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TOPIC HIGHLIGHT

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Therapy of gallstone disease: What it was, what it is, what it will be

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Abstract

Cholesterol gallstone disease is a common clinical condition influenced by genetic factors, increasing age, female gender, and metabolic factors. Although laparoscopic cholecystectomy is currently considered the gold standard in treating patients with symptomatic gallstones, new perspectives regarding medical therapy of cholelithiasis are currently under discussion, also taking into account the pathogenesis of gallstones, the natural history of the disease and the analysis of the overall costs of therapy. A careful selection of patients may lead to successful non-surgical therapy in symptomatic subjects with a functioning gallbladder harboring small radiolucent stones. The classical oral litholysis by ursodeoxycholic acid has been recently paralleled by new experimental observations, suggesting that cholesterol-lowering agents which inhibit cholesterol synthesis (statins) or intestinal cholesterol absorption (ezetimibe), or drugs acting on specific nuclear receptors involved in cholesterol and bile acid homeostasis, might be proposed as additional approaches for treating cholesterol gallstones. In this review we discuss old, recent and future perspectives on medical treatment of cholesterol cholelithiasis.

Key words: Gallstones; Dissolution therapy; Cholecystectomy; Bile acids; Ezetimibe; Statins; Gallbladder; Bile; Nuclear receptors

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INTRODUCTION

The prevalence of gallstones increases with age, and is associated with a number of major risk factors (Table 1)[1-3].

In westernized countries, well known risk factors are: obesity, type 2 diabetes, dyslipidaemia, and hyperinsulinaemia, which are often components of the metabolic syndrome[4-8]. Although the majority of stones in the gallbladder remain "silent" and do not require medical or surgical treatment, gallstone disease is still one of the most common digestive diseases requiring hospital admission and financial resources, since its prevalence ranges from 10% to 15% in adults and medical expenses for gallstone treatment exceeded $6 billion in the year 2000 in the United States[1,9-11].

To know exactly the composition of gallstones is an essential step to select patients responsive to oral litholysis with bile acids (see below). In principle, the only gallstones amenable to litholysis are cholesterol-enriched, calcium-free stones. Cholesterol gallstones represent about 75% of the gallstones in westernized countries[12-14] and can be dissolved when no calcium has deposited in the stones[1,15]. Historically, the Renaissance physician, botanist, alchemist and astrologer Paracelsus (Philippus Aureolus Theophrastus Bombastus von Hohenheim) was the first one to hypothesize that gallbladder concrements were originating from the precipitation of solid material made of tartaric acid[16,17]. To date, we know that specific pathogenetic factors contributing to the formation of cholesterol gallstones must include: hepatic hypersecretion of cholesterol into bile leading to a supersaturated bile, accelerated nucleation/crystallization of cholesterol, defective gallbladder motility (a form of leiomyopathy) leading to gallbladder stasis, increased absorption of intestinal cholesterol, and influence of *LITH* genes[1,18-24]. The remaining gallstones are pigment stones that contain less than 30% cholesterol, i.e., black pigment stones which are 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 which are about 5% of all gallstones, found in bile ducts (containing calcium bilirubinate, calcium palmitate, stearate and cholesterol)[25].

Patients presenting with a typical colicky pain ("symptomatic") do need treatment because of the high rates of complications (e.g., acute cholecystitis, acute biliary pancreatitis or cholangitis), and early recurrence of symptoms. The high costs of both surgical and medical therapeutic interventions and the natural history of the disease indicate restricting the treatment to a subgroup of symptomatic patients with specific symptoms[1,23,26].

The first cholecystectomy was performed in 1882 by Carl Langenbuch in Berlin[27,28], which was the first milestone in the treatment of gallstones. Initial experiments on the dissolution of gallstones were already happening at the end of the 19th century[29,30] and in the first half of the 20th century[31]. However, it was Danzinger *et al*[32] in 1972 who reported that the primary bile acid chenodeoxycholic acid (CDCA) could dissolve cholesterol gallstones in humans when given orally for 6 mo. These days, oral litholysis by ursodeoxycholic acid (UDCA) plays a limited role in cholesterol gallstone treatment. However, some novel and interesting therapeutic options have been suggested by data from pathogenetic and pharmacological studies[1], in particular in subjects permanently or temporarily at risk for gallstone disease (Table 1). Experimental data on the capacity of the Niemann-Pick C1-like 1 (NPC1L1) protein inhibitor ezetimibe to reduce intestinal absorption of cholesterol[33], the effects of statins to inhibit cholesterol synthesis[34], or drugs acting on specific nuclear receptors (NRs) involved in cholesterol and bile acid homeostasis[35] may offer an integrate, potent and innovative strategy for the medical treatment of cholesterol gallstones[36]. Major updated therapeutic aspects in patients with gallstones will be reviewed in this paper.

MANAGING GALLSTONE DISEASE

The therapeutic option of gallstone disease is based on few crucial steps, i.e., presence/absence of typical symptoms (i.e., colicky pain), presence of complications, and gallbladder function, as well as composition and size of gallstones (Figure 1).

Bearing in mind data on epidemiology and overall costs of both medical and surgical therapies, it is not routinely recommended to treat asymptomatic gallstone patients[37-39]. Thus, an expectant management (medical attention) is currently considered the most appropriate choice in patients with gallstones of any type without specific symptoms (i.e., biliary colic). Indeed, approximately 60%-80% of patients with gallstones are completely asymptomatic[40-42] and stones are frequently found during routine abdominal ultrasonography[40-42]. In general, the risk of developing typical biliary pain is low (2.0%-2.6% per year[43-46]) although microlithiasis or biliary sludge in the gallbladder lumen puts patients at risk for colicky pain or acute pancreatitis[47,48]. Nevertheless, the overall risk rate for complications (yearly incidence 0.3%) and gallbladder cancer (0.02%) are very low[49,50]. If biliary pain and/or complications are present, cholecystectomy represents the gold standard (see below), as oral litholysis with hydrophilic bile acids have a limited role, and are reserved to symptomatic patients with small radiolucent gallstones in a well functioning gallbladder with a patent cystic duct[1,23]. Before cholecystectomy, however, careful medical attention and analgesia are often required. Major features of the uncomplicated biliary colic are depicted in Table 2, concerning pathogenesis, onset, intensity, localization, duration, radiation, associated features, relief of pain, and therapeutic aspects. The chemical formula of drugs currently used to induce analgesia in patients with colicky pain is depicted in Figure 2.

Cholecystectomy

Cholecystectomy can be performed by laparoscopy, by a small-incision (< 8 cm in length), or by open operation, and several meta-analyses indicate surgical procedures as the gold standard for the treatment of symptomatic gallstones[51-53]. Laparoscopic cholecystectomy, or alternatively, small incision cholecystectomy[53], are both safe with

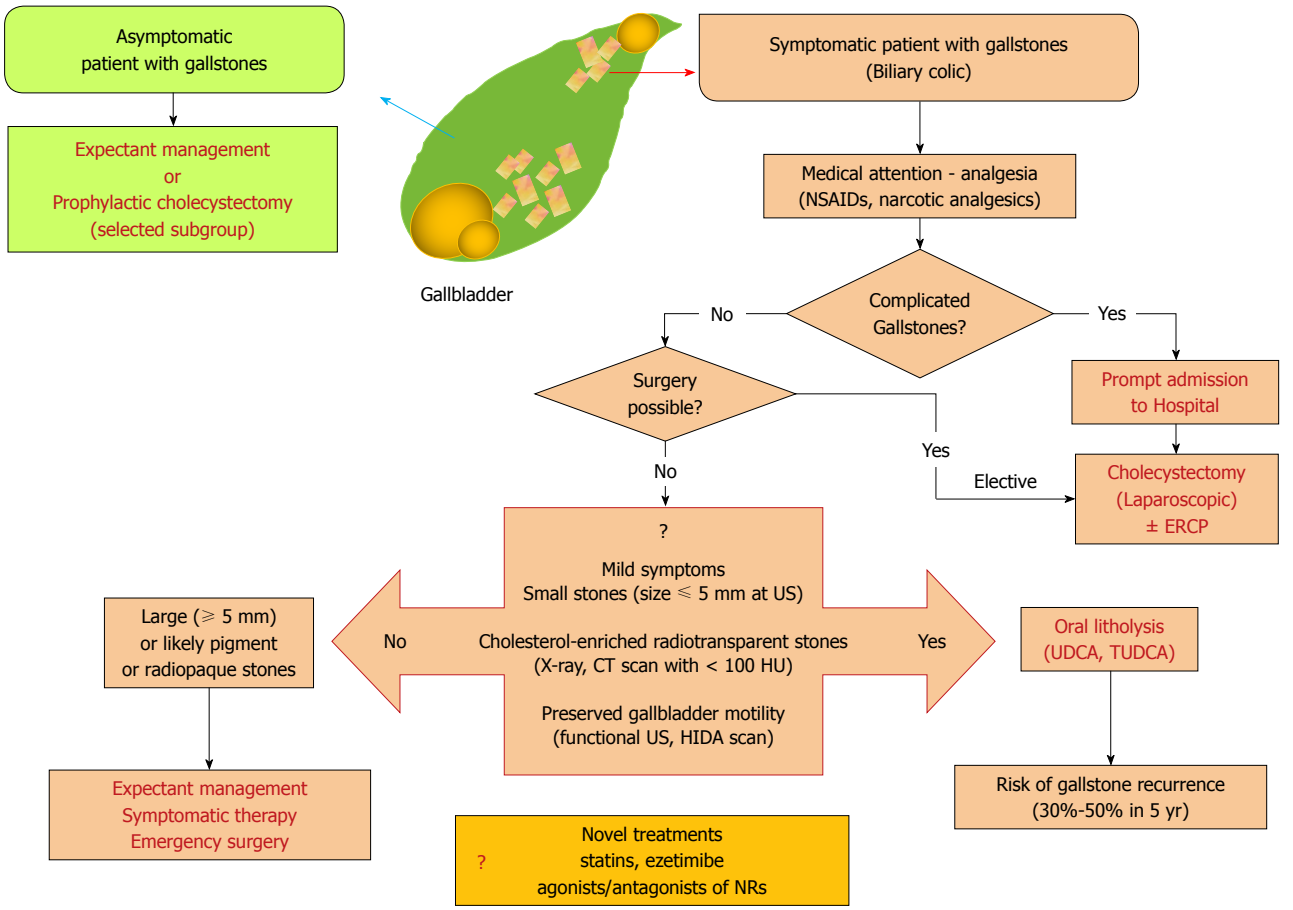

Figure 1 Flow-chart depicting the standard therapies of gallstone disease (adapted from Portincasa *et al*[1,15,23,148]). As a starting point, at the top the gallbladder containing "supersaturated" biliary cholesterol is depicted. Typical solid plate-like monohydrate cholesterol crystals form first and aggregate after, to grow as cholesterol stones. Left: flow-chart reserved to asymptomatic patients with gallstones (i.e., when stones/crystal aggregates are not impacted within the cystic duct). Best choice is expectant management, while few indications for prophylactic cholecystectomy exist and are reported in Table 2; Right: the complex flow-chart reserved to symptomatic gallstone patients is shown. This is the case when stones/crystal aggregates are impacted within the cystic duct. A key step is to identify the "symptomatic" patients with or without complications. In this respect, documenting the presence of biliary colic is of key importance. Meta-analyses indicate that surgery (cholecystectomy) is the gold standard for treating symptomatic gallstones[51-53]. For treatment of uncomplicated and complicated biliary colic, see also Tables 3 and 4. CT: Computed tomography; ERCP: Endoscopic retrograde cholangiopancreatography; HIDA: 99mTc-N-(2,6-dimethylacetanilide)-iminodiacetic acid; HU: Hounsfield Unit; NSAIDs: Non-steroidal anti-inflammatory drugs; NRs: Nuclear receptors; TUDCA: Tauroursodeoxycholic acid; UDCA: Ursodeoxycholic acid; US: Abdominal ultrasonography. The HU is an arbitrary unit of X-ray attenuation used for CT scans. Each voxel is assigned a value on a scale in which air has a value of -1000; water, 0; and compact bone, +1000.

Table 1 Non-genetic risk factors for gallbladder stones

Age
Female gender
High-calorie, low-fiber diet
High-carbohydrate diet, dietary glycemic load
Obesity
Physical inactivity
Rapid weight loss/surgery for obesity
Total gastrectomy with lymph node dissection
Spinal cord injury
Infections: enterohepatic *Helicobacter* species, malaria
Biliary strictures
Drugs: estrogens, calcineurin inhibitors, fibrates, octreotide, ceftriaxone
Total parenteral nutrition
Duodenal diverticulum
Extended ileal resection (black pigment stones)
Vitamin B_{12}/folic acid deficient diet (black pigment stones)
Pancreatic insufficiency
Cholangitis (brown pigment bile duct stones)

Adapted from Portincasa *et al*[1] and Grünhage *et al*[160] with permission.

a similar mortality rate ranging from 0.1% to 0.7%[52,54]. Both these procedures are cost-effective compared with open cholecystectomy[52]. Hospital stay and convalescence are shorter, as is the total cost lower for laparoscopic cholecystectomy compared with open cholecystectomy[54]. The overall incidence of bile duct injuries requiring corrective surgery varies between 0.1% and 0.3%[55-57] and both laparoscopic and open cholecystectomies yield similar complication rates[52,54].

Principally due to the low rate of complications, it is currently under discussion if cholecystectomy may be suggested also for patients with asymptomatic gallstones, but it is generally conceived that surgical procedures are not recommended routinely in symptom-free patients (Figure 1). Few indications for prophylactic cholecystectomy in asymptomatic patients with gallstones are reported in Table 3. For example, cholecystectomy should be considered in children with asymptomatic gallstones[58] (in particular with sickle cell disease[59,60], spherocytosis,

Table 2 Major features of the uncomplicated biliary colic

Pathogenesis	Visceral pain caused by the impaction of the stone in the cystic duct or the ampulla of Vater, followed by distension of the gallbladder and/or biliary tract with activation of visceral sensory neurons[161]
Onset	Not exclusively postprandial, typically intermittent
Intensity	Mean visual analogue scale of 9 cm on a 0-10 cm scale
Localization	Most frequently right upper quadrant of the abdomen and/or the epigastrium (representative dermatomes T8/9)
Duration	Generally longer than 15-30 min. Can last several hours and be associated non-specific symptoms of indigestion
Radiation	Angle of the right scapula and/or shoulder (about 60% of cases), retrosternal area (less than 10% of cases)
Associated features	Urgency to walk[162] (two-third of patients), nausea or vomit[42,161,162]
Relief	If the stone returns into the gallbladder lumen, passes through the ampulla of Vater into the duodenum or migrates back to the common bile duct[26]
First-line therapy	Fast-acting narcotic analgesics (meperidine[163]) or non-steroidal anti-inflammatory drugs (NSAIDs) (im or iv ketorolac or ibuprofen po) which could also reduce the risk of evolution towards acute cholecystitis[164-167]
Second-line therapy	Antispasmodic (anticholinergic) agents like hyoscine (scopolamine). Less effective than NSAIDs[164]
Recommendations	Fasting, to avoid release of endogenous cholecystokinin and further gallbladder contraction

Adapted from[25,63,148] with permission.

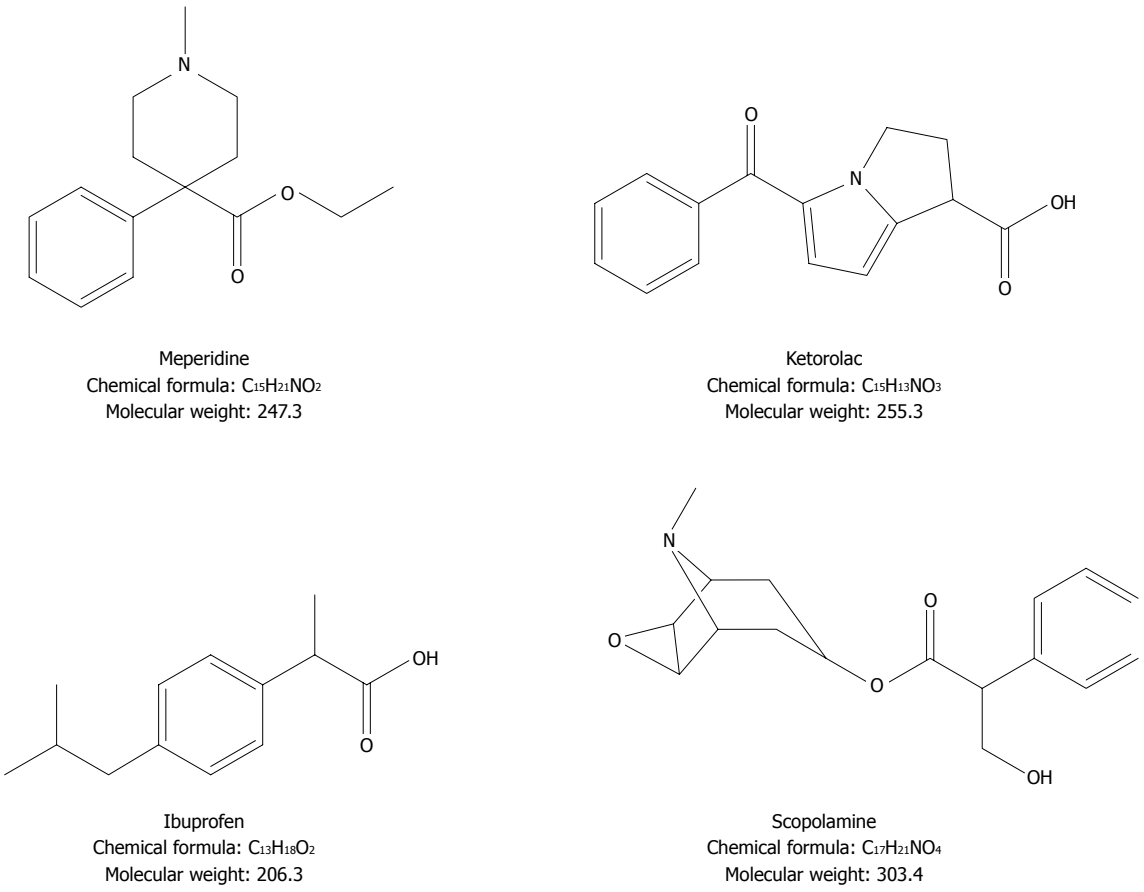

Figure 2 Chemical formula of drugs currently used to induce analgesia in patients with colicky pain. The three categories are: narcotic analgesics, non-steroidal anti-inflammatory drugs, and antispasmodics.

and elliptocytosis[60]) who are exposed to the risk of pain and complications. In this group the natural history of gallstones is not well known[61], although a recent study suggests that clinically silent gallstones in children and infants are associated with low rates of complications and can be therefore managed conservatively[60], as in adults. Other groups in which prophylactic cholecystectomy must be considered are the morbidly obese undergoing bariatric surgery, patients at high risk for gallbladder cancer, patients with sickle cell anemia, and coexistence of small gallstones and gallbladder dysmotility[47,62-68]. A totally different approach is necessary in the case of complicated biliary colic, as also shown in Table 4.

OLD AND NEW NON-SURGICAL OPTIONS

There is no established medical therapy for dissolution of pigment stones or calcified stones of any type. For

Table 3 Indications for "prophylactic" cholecystectomy (i.e., asymptomatic gallstone patients bearing a high risk of becoming symptomatic)

Children (because they are exposed to the long-term physical presence of stones[58])
Morbid obese patients undergoing bariatric surgery (high risk to become symptomatic during rapid weight loss[62])
Increased risk for gallbladder cancer[63]
Patients with large gallstones (greater than 3 cm)[64,65]
A "porcelain" gallbladder[66] or gallbladder polyps rapidly growing or larger than 1 cm
Native Americans with gallstones (risk of gallbladder cancer 3 to 5 percent)[67]
Gallstone patients with sickle cell anemia (formation of calcium bilirubinate gallstones due to chronic hemolysis. Patients may become symptomatic with recurrent episodes of abdominal pain[68])
Coexistence of small gallstones and gallbladder dysmotility (increased risk of pancreatitis[47])

Adapted from[25,63,148] with permission.

Table 4 Major features of the complicated biliary colic

Additional findings compared to uncomplicated biliary pain	Leukocytosis, nausea, jaundice, vomiting, fever
Underlying potential complications	Acute pancreatitis, acute cholecystitis, biliary obstruction and cholangitis, gallbladder perforation, abscess formation, mucocele of the gallbladder
Decision	Quick admission to the hospital
Therapies	Antibiotics or invasive procedures with or without surgical procedures (Figure 1)
	Early laparoscopic cholecystectomy recommended between 2 and 4[168] in mild and moderate acute cholecystitis

Adapted from[25,63,148] with permission.

cholesterol gallstones, current medical treatment includes oral litholysis with bile acids (see below). Medical therapies alternative to oral bile acids have been proposed in the past, including direct stone dissolution with methyl tert-butyl ether (MTBE), a potent organic cholesterol solvent[69], extracorporeal shock-wave lithotripsy (ESWL)[70], or in combination[71], followed by oral litholysis with bile acids. The interest in such options, however, has vanished due to their invasiveness, potential toxicity (MTBE) or traumatic (ESWL) side effects and, for both, the high post-dissolution recurrence rate[1,72,73]. Novel treatments to be discussed include statins, ezetimibe, and agonists/antagonists of NRs.

Oral dissolution therapy

The first successful dissolution of cholesterol gallstones was achieved in 1972 by oral administration of the natural primary tri-hydroxy bile acid CDCA[32] (Figure 3). The use of CDCA was abandoned because side effects were noticed, including a dose-dependent increase in serum liver enzymes, an increase in serum low-density lipoprotein (LDL) cholesterol, and diarrhea.

A further step was to use the more hydrophilic tri-hydroxy bile acid UDCA[74]. UDCA is more hydrophilic and less toxic than CDCA, and is currently employed for oral litholysis of small cholesterol gallstones in patients with a functioning gallbladder (Figure 3). This bile acid, in a dose of 10-14 mg/kg per day, increases its proportion in the bile acid pool (it originally accounts for less than 8%-10% of the biliary bile acid pool in healthy subjects), inducing a decreased hepatic secretion of biliary cholesterol and the formation of unsaturated gallbladder bile (cholesterol saturation index of less than 1)[75-77], the key factor which promotes the dissolution of cholesterol crystals and gallstones.

The fine mechanisms involved in UDCA-induced dissolution of cholesterol stones are rather complex. The so-called ternary phase diagram is used to explain the molecular effects of UDCA on bile composition and cholesterol solubility[78]. A group of the equilibrium phase diagram of cholesterol-lecithin-taurine-conjugated bile acid systems (37 ℃, 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 acid hydrophobicity, with the lipid components being expressed in moles percent. At the bottom, the one-phase micellar zone exists (i.e., high bile acid-lecithin moles percent), while above this zone two-phase zones exist on both sides from a central three-phase zone. The study of solid and liquid crystallization sequences present in bile shows that different regions exist within each zone, namely A, B in the left two-phase, C, D in the central three-phase regions, and E in the right 2-phase zone. The number of phases given represents the equilibrium state and develop as 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[78]. As the bile acid hydrophobicity decreases, 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 ex-

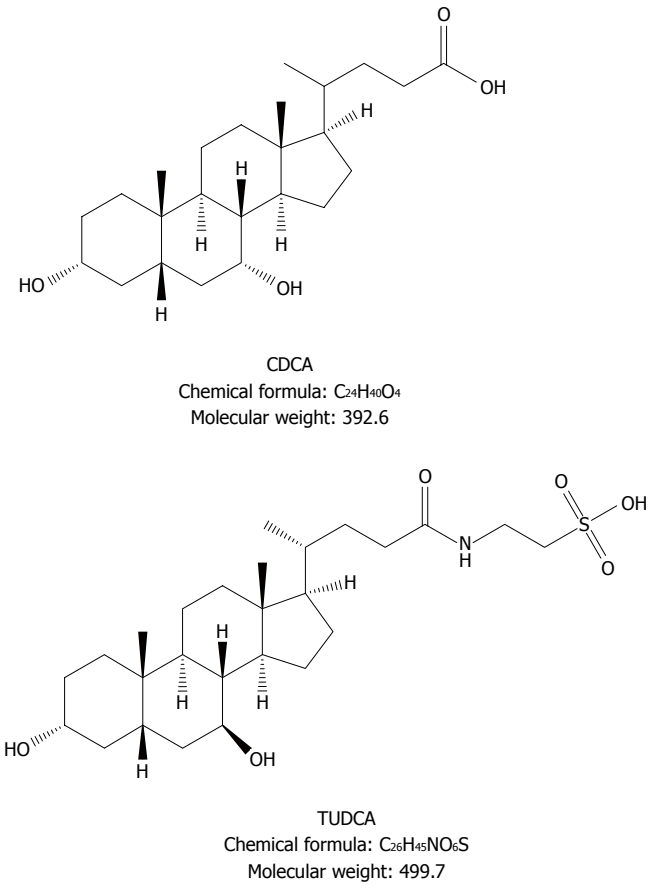

Figure 3 Chemical formula of bile acids used for oral litholysis of small, radiotransparent, cholesterol-enriched gallstones in a functioning gallbladder with a patent cystic duct of patients with symptomatic gallstones. CDCA: Chenodeoxycholic acid; UDCA: Ursodeoxycholic acid; TUDCA: Tauroursodeoxycholic acid.

emplified 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.

A bedtime administration of UDCA or TUDCA, is recommended since it maintains hepatic bile acid secretion rate overnight, thus reducing secretion of supersaturated bile and increasing the dissolution rate[79,80]. The hydrophilic bile acid UDCA is also able to act as a litholytic agent through the reduction of intestinal cholesterol absorption[81-83] and as a possible "prokinetic" agent capable of ameliorating postprandial gallbladder emptying as suggested by observations *in vitro* on isolated gallbladder smooth muscle strips from both animals and gallstone patients[84,85]. The improvement of gallbladder smooth muscle contractility probably also results from the prevention of the impairment of smooth muscle contractility induced by the more hydrophobic and toxic deoxycholate[86,87].

However, although the majority of gallstones (about two-thirds) in westernized countries are mainly composed of cholesterol, only a minority of patients (less than 10% of total) with cholesterol-enriched gallstones is amenable to oral dissolution therapy with UDCA or with its taurine-conjugates TUDCA[1,26]. In fact, dissolution therapy with oral bile acids can be only suggested to symptomatic gallstone patients who are unfit for surgery and have small (equal to or less than 5 mm in size), uncalcified (radiolucent), and cholesterol-enriched (i.e., more than 80%) stones in a functioning gallbladder with a patent cystic duct[88]. A number of diagnostic techniques provide essential information for appropriate selection of patients.

Gallbladder ultrasonography allows the accurate visualization of gallstone number, size, burden, biliary sludge[39,89,90] and explores the morphology and contractile property of the gallbladder, the features of the gallbladder wall with respect to the (acute-chronic) inflammatory status, and the patency of the cystic duct[91-97]. An abdominal plain radiography or a computed tomography (CT) scan[98,99] are needed to exclude the presence of calcified stones[25]. By CT scan, in particular, values of < 100 Hounsfield Units predict radiolucent cholesterol rich, dissolvable stones[100] (see also Figure 1 for explanation).

An accurate selection of gallstone patients with the characteristics described above offers a higher chance of successful oral litholysis alone or after ESWL inducing stone fragmentation[93-96,101], with an expected dissolution rate of about 1 mm decrement in stone diameter per month[102].

The complete disappearance of stones with a diam-

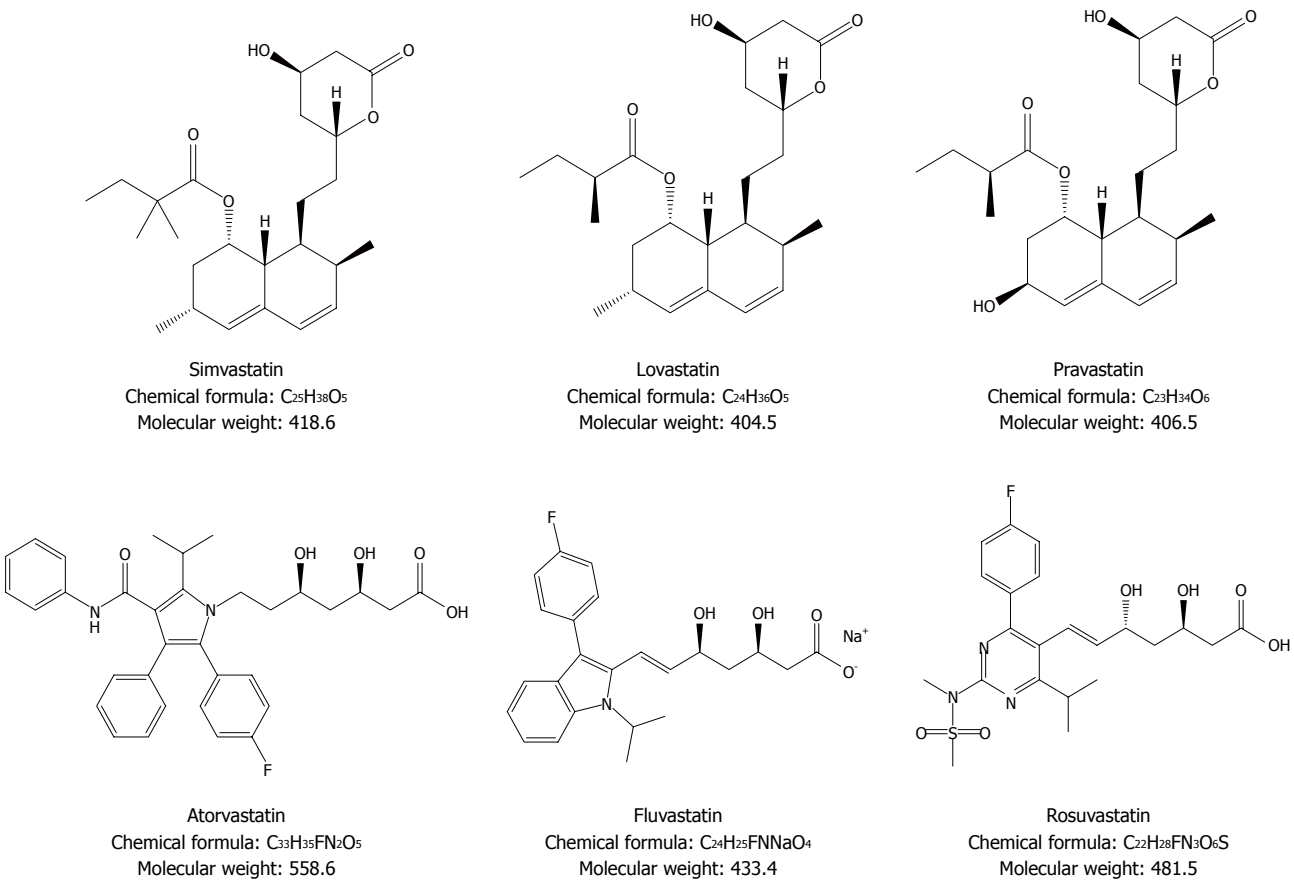

Figure 4 Chemical formula of different statins used to inhibit hepatic cholesterol synthesis[119-122].

eter of less than 5 mm has been described after 6 mo of UDCA administration in about 90% of cases[103]. The chance of dissolution is significantly lower (less than 40%-50% after 1 year of the treatment) in patients with larger or multiple stones[49,104].

Main limits of the dissolution therapy by oral bile acids are the possibility of gallstone recurrence (about 10% per year up to 5 years[105,106]) and the risk of appearance of a surface calcification on cholesterol gallstones during bile acid therapy in about 10% of cases[107]. A recurrence rate of 30%-50% at 5 years is seen after bile acid therapy or lithotripsy[94,108-110], particularly in patients with multiple gallstones[109]. After gallstone disappearance, the persistence of the same pathogenetic factors inducing gallstone formation is principally responsible for their recurrence[1]. It has to be underlined, however, that recurrent gallstones respond well to a re-treatment[99,111].

Although limited to a relatively small subgroup of patients, the dissolution therapy with UDCA or TUDCA still remains at present an interesting tool in patients who form gallstones as a consequence of transient and non-genetic risk factors (i.e., pregnancy, convalescence from abdominal surgery, obese patients during rapid weight loss[1,112-114], Table 1) and, thus, have a minimum risk of recurrence. Early non-randomized or placebo-controlled studies[115-117] suggested that UDCA might also reduce the risk of biliary colic. A large randomized, double-blind, placebo-controlled trial on the effects of UDCA in highly symptomatic gallstone patients scheduled for cholecystectomy, however, found that UDCA was ineffective on biliary colic. In fact, the likelihood of remaining colic-free is comparable in patients with strong or weak baseline gallbladder contraction as determined by ultrasonography after a standard mixed meal[118].

CHOLESTEROL LOWERING AGENTS

Bile supersaturation with cholesterol is a key factor for cholesterol gallstone formation, and it is principally related to a sustained hepatic hypersecretion of cholesterol depending on the source; from hepatic cholesterol biosynthesis, intestinal cholesterol absorption and HDL-derived cholesterol[18]. As a consequence, all drugs targeting these steps are potentially able to influence both cholesterol gallstone formation and dissolution. Statins and ezetimibe have interesting effects.

Statins are competitive inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, a rate-limiting enzyme for cholesterol biosynthesis, and they are able to reduce biliary cholesterol independently of their ability to suppress hepatic cholesterol synthesis[119-122]. Several statins are being used (Figure 4) and their pharmacological properties modulate cholesterol homeostasis both in bile and in the liver, potentially leading to a reduction of

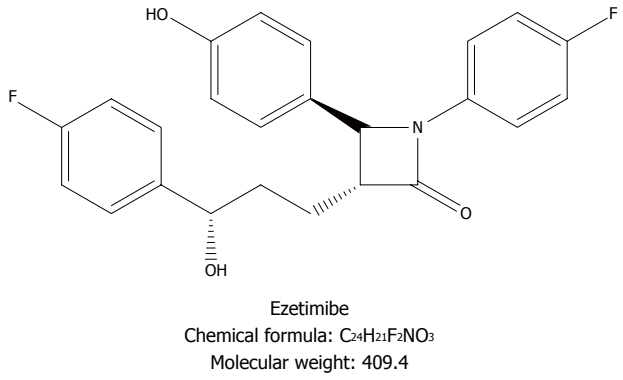

Figure 5 Chemical formula of ezetimibe, the specific inhibitor of the Niemann-Pick C1-like 1 protein.

cholesterol gallstone formation[123-125], as clearly demonstrated by animal studies[126,127]. In humans, by contrast, the potential beneficial effects of statins on cholesterol gallstones are not so clear.

The risk of cholecystectomy decreased slightly in a cohort of US women self-reporting long-term use of statins[128]. Similar results were suggested by a case-control analysis using the UK-based General Practice Research Database and evaluating incident patients between 1994 and 2004. In this study the long-term use of statins (1 to 1.5 years) was associated with a decreased risk of gallstones followed by cholecystectomy, compared with patients without statin use[129]. Furthermore, a recent population-based control study using medical databases from northern Denmark showed a decreased odds ratio for gallstone disease in current statin users (1-2 years of statin use), as compared with nonusers[130]. However, experimental studies show controversial results, since a decreased biliary cholesterol concentration, a reduced gallstone formation, or gallstone dissolution has been found by some[131-134] but not all studies[124,135-138].

Recent advances underscore the role of intestinal factors as a key factor for cholesterol absorption, biliary secretion and cholesterol gallstones[1,139]. In fact, it has been found experimentally that if dietary cholesterol is absent, all biliary cholesterol derives mainly from a limited *de novo* synthesis (less than 15%). Thus, the small intestine must be seen as a unique organ providing dietary and reabsorbed biliary cholesterol to the body[139]. This step plays a crucial role in cholesterol gallstone pathogenesis, since animal studies demonstrate that there is a significant positive correlation between the efficiency of intestinal cholesterol absorption and the prevalence of cholesterol gallstone formation[21].

Ezetimibe, in this respect, is an interesting drug since it has novel hypocholesterolemic effect[140] (Figure 5). Ezetimibe has a strong inhibitory effect on intestinal cholesterol absorption; cholesterol is indeed the most effective substrate of the NPC1L1 protein, the protein that governs intestinal absorption of cholesterol by recycling between the endocytic recycling compartment and plasma membrane[141]. NPC1L1 is highly expressed in the small intestine and localized along the brush border in both humans and mice[142,143], but also present in the human liver[143,144]. In mice, ezetimibe largely reduces cholesterol, and to some extent phospholipid content, but not the bile acid content in gallbladder bile. However, all crystallization pathways and phase boundaries on the bile phase diagram are essentially similar, regardless of whether animals are treated with or without ezetimibe[36]. By inhibiting both the cholesterol absorption in the intestine and the hepatic uptake of chylomicron remnants, ezetimibe might lower biliary cholesterol secretion and saturation[145]. Furthermore, it has been also demonstrated that increasing doses of ezetimibe lead the relative lipid composition of gallbladder bile to a progressive shift down and to the left of the phase diagram, which goes into the one-phase (protective) micellar zone, with an abundance of unsaturated micelles but never solid cholesterol crystals or liquid crystals. As a consequence, in gallbladder bile the micellar cholesterol solubility is increased, with more cholesterol molecules transferred from the cholesterol monohydrate surface into unsaturated micelles. In this environment, gallstones are reduced in size and can be completely dissolved[36,146]. Ezetimibe might therefore act as a new tool in treating/preventing cholesterol gallstones[147] but also induce amelioration of gallbladder motility, as a consequence of bile desaturation[36]. Ezetimibe is also effective in humans, since it has been demonstrated in a Mexican population that this drug in a dosage of 20 mg po/d for 1 mo, is able to significantly reduce cholesterol saturation and cholesterol saturation index and to retard cholesterol crystallization in gallstone patients[36].

In the near future, well designed experimental studies might confirm the efficacy of statins and ezetimibe, alone and/or in association with hydrophilic bile acids, in symptomatic patients without genetic risk of gallstone formation but in the presence of several predisposing conditions (Table 1). Obesity, in particular, is associated with an increased cholesterol biosynthesis in the liver, mostly due to higher levels of HMG-CoA reductase activity. Thus, in obese patients, the administration of statin might be potentially useful to prevent gallstone formation[148]. It may be also useful in patients with rapid weight loss, a condition characterized by an increased hepatic secretion of biliary cholesterol, an increase in mucin production by the gallbladder epithelium, and a significant impairment of gallbladder motility[149].

AGONISTS AND ANTAGONISTS OF NRS

Multiple physiological, developmental, and toxicological processes in the body are regulated by sets of genes, which are coordinated and activated by ligand-activated transcription factors, the NRs[150]. Lipid sensing NRs drive lipid homeostasis in the hepatobiliary and gastrointestinal systems. A key function is exerted by the oxysterol receptor liver X receptor (LXR) and by the bile acid receptor farnesoid X receptor (FXR); both are involved in the

molecular regulation of hepatic and biliary lipid metabolism, and modulate bile flow and cholesterol gallstone formation. LXR acts as the intracellular "sensor" of cholesterol[151], while FXR is the intracellular sensor of bile acids[152,153]. To maintain lipid homeostasis, cells synthesize oxysterols under conditions of cholesterol overload, and oxysterols, in turn, bind and activate LXR, which acts to reduce the systemic cholesterol burden[154]. In the enterohepatic system, FXR highly determines expression levels of genes involved in the maintenance of cholesterol, bile acid and triglyceride homeostasis[155].

FXR also up-regulates hepatic expression of bile acid and lipid transporters on the canalicular membrane of hepatocytes and increases activity of regulatory enzymes responsible for bile acid detoxification. These biochemical properties characterize FXR as a potential suitable target for drugs to be employed in the treatment of both cholestasis and cholelithiasis[156]. Animal studies confirmed a direct role of LXR and FXR in the processes leading to cholesterol precipitation in bile. FXR-null mice are prone to cholesterol gallstone formation, while the activation of FXR *via* specific synthetic ligands such as GW4064 restores a normal homeostasis between cholesterol, bile acids and phospholipids in bile[157]. This mechanism depends on FXR-induced activity of the energy-dependent ATP-Binding Cassette (ABC) transporters ABCB11 for bile acids and ABCB4 for phospholipid[158] and it is linked to a better cholesterol solubilization in bile, thus preventing the formation of cholesterol crystals and gallstones. The activation of FXR promotes an increase in cholesterol secretion by a direct up-regulation of the main hepatocyte canalicular transporters (ABCG5 and ABCG8) leading to increased biliary cholesterol saturation and precipitation of cholesterol crystals, gallstone formation and growth[159]. Such innovative and intriguing results from animal studies have not been confirmed in humans, so far. Future studies are required to assess the usefulness and safety of synthetic, liver-specific FXR agonists and LXR antagonists in humans, not only targeting gallstone disease but also type Ⅱ diabetes, dyslipidaemia and several cancers[35].

CONCLUSION

The gold standard for treating symptomatic gallstones remains laparoscopic cholecystectomy. Oral litholysis (basically restricted to few oral hydrophilic bile acids) has a limited role in a scant subgroup of selected patients with symptomatic cholesterol gallstones, but is complicated by the high rate of gallstone recurrence after dissolution treatment and a negative cost-benefit balance. As a consequence of novel and recent animal and human studies, the research agenda in the field of non-surgical therapy of cholesterol cholelithiasis is filled with several possibilities. Drugs affecting cholesterol synthesis and intestinal absorption (i.e., statins, ezetimibe) and agonists/antagonists of the NRs FXR/LXR involved in biliary lipid secretion may offer, in the near future, promising agents to treat cholesterol gallstones or to prevent their formation in populations at risk.

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