Complete dissolution of gallstones by bile acids was first documented by Rewbridge in 1937 (74), although initial reports were published in 1873 and 1876 (75;76). The bile acid chenodeoxycholic acid (CDCA) was first used in the 1970s (77) but was associated with a dose-dependent increase in serum aminotransferases, serum low-density lipoprotein (LDL) cholesterol levels, and diarrhea. In 1975 Makino *et al.* identified UDCA as a more hydrophilic bile acid which could replace CDCA without side effects (78). Dissolution of cholesterol gallstones by UDCA following fragmentation by extracorporeal shock wave lithotripsy was introduced first by Paumgartner *et al.* in Munich in 1986 (60). The bile acid UDCA is currently employed for oral dissolution at a dosage of 10-14 mg/kg body weight per day. Bedtime administration is suggested because it maintains hepatic bile acid secretion rate overnight, thus reducing secretion of supersaturated bile and increasing the dissolution rate (79;80) (**Grade A**). Oral UDCA (at least 10 mg/kg/day) results in increased proportion of biliary UDCA in bile (from less than 8-10% of biliary bile acid pool to about 40%). Increasing biliary UDCA, in turn, results in a decreased hepatic secretion of biliary cholesterol and the formation of unsaturated gallbladder bile (i.e. containing less cholesterol in solution) with a cholesterol saturation index of less than 1 (81-83) (Figure 2). This step represents a key factor in initiating the process of dissolution of cholesterol crystals and gallstones. During UDCA treatment, cholesterol crystallization can be prevented since more cholesterol can be transported within vesicles which contain mainly phospholipids and cholesterol and little bile acids (84). Also, oral therapy with UDCA is associated with the reduction of intestinal absorption of cholesterol (85-87), as well as with a better contractility of the stimulated gallbladder smooth muscle, as shown by *in vitro* studies in animals and gallstone patients (88;89). By decreasing cholesterol saturation of bile, UDCA might counteract the impaired contractility due to incorporation of excessive luminal cholesterol into the plasmalemma of gallbladder smooth muscles (88-91). UDCA might also counteract the detrimental effects of the hydrophobic bile acid deoxycholate on the gallbladder smooth muscle contractility (91;92), and have effect on local oxidative stress (89;93) and risk of acute cholecystitis (71). Excess biliary cholesterol might provide the basis for stimulation of inflammatory cells in the gallbladder, since cholesterol monohydrate crystals induce expression of T-cell-dependent proinflammatory cytokines in murine model of cholesterol cholelithogenesis (94).

Patients suitable for medical dissolution of cholesterol gallstones need to be carefully selected. Well-selected patients are those who have the higher chance for successful oral litholysis alone or after extracorporeal shock wave lithotripsy for stone fragmentation (95-99). Ultrasonography of the right upper quadrant is still the best and more convenient diagnostic tool for detecting gallstones (58;100), as well as for assessing both gallstone size and burden (101). “Functional” ultrasonography, i.e. the study of time-dependent changes of both fasting and postprandial gallbladder volumes following a standard test meal, although not routinely employed, provides additional information about gallbladder size, emptying, and bile duct patency (36;95-98;102-105). Abdominal plain radiography (not routinely used) and the computerized tomographic (CT) scan (106;107) detect only calcified stones (as radiopaque bodies) in the right upper quadrant. Such stones are obviously unfit for dissolution because they are either calcified cholesterol stones or stones made of pigment calcium bilirubinate (9). Oral cholecystography might disclose the presence of floating (cholesterol) stones in the gallbladder and a preserved cystic duct patency. The expected dissolution rate following UDCA at the standard dosage is estimated to about a 1 mm decrement in stone diameter per month (108). In patients with a gallstone diameter less than 5 mm, the complete disappearance of stones assessed by ultrasonography is expected to be reached in about 90% of cases by 6 months of UDCA administration (109). A series of conditions might impede the dissolution of cholesterol gallstones by UDCA or TUDCA. In patients with larger and/or multiple stones the dissolution rate approaches 40-50% after one year of the treatment (54;110), while the appearance of a surface calcification on cholesterol

gallstones during oral dissolution therapy with UDCA, CDCA, or TUDCA has been reported in about 10-12% of patients. This event would obviously impede any further dissolution of the calcified stone (111;112). Another issue is the possibility that gallstones will recur sometime after dissolution with bile acids, and this is a major limitation of oral dissolution therapy. Overall, recurrence might be as high as 10% per year, i.e. about 30-50% of cases 5 years after bile acid therapy or lithotripsy (96;113-117). Recurrence rate is higher particularly in subjects with multiple gallstones (116). Whereas recurrent gallstones respond well to re-treatment (107;118), it is logical to speculate that such an high recurrence rate is dependent on persistent pathogenetic conditions (1). Oral dissolution therapy of cholesterol gallstones with bile acids might still represent the option in patients who are at minimal risk of gallstone recurrence or have transient risk factors including rapid weight loss (i.e. obese patients following bariatric surgery), pregnancy, and convalescence from abdominal surgery, (1;119-121)). Major limitations for oral litholysis with bile acids, by contrast, are the small number of suitable patients and the high rate of gallstone recurrence (37;122;123).

Novel Medical Treatments

The presence of a lithogenic bile is primarily due to a sustained hypersecretion of biliary cholesterol which has two key components: hepatic and intestinal (31). In principle, drugs influencing hepatic synthesis and/or secretion of cholesterol (i.e. statins) and/or intestinal absorption of cholesterol (i.e. EZT), are potentially able to influence the formation of cholesterol gallstones and to promote dissolution of gallstones.

Inhibition of hepatic cholesterol synthesis by statins

Statins are competitive inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, the rate-limiting step in cholesterol biosynthesis. They occupy a portion of the binding site of HMG-CoA, blocking access of this substrate to the active site on the enzyme (124). Currently available statins in the United States include lovastatin, pravastatin, simvastatin, fluvastatin, atorvastatin, and rosuvastatin. Statins appear also to reduce cholesterol secretion and concentration in bile independently of their ability to block hepatic cholesterol synthesis (125-128). Such combined effects of statins on cholesterol homeostasis in the liver and bile might be potentially able to lower the risk of cholesterol gallstones (129-131). Indeed, beneficial effects of statins in preventing gallstone formation have been reported in animal studies (132;133). In humans the effect of statins on gallstone disease has been controversial: reduced gallstone formation, decreased cholesterol concentration in bile, and gallstone dissolution following therapy with statins have been reported by some (134-137), but not all studies (130;138;139). Another two small studies have also been conflicting with either no association between statin use and the risk of gallstones (140) or with an effect of statin on gallstones, although the statistical power was small (141). More recently, two studies have re-assessed the problem of statin use and risk of gallstone disease, and opened new perspectives. In a cohort of US women self-reporting long-term use statins, the risk of cholecystectomy was found to decrease slightly (142). In a case-control analysis using the UK-based General Practice Research Database and evaluating incident patients between 1994 and 2004, long-term use of statins (1 to 1.5 yrs) was associated with a decreased risk of gallstones followed by cholecystectomy, compared with patients without statin use (143). Whether statin use will be part of the medical therapeutic *armamentarium* in a subgroup of patients with gallstone disease or to prevent gallstone disease in selected patients at risk, needs to be investigated further by appropriate clinical studies.

Inhibition of intestinal cholesterol absorption by ezetimibe

The importance of intestinal factors in the pathogenesis of cholesterol gallstones has recently raised the interest of several research groups (1;144). Animal studies have shown

that when no dietary cholesterol is available, all biliary cholesterol is mainly derived from hepatic *de novo* synthesis with a limited contribution (less than 15%) to biliary cholesterol secretion. Rather, the small intestine is the site which solely provides the absorption of dietary cholesterol, as well as re-absorption of biliary cholesterol (144). The importance of intestinal absorption of cholesterol for gallstone pathogenesis is supported by the fact that a positive correlation exists between the efficiency of intestinal cholesterol absorption and the prevalence of cholesterol gallstone formation in several strains of inbred mice (34). The protein Niemann-Pick C1-like 1 (NPC1L1) is highly expressed in the small intestine and localized along the brush border of the enterocytes in both humans and mice (145;146). There is also a significant amount of NPC1L1 in the human liver but not in the mouse liver (146;147). Of note, cholesterol is the most effective substrate of NPC1L1 (148) which governs intestinal absorption of cholesterol (146) by recycling between endocytic recycling compartment and plasma membrane (148) (Figure 3). Thus, inhibition of cholesterol absorption in the intestine or hepatic uptake of chylomicron remnants has become an attractive possibility to decrease biliary cholesterol secretion and saturation [100]. More interestingly, similar to humans, the abundance of Niemann-Pick C1-like 1 (NPC1L1) in the small intestine far exceeded that in other regions of the gastrointestinal tract, liver, and gallbladder in the Golden Syrian hamster (149). Ezetimibe-induced reduction in intestinal cholesterol absorption is coupled with a decrease in the absolute and relative cholesterol levels in bile in hamsters fed a high cholesterol and high fat diet. These results are consistent with the recent finding that ezetimibe treatment significantly reduced biliary cholesterol saturation in patients with gallstones (47). EZT belongs to the new class of 2-azetidinones approved as a novel hypocholesterolemic drug (150) with a potent inhibitory effect on intestinal cholesterol absorption by specifically suppressing the NPC1L1. EZT might therefore play a primary role in the medical treatment or prevention of cholesterol gallstones, as suggested by studies from our group (Figure 4). In mice, EZT reduces cholesterol and partly phospholipid but not bile salt content in gallbladder bile; all crystallization pathways and phase boundaries in the bile phase diagram remain similar, with or without EZT (47). If EZT is increased, the relative lipid compositions of pooled gallbladder bile samples are progressively shifted down and to the left of the phase diagram, and enter the one-phase (protective) micellar zone (which contains an abundance of unsaturated micelles but never solid cholesterol crystals or liquid crystals). Thus, the micellar cholesterol solubility is increased in gallbladder bile with more cholesterol molecules transferred from the cholesterol monohydrate surface into unsaturated micelles. In this environment, gallstones can dissolve (47;151). EZT also protected gallbladder motility function by desaturating bile (47). It must be noted that the physical-chemical mechanisms underlying the beneficial effects of EZT on supersaturated bile, cholesterol crystals, cholesterol stones differ from those of hydrophilic bile acids such as UDCA, TUDCA and β-muricholic acid. These hydrophilic bile acids enhance dissolution of cholesterol gallstones by promoting the formation of a vesicle-enriched liquid crystalline mesophase (152). Translational studies have shown that EZT (20 mg p.o./day for one month) significantly reduced cholesterol concentration and cholesterol saturation index and retarded cholesterol crystallization in gallstone patients (47).

In conclusions, both animal and preliminary human studies show that EZT by inhibiting NPC1L1-mediated intestinal cholesterol absorption, also lowers biliary cholesterol secretion, desaturates bile, and preserves gallbladder motility function, even under conditions of high dietary cholesterol loads (47;151;153). Whether EZT will become a novel and effective cholelitholytic agent (alone or combined with statins and/or hydrophilic bile acids), is a matter of research in future well-designed, controlled, long-term clinical studies.

Agents effective on nuclear receptors

Coherent and coordinate activation of sets of genes involved in multiple cellular activities depend on nuclear receptors (NRs), which are ligand-activated transcription factors (154). Lipid sensing NRs govern lipid homeostasis in the hepatobiliary and gastrointestinal systems. Both hepatic and biliary lipid metabolism are involved in lipid secretion by the hepatocytes, and are controlled by the oxysterol receptor LXR (the intracellular “sensor” of cholesterol (155)) and the bile acid receptor farnesoid X receptor (FXR) (the intracellular “sensor” of bile acids (156;157)). The subtle cellular mechanisms governing lipid homeostasis imply that cells synthesize oxysterols under conditions of cholesterol overload, and oxysterols in turn bind and activate LXR which acts to reduce systemic cholesterol burden (156-158). In the enterohepatic system FXR is highly expressed and regulates the expression of genes involved in the maintenance of cholesterol, bile acid and triglyceride homeostasis (159). Of note, liver FXR might become an interesting pharmacological target for the treatment of cholestasis and cholelithiasis (160), since this NR up-regulates the expression of bile acid transporters in the canalicular membrane and of enzymes responsible for bile acid detoxification. Interestingly, manipulation of NRs in the animal model has disclosed novel potential approaches to the problem of cholesterol gallstone disease. FXR is a promising therapeutic target for treating or preventing cholesterol gallstone disease. FXR null mice are susceptible to cholesterol gallstone formation; activating FXR with the compound GW4064, a specific synthetic ligand, by contrast, increase biliary bile salt and phospholipid concentrations (161). The effect is tightly dependent on FXR-induced regulation of the energy dependent ATP-Binding Cassette (ABC) transporters ABCB11 (for bile salts) and ABCB4 (for phospholipid) (162) and is associated with better solubilization of cholesterol, and prevention of solid plate-like crystals and stones. Looking at the other side, increased propensity to cholesterol crystallization and stone formation in bile has been described in mice following the activation of hepatic LXR and direct up-regulation of the major hepatocyte cholesterol canalicular transporters ABCG5 and ABCG8 which together for an heterodimer (163). Again, future studies need to test if liver-specific FXR agonists and LXR antagonists might be safe and effective in human gallstone disease, as is the case for dyslipidemia, type II diabetes and several cancers.

Conclusions

The advent of laparoscopic cholecystectomy has moved the interest away from the pharmachological treatment of gallstones. Currently, medical therapy is restricted to a scant group of symptomatic (colicky pain) well-selected patients, in which both the unfavorable cost-benefit analyses and a high rate of gallstone recurrence play a negative role. Following early cholelitholytic therapies with the oral bile bile acid UDCA, recent studies indicate that the research agenda should include studies on the role of gallstone (*LITH*) genes, as well as the mechanisms of intestinal absorption of cholesterol and pathways of liver synthesis-secretion of cholesterol. Promising agents might include, alone or in combination, statins (competitive inhibitors of HMG-CoA reductase, the rate-limiting step in cholesterol biosynthesis), ezetimibe (specific inhibitor of the intestinal cholesterol transporter protein NPC1L1), and liver-specific agonists/antagonists of the nuclear receptors FXR/LXR involved in biliary lipid secretion.

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List of abbreviations

CDCA	chenodeoxycholic acid

EZT	ezetimibe
FXR	farnesoid X receptor
LXR	liver X receptor
NPC1L1	Niemann-Pick C1-like 1
NR	nuclear receptors
NSAIDs	non-steroidal anti-inflammatory drugs
TUDCA	tauroursodeoxycholic acid
UDCA	ursodeoxycholic acid

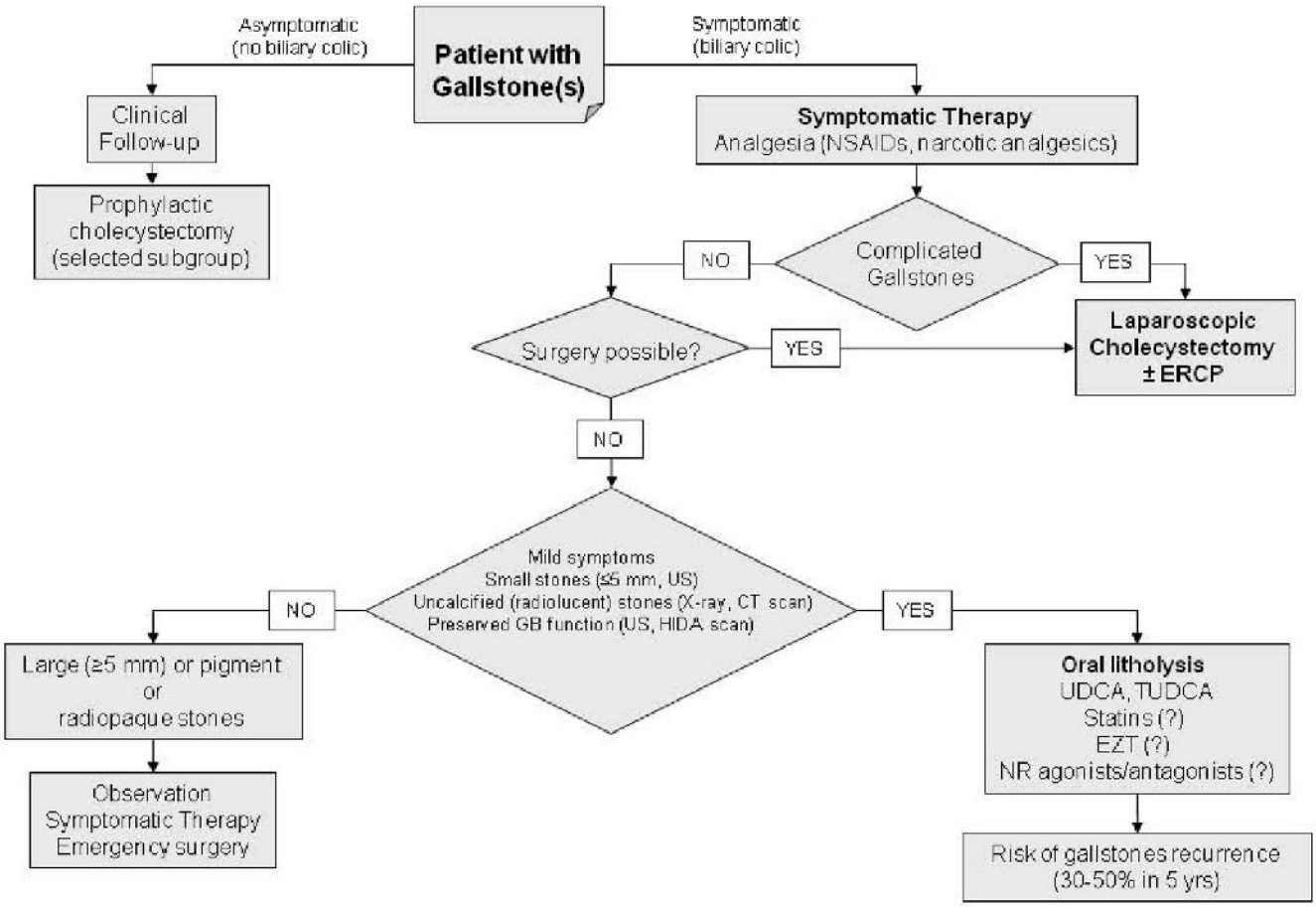

Figure 1.

Current therapies of gallstone disease, including cholesterol gallstones (adapted from P. Portincasa *et al.* (1;36;123)). Novel and potentially effective medical therapies are denoted by the symbol (?). See text for details. Results from meta-analyses indicate surgery as the gold standard for the treating symptomatic gallstones (164-166). Laparoscopic cholecystectomy and small incision cholecystectomy (166), are safe and have similar mortality rate (from 0.1% to 0.7%) (122;165). Both approaches are cost-effective, if compared with open cholecystectomy (165). Compared with open cholecystectomy, both convalescence and hospital stay are shorter and total cost is lower for laparoscopic cholecystectomy (122). Complication rates (including bile duct injuries) are similar between laparoscopic and open cholecystectomy (122;165). When looking at surgical options, a "prophylactic" cholecystectomy can be taken into account in a subgroup of asymptomatic patients bearing a high risk of becoming symptomatic: children (who are exposed to long-term physical presence of stones (167)), morbid obese patients undergoing bariatric surgery (who are at high risk to became symptomatic during rapid weight loss (168)), patients at increased risk for gallbladder cancer (169) (i.e. those with large gallstones, greater than 3 cm) (170;171), a "porcelain" gallbladder (172) or gallbladder polyps rapidly growing or larger than 1 cm). Prophylactic cholecystectomy should also be considered in Native Americans with gallstones, who are at increased risk of gallbladder cancer (3 to 5 percent) (173), and asymptomatic gallstone patients with sickle cell anemia, who form calcium bilirubinate gallstones due to chronic hemolysis and may become symptomatic with recurrent episodes of abdominal pain (174). Prophylactic cholecystectomy has also been proposed in patients with small gallstones and gallbladder dysmotility, since the coexistence

of these conditions increases the risk of pancreatitis (51). Abbreviations: CT, computerized tomography; ERCP, endoscopic retrograde cholangiopancreatography; EZT, ezetimibe; HIDA, 99mTc-N-(2,6-dimethylacetanilide)-iminodiacetic acid; GB, gallbladder; GS, gallstones; NR, nuclear receptors; NSAIDs, non-steroidal anti-inflammatory drugs; TUDCA, tauroursodeoxycholic acid; UDCA, ursodeoxycholic acid; US, abdominal ultrasonography.

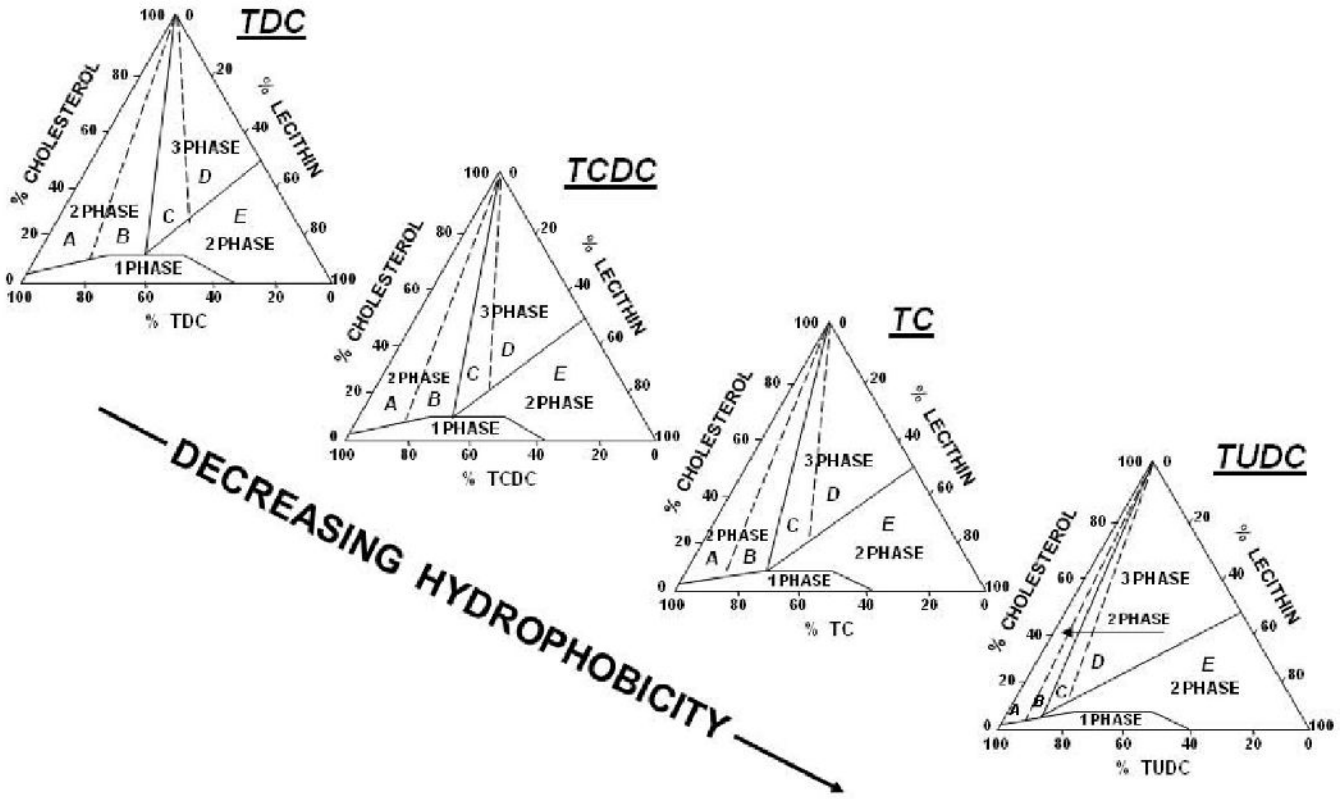

Figure 2.

Effects of UDCA on bile composition and cholesterol solubility are explained by using the ternary phase diagram (175). A group of the equilibrium phase diagram of cholesterol-lecithin-taurine-conjugated bile saltacid systems (37°C, 0.15 M NaCl, pH 7.0, total lipid concentration 7.5 g/dL) are drawn to display varied positions and configuration of crystallization regions due to decreasing bile salt hydrophobicity. The lipid components are expressed in moles percent. The one-phase micellar zone at bottom is enclosed by a solid curved line. Above it, two solid lines divide the two-phase zones from a central three-phase zone. Based upon the solid and liquid crystallization sequences present in the bile, the left two-phase and the central three-phase regions are divided by dashed lines into regions A to D. The number of phases given represents the equilibrium state. They are cholesterol monohydrate crystals and saturated micelles for crystallization regions A and B. Cholesterol monohydrate crystals, saturated micelles and liquid crystals for regions C and D, and liquid crystals of variable compositions and saturated micelles for region E (175). As the bile acid hydrophobicity decreased, the maximum micellar cholesterol solubility is reduced and crystallization pathways A-E move to the left. This change results in an enlarged region E that extends to the left and overlaps pathophysiological compositions as exemplified in the tauroursodeoxycholate (TUDC)-lecithin-cholesterol system. This event induces a greatly reduced chance for the formation of solid plate-like cholesterol monohydrate crystals in bile. Adapted and reproduced with permission from (175) and (123).

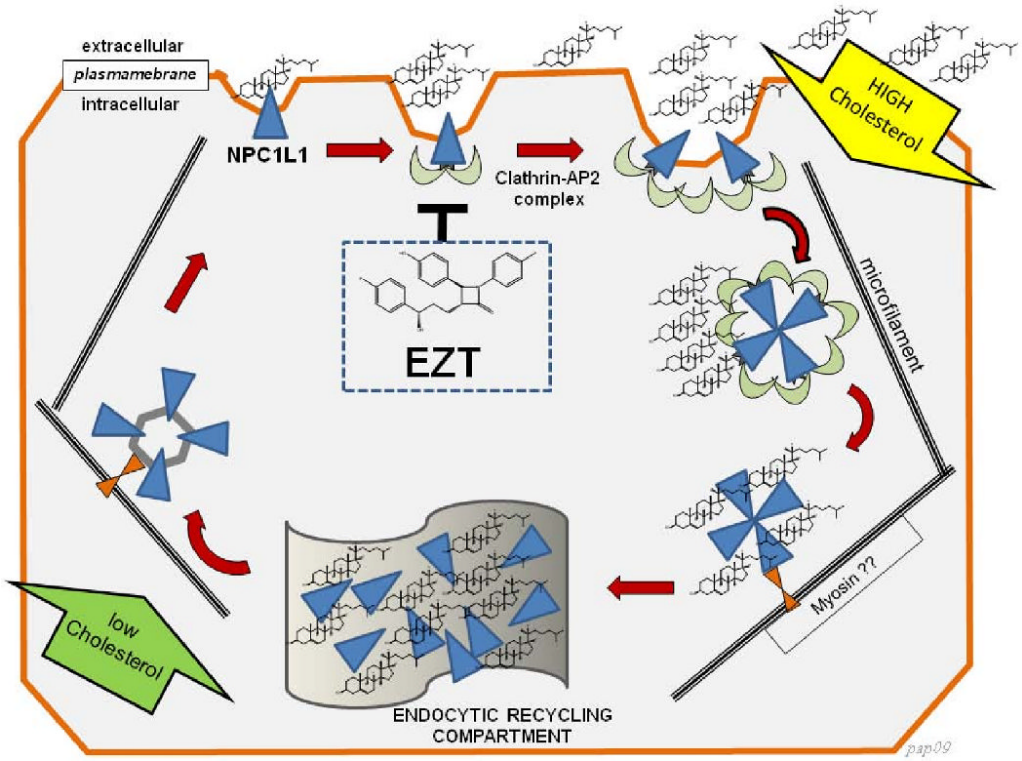

Figure 3.

Mechanisms for cholesterol uptake mediated by the NPC1L1 according to the model proposed by Ge *et al*. (148). Adapted and reproduced with permission, Copyright Bentham Science Publisher, 2009 (123). The NPC1L1 protein recycles between the plasma membrane facing the extracellular space and the endocytic recycling compartment. If extracellular cholesterol concentration is high, cholesterol is incorporated into the plasma membrane and is sensed by cell surface-localized NPC1L1. Both NPC1L1 and cholesterol are then internalized together through clathrin/AP2-mediated endocytosis. The clathrin-coated globular vesicles are transported along microfilaments to the endocytic recycling compartment. The role of myosin in this process is unclear. Large quantities of cholesterol and NPC1L1 are subsequently stored within the endocytic recycling compartment. If the intracellular cholesterol level is low, endocytic recycling compartment-localized NPC1L1 moves back to the plasma membrane along microfilaments and new cholesterol is absorbed. The key role of the NPC1L1 inhibitor ezetimibe (EZT) is shown at the center of the cell. EZT prevents NPC1L1 from entering the AP2-mediated clathrin-coated vesicles. At this stage, the endocytosis of NPC1L1 is inhibited and cholesterol absorption is decreased.

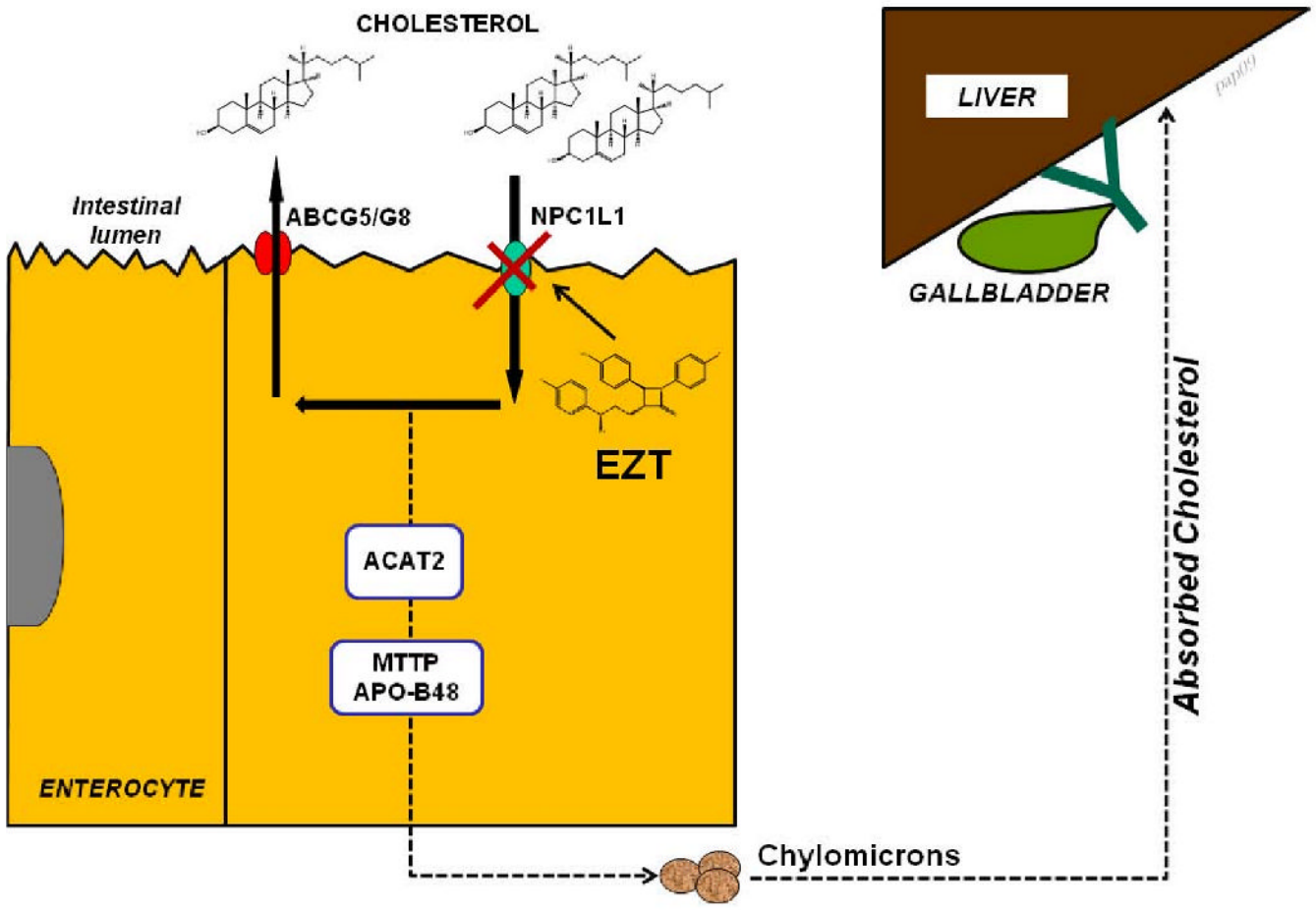

Figure 4.

Pathways underlying absorption of cholesterol from the intestinal lumen and its delivery to the liver. High dietary cholesterol through the chylomicron pathway could provide an important source of excess cholesterol molecules for secretion into bile, thereby inducing cholesterol-supersaturated bile and enhancing cholesterol gallstone formation (31;47). Ezetimibe (EZT) significantly suppresses cholesterol absorption from the small intestine via the Niemann-Pick C1-like 1 (NPC1L1) pathway (47). This effect should diminish the cholesterol content of the liver, which in turn decreases bioavailability of cholesterol for biliary secretion. Abbreviations: ABCG5/G8, ATP-binding cassette (transporter); ACAT2, acyl-CoA:cholesterol acyltransferase isoform 2; APO-B48, apolipoprotein B48; MTTP, microsomal triglyceride transfer protein. See text for details.