Digestive and Liver Disease 42 (2010) 409–418

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Mini-Symposium

Bile salts and cholestasis

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ARTICLE INFO

Article history:
Received 13 March 2010
Accepted 13 March 2010

Keywords:
Bile acids
Chenodeoxycholate
Primary biliary cirrhosis
Primary sclerosing cholangitis
Ursodeoxycholate

ABSTRACT

Bile salts have a crucial role in hepatobiliary and intestinal homeostasis and digestion. Primary bile salts are synthesized by the liver from cholesterol, and may be modified by the intestinal flora to form secondary and tertiary bile salts. Bile salts are efficiently reabsorbed from the intestinal lumen to undergo enterohepatic circulation. In addition to their function as a surfactant involved in the absorption of dietary lipids and fat-soluble vitamins bile salts are potent signaling molecules in both the liver and intestine.

Under physiological conditions the bile salt pool is tightly regulated, but the adaptive capacity may fall short under cholestatic conditions. Elevated serum and tissue levels of potentially toxic hydrophobic bile salts during cholestasis may cause mitochondrial damage, apoptosis or necrosis in susceptible cell types.

Therapeutic nontoxic bile salts may restore impaired hepatobiliary secretion in cholestatic disorders. The hydrophilic bile salt ursodeoxycholate is today regarded as the effective standard treatment of primary biliary cirrhosis and intrahepatic cholestasis of pregnancy, and is implicated for use in various other cholestatic conditions. Novel therapeutic bile salts that are currently under evaluation may also prove valuable in the treatment of these diseases.

1. Introduction

Bile salt synthesis, secretion and recycling represent crucial functions of the liver, the central organ for the maintenance of metabolic homeostasis. Bile salts form two thirds of organic compounds in mammalian bile and are continuously recycled in the body by undergoing a highly efficient enterohepatic circulation. In the small intestine, the amphipathic molecules function as micellar solubilizers, mediating the intestinal uptake of dietary fats. In addition, bile salts represent potent signaling molecules in liver and intestine: in the small intestine, they strengthen the defence against microbes by farnesoid X receptor (FXR)-dependent mechanisms and modulate hepatobiliary bile formation by FXR-controlled ileal release of the peptide hormone fibroblast growth factor 19 (FGF19) [1,2]. In the liver, bile salts directly modulate their hepatocellular uptake, synthesis and biliary secretion at both the transcriptional level via activation of nuclear receptors and at the posttranslational level via modulation of cytosolic signaling cascades [3–5].

The adaptive functions of bile salts may decompensate under the pathological condition of bile secretory impairment. Bile salts are suspected to be the main causative agents of cellular damage to hepatocytes and cholangiocytes during cholestasis [6].

The dual nature of bile salts, with their various biological functions on one hand and their potentially cytotoxic effects when accumulating in cholestasis on the other hand, are the focus of this review. Therapeutic effects of bile salts like ursodeoxycholate in the treatment of several of these cholestatic diseases are also addressed.

2. Evolutionary perspective on bile salts

The chemical structure of bile salts differs markedly between vertebrate species, although all are variants of the same type of molecule. Among the different animal orders, but less so among families, genera and species, evolutionary processes have selected a range of distinct bile salts. *'The end products of bile salt biosynthesis in lower vertebrates are the precursors of end products of bile salt biosynthesis in higher vertebrates'* [7]. New bile salts are discovered regularly as mass high-resolution chemical techniques are applied to bile from animal species that were not previously analysed [8].

As natural selection drove the generation of novel bile salts, it concurrently favoured the evolution of bile salt receptors. Co-evolution between bile salts and bile salt receptors was shown for the farnesoid X receptor (FXR) and made probable for pregnane X receptor (PXR), while the ability of the vitamin D receptor (VDR) to use bile salts as ligands may be a recent evolutionary event restricted to the mammalian lineage [9].

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1590-8658/$36.00 © 2010 Published by Elsevier Ltd on behalf of Editrice Gastroenterologica Italiana S.r.l.
doi:10.1016/j.dld.2010.03.015

3. Biochemical cycle of bile salts

3.1. Synthesis and chemical properties

Primary bile salts – cholate (C) and cheonodeoxycholate (CDC) in humans – are synthesized by hepatocytes via enzymatic modification of cholesterol, adding hydroxyl groups and oxidizing the side chain. The thus formed bile salt is an amphipathic molecule, much more hydrophilic than cholesterol, which empowers it to efficiently form micelles [3,10]. These characteristics are necessary for the physiological function of bile salts during fat uptake from the intestine but also make them potentially dangerous for cell membrane integrity under cholestatic conditions. Biotransformation of bile salts by bacteria in the intestine results in the formation of secondary bile salts, such as deoxycholate (DC) and lithocholate (LC). Bacterial and hepatic biotransformation contribute to the formation of tertiary bile salts such as ursodeoxycholate (UDC) [11].

Unconjugated bile salts have a pKa of approximately 5. Hydroxylation and conjugation with taurine or glycine reduces the pKa of bile salts, improving water solubility and reducing their ability to cross lipid membranes [12,13]. C_{27} bile alcohols are conjugated with sulfate. C_{27} bile salts are conjugated with taurine. C_{24} bile salts are mainly conjugated with taurine or glycine and to a lesser extent with glucuronate, N-acetylglucosamine, or sulfate.

At physiological pH, bile salts are mostly present in their deprotonated salt form, but under more acidic circumstances the protonated bile acid becomes more prevalent. The ionized bile salt is more amphipathic, better water-soluble and a stronger emulsifier than the protonated bile acid thereby strengthening the formation of mixed micelles. Conjugation increases the ratio of ionized bile salt over protonated bile acid molecules at the prevalent pH of 5–7 in the duodenum. Taurine and glycine conjugates of bile salts are resistant to enzymatic cleavage by pancreatic esterases.

3.2. Plasticity of the bile salt pool

Influenced by both primary bile salt synthesis in the liver and bile salt metabolism in the liver and intestine, the composition of the bile salt pool of an individual is dynamic. The relative contributions of different primary and secondary bile salts can vary, dependent on regulatory mechanisms and environmental factors.

For example, ursodeoxycholate (UDC) is a primary bile salt in most members of the bears (Ursidae) family, and comprises 30–60% of total serum bile salts in these animals [14–16]. During hibernation the percentage is increased [15,16], probably an adaptation that is protective to the biliary tree during the long fasting period. In humans, UDC is formed by colonic bacteria from the primary bile salt, CDC, via the intermediate 7-ketolithocholate, and thus strictly speaking a tertiary bile salt. Typically it accounts for about 1–3% of total bile salts. In cholestatic patients treated with UDC at daily doses of 15 mg/kg, the contribution of this bile salt may rise up to 65% of total serum bile salts [17].

Physiological changes in the composition of the bile salt pool occur during human life. The bile salt pool of the fetus and newborn is markedly different from the adult bile salt pool, with more hydrophilic bile salts due to hydroxylation of positions C1, C4 and C6. The adult bile salt pool size is maintained at 3–5 g mainly by a negative feedback loop of bile salts on their own synthesis. The key enzymes of bile salt synthesis, CYP7A1 and CYP8B1, are tightly regulated at the transcriptional and posttranscriptional level.

The bile salt pool is influenced by the diet: vegetarians have lower concentrations of bile salts in their stools, especially lithocholate [18,19]. A high-fat diet was shown to increase the cholate to total bile salt molar ratio as opposed to the effects of a very low-fat diet [20]. The change in nutrition habits in western societies might thus have modified the average bile salt pool and biliary and intestinal bile salt pattern [21].

3.3. Hepatocellular bile salt transport

Biliary bile salts originate from two sources: direct synthesis by hepatocytes and recycling via the enterohepatic circulation, delivering bile salts via the portal vein to the basolateral side of the hepatocyte. Bile salts are taken up either via the Na^+ taurocholate cotransporting protein (NTCP) or via sodium-independent uptake by one of the organic anion-transporting polypeptides (OATPs, particularly OATP1B1 and OATP1B3) [22]. Upon delivery to the apical hepatocellular membrane most likely via bile salt binding proteins, bile salts are secreted by the bile salt export pump (BSEP/ABCB11) [22,23].

3.4. Bile salts in bile

Biliary bile contains a mix of 3–10 individual bile salts or bile alcohols in significant amounts [8,24]. In addition, bile contains inorganic molecules such as ions and bicarbonate, as well as organic compounds such as phospholipids, cholesterol, bilirubin, adenosine nucleotides [25] and proteins such as albumin and immunoglobulins [26–28]. Still, hepatic bile contains relatively little protein, while the gallbladder bile is richer in protein, probably due to mucin secretion [29,30].

3.5. Intestinal absorption and hepatobiliary resecretion

Bile salts and bile alcohols are recycled after secretion via bile to the intestine. In the process of enterohepatic cycling, bile salts are taken up from intestinal lumen and transported to the liver via the portal vein, where these molecules are reabsorbed by the hepatocytes, to be secreted into bile again [31]. Enterocytes mainly of the terminal ileum, but in humans at markedly lower amounts also the upper small intestine express an apical sodium-dependent bile salt cotransporter, ASBT, the key mediator of intestinal bile salt uptake. In cholestatic patients, duodenal ASBT expression was found to be markedly impaired in comparison to control patients, and mRNA levels were inversely correlated to plasma bile salt and bilirubin concentrations [32]. This effect mirrors the transcriptional regulation of ASBT expression by the bile salt sensor, farnesoid X receptor, FXR. Ostα-Ostβ and MRP3/ABCC3 are two transporters in the basolateral membrane of enterocytes that are thought to further channel the resorbed bile salts towards mesenteric venous blood and, thereby, enterohepatic recycling [33,34].

Circadian rhythms influence bile salt cycling, and disruption of the circadian clock affects bile salt control and leads to hepatic cholestasis in mice [35]. During long periods of fasting the turnover of this cycle can be diminished by storage of bile salts in the gallbladder. In patients after cholecystectomy and in animals without a gallbladder, such as horses, elephants and rats, bile salts are most probably retained in the intestine during fasting to achieve a similar effect but the precise regulation of the proposed slowdown of the enterohepatic circulation of bile salts remains to be further unraveled [3].

Bile salts may also be absorbed by the biliary epithelium, probably by ASBT, and transported back to the hepatocyte in a process called cholehepatic shunting [36,37]. The physiological relevance of this mechanism is unclear as bile salt transport via cholangiocellular ASBT does not appear to be a high throughput system for physiological bile salts. Thus, it could be speculated that cholangiocyte bile salt uptake may serve a purpose of signaling rather than bile salt conservation via cholehepatic shunting and may represent a crucial step in the regulation of cholangiocyte secretory activity.

Stimulation of cholehepatic shunting by bile salts has been particularly observed with C_{23} bile salts like norUDC (see 6.3). Whether stimulation of cholehepatic shunting is instrumental to the anticholestatic and antifibrotic effect of norUDC as observed in an experimental model of fibrosing cholangitis, the Mdr2−/− mouse [38], is unclear. NorUDC is being evaluated as a possible therapeutic strategy in cholestatic diseases [39].

3.6. Modification by gut bacteria

The intestinal flora has the ability to modify luminal bile salts before mucosal resorption. Various species of bacteria are known to deconjugate bile salts. The rate of deconjugation is dependent on the bacterial load. For example, jejunal bacterial overgrowth induced by gastric proton pump inhibitor (omeprazole) treatment was shown to speed up this process [40], and doxycycline treatment lowers the rate of bacterial modification of bile salts [41]. Treatment of mice with antibiotics resulted in enhanced levels of TLC in the liver, which was not observed in germfree mice, stressing the impact of the intestinal flora on the composition of the bile salt pool [42]. Administration of specific strains of bacteria was shown to modify the bile salt pool in an animal model. Thus, this type of bile salt pool modification might become a future therapeutic approach in case bile salt pool composition will turn out to affect the course of hepatobiliary and possibly gastro-intestinal diseases [41,43].

3.7. Fecal excretion

The rectal concentration of bile salts is approximately twenty times lower than the concentration high in the small intestine, due to an ~95% reuptake of bile salts in the gastro-intestinal tract. About 5% of the secreted bile salts are thus lost via the faeces under physiological conditions.

4. Beneficial effects of bile salts

4.1. Induction of bile flow

Bile salts are the major osmotic driving force for bile formation. Active secretion of bile salts against a steep concentration gradient across the apical hepatocyte membrane leads to passive water movement into bile, forming the bile salt-dependent fraction of bile flow.

4.2. Detoxification

Liver and small intestine are the main sites of detoxification in the body, and bile is the primary excretory route for substances such as bilirubin, cholesterol and waste products. Bile salts help solubilise lipophilic xenobiotic molecules and may bind heavy metal cations that are excreted via the biliary route [3]. Different hydrophobic bile salts also act as potent transcriptional inducers of uptake carriers (biotransformation phase 0), biotransformation enzymes (phase 1 and 2) and export pumps (phase 3) in liver and probably intestine, by activating nuclear receptors such as PXR, FXR and VDR [5,44].

4.3. Food digestion

Bile salts act as tensioactives for the absorption of dietary fats. Upon meal ingestion bile is secreted into the duodenal lumen after contraction of the gall bladder in response to cholecystokinin, and by enhanced bile production from the liver. Secretin released by cells in the duodenum stimulates HCO_3^--rich bile flow via recruitment of the Cl^-/HCO_3^- exchanger, AE2, the cAMP-sensitive chloride channel, CFTR, and the aquaporin, AQP1, to the apical membrane of cholangiocytes [45]. Bile formation is tightly regulated at the level of the duodenum where increasing bile salt levels activate mucosal FXR and its executer, small heterodimer partner (SHP), which subsequently suppresses duodenal secretin expression via inhibition of the transcription factor beta-cell E-box transactivator 2 (neurogenic differentiation factor 1 in mice) [46] to complete the feedback loop.

4.4. Protection against bacterial overgrowth

Bile is an important modulator of the intestinal flora [1]. Although not germfree, the small intestine contains markedly less bacteria than the large intestine. Failure to adequately secrete bile salts to the duodenum leads to bacterial overgrowth as observed in cirrhotic patients [47,48] or after bile duct obstruction in animal experiments [49]. The exact mechanism of bile salt-mediated control of the intestinal flora remains to be further unraveled, but probably includes both direct effects on the growth of bacteria via accumulation of bile salts in the organisms [2,50], and indirect effects via bile salt-induced, FXR-mediated [51], antibacterial actions of the intestinal mucosa [1,4,51].

In the biliary tract, bile salts probably confer crucial protection against microbes. Under normal circumstances the biliary tract is practically free from bacteria, bile salts are thought to make an important contribution to the defence against colonisation of the biliary tract, again both via direct antimicrobial properties and via induction of local defence mechanisms [52–54].

4.5. Signaling

Bile salts are increasingly recognized as important signaling molecules in liver, bile ducts and the intestine [3,4,55,56]. They can alter transcriptional activity by stimulating nuclear receptors like FXR, PXR and VDR [57]. Various bile salts can also modify intracellular signaling pathways, including $[Ca^{++}]_i$-, protein kinase C (PKC)-[58–63] and phosphatidylinositol-3 kinase (PI3K)/AKT-dependent [64–66] pathways and differentially activate the mitogen-activated protein kinases (MAPK), ERK 1/2, $p38^{MAPK}$, and JNK [67]. Bile salts thereby modulate their synthesis, expression and targeting of transport proteins and biliary secretion, but also various other metabolic processes by transcriptional and posttranscriptional mechanisms.

4.6. Glucose metabolism

The ability of bile salts to activate the PI3K/AKT pathway that among others has an effect on the insertion of the glucose 4-transporter (GLUT4) to the plasma membrane illustrates the effect of bile salt signaling on glucose metabolism [4,68–70]. With their activating effect on the AKT pathway bile salts function in a way which is similar to insulin, thus regulating the uptake of insulin from the blood into the hepatocytes. Further modulation of the glucose metabolism is effectuated through activation of SHP transcription via FXR, which then inhibits gluconeogenesis by the binding transcription factors FOX01, CEBPα and HNF4α. The FXR agonist GW4064 was shown to counteract insulin resistance and hyperglycaemia in animal studies [71], and INT-747, another FXR agonist, is currently under evaluation for use in the treatment of type 2 diabetes and non-alcoholic fatty liver disease.

4.7. Endocrine signaling

Bile salts have only recently been recognized as modifiers of hormonal signaling [72,73]. Thyroid hormone signaling is altered by bile salts, as they stimulate the production of active T3 in mouse

brown fat tissue. While humans have only small amounts of brown fat, bile salts were implicated to have similar stimulating effects on the metabolic rates of human cell cultures [74]. It was earlier shown in mice that bile salts could counteract obesity induced by high-fat diets [75].

4.8. Immunological signaling

The immune system is implicated in many diseases, both by its protective functions in, e.g. bacterial infection and as a potential cause of harm in, e.g. autoimmune diseases. Bile salts have been claimed to influence immune functions whereby the molecular mechanisms involved remain largely unknown so far. Particularly secondary bile salts are recognized as weak ligands for the Vitamin D receptor, a regulator of calcium homeostasis that is also involved in immunity [76,77]. Bile salts also directly affect cellular immunity. At supraphysiological concentrations, they were shown to suppress the cytokine production of monocytes, probably via TGR5, and may inhibit phagocytosis and reactivity to LPS stimulation [78]. Bile salts can furthermore induce degranulation of eosinophils [79]. How all these experimental observations can be translated to the clinical setting remains unclear [80].

5. Pathological effects of bile salts

Bile salts are potentially toxic to living cells when present at supraphysiologic levels. At low micromolar concentrations, unconjugated more than conjugated hydrophobic bile salts such as LC, CDC, or DC can cause damage particularly of mitochondria, without affecting integrity of plasma membranes. At high micromolar and millimolar concentrations they may even dissolve plasma membranes [81,82]. The more hydrophilic conjugated bile salts like GUDC generally only yield toxic effects at doses above their critical micellar concentration in the millimolar range [83,11].

Under physiological circumstances the intracellular concentration of unbound bile salts is tightly controlled to prevent damage to cell organelles. Under cholestatic conditions due to impaired hepatocellular or cholangiocellular secretion and/or biliary obstruction, intracellular bile salt levels can rise to the point where glycine- and taurine-conjugated hydrophobic bile salts such as GCDC, TCDC, GDC and TDC inflict damage to cell organelles like the mitochondria [82,84,85]. In the liver, both hepatocytes and cholangiocytes are targets of bile salt-induced damage. The exact mechanisms of bile salt-induced damage are still under debate, but bile salts appear to cause both mitochondrion-mediated damage [84] and tissue stress due to the induction of inflammatory processes [11].

5.1. Plasma membrane damage

High concentrations of hydrophobic bile salts in the millimolar range *in vitro*, especially above the critical micellar concentration, may solubilise membrane components. Hydrophobic more than hydrophilic bile salts bind to the lipid bilayer, and may cause holes in the plasma membrane [86]. Cholangiocytes are directly exposed on their apical side to millimolar levels of bile salts. However, cholangiocyte plasma membrane damage is not observed *in vivo*, suggestive of protective mechanisms under physiological circumstances [82]. Thus, plasma membrane damage appears to play no major role in bile salt-induced liver injury *in vivo*.

5.2. Mitochondrial damage

The mitochondrion is particularly vulnerable to bile salt-induced damage. Elevated intracellular levels of hydrophobic bile salts can trigger the loss of the integrity of the mitochondrion. This can lead to the permeabilisation of mitochondrial membranes (the so-called mitochondrial permeability transition) [87–91], which provokes depolarization of the organelle, uncoupling of oxidative phosphorylation and mitochondrial swelling. Ultimately this leads to mitochondrial collapse, cytochrome c release, and activation of apoptosis. Mitochondrial damage caused by hydrophobic bile salts appears to be at least partially caused by the generation of radical oxygen species [92,93], providing a rationale for antioxidant treatment in cholestasis [90]. Still, *in vivo* no evidence for the benefit of antioxidant therapy in the management of cholestasis exists [94,95].

5.3. Induction of apoptosis

Toxic bile salts such as GCDC, the predominant human dihydroxy bile salt under cholestatic conditions, may induce apoptosis via generation of reactive oxygen species followed by epidermal growth factor receptor-dependent tyrosine phosphorylation of Fas. This Fas death receptor-dependent process is independent of Fas ligand, but requires oligomerization of Fas by increasing cell surface trafficking of this death receptor [96,97]. GCDC can also directly induce the intrinsic pathway of apoptosis by a Fas-independent mechanism, which might represent an escape mechanism, when the faster extrinsic pathway is not available [98,99].

5.4. Endoplasmic reticulum-mediated apoptosis

Apoptosis can also be initiated in the endoplasmic reticulum (ER). The concept of ER stress was only recently established, but appears to be an important cause for the initiation of programmed cell death, making it a target for novel therapeutics [100]. Clogging of the ER with misfolded proteins may result in the loss of integrity of the ER membrane, leading to Ca^{2+} release into the cytosol and subsequent activation of intrinsic apoptotic cascades [101]. Accumulating bile salts induce ER stress, which in turn can activate programmed cell death pathways as shown in hepatocytes *in vitro* treated with hydrophobic bile salts [102,103], and in an *in vivo* model of cholestasis [104].

6. Therapeutic effects of bile salts

6.1. Ursodeoxycholate (UDC)

Extensive experimental evidence of the past 25 years [105] indicates that UDC improves impaired hepatocellular [106] as well as cholangiocellular [107] secretion by mainly posttranscriptional mechanisms as reviewed previously [14,108–110]. In cholestatic hepatocytes, Ca^{2+}/cPKCα/PKA-dependent [14,106,111,112] or possibly MAPK-dependent [67] targeting of apical transporters such as the bile salt and conjugate export pumps, Bsep and Mrp2, may contribute to the anticholestatic effect of UDC. In cholangiocytes, UDC-induced stimulation of biliary HCO_3^- secretion may depend on purinergic signaling [113–115] and apical targeting of AE2 and CFTR possibly analogous to the mechanism described in cholestatic hepatocytes [14,106]. Thereby, UDC may also decrease bile toxicity. In addition, UDC has been shown to exert potent antiapoptotic [116] and otherwise cytoprotective effects [117–119] in hepatocytes and cholangiocytes [14,108–110].

Bile salts modulate cellular functions after entry via specific bile salt carriers like NTCP in hepatocytes and ASBT in cholangiocytes and ileocytes or via the membrane receptor, TGR5, which is expressed by various cell types except hepatocytes within and outside the liver. UDC is a poor/negligible ligand of TGR5. Thus, it is not surprising that beneficial effects of UDC have mainly been observed at the level of hepatocytes and cholangiocytes under pathophysiological conditions.

The therapeutic effects of UDC in various cholestatic disorders are summarized below.

Primary biliary cirrhosis (*PBC*): UDC (13–15 mg/kg/day) is today regarded as the treatment of choice for patients with PBC based on placebo-controlled trials and long-term case-control studies [120]. UDC improved serum liver tests including bilirubin, AP, γGT, AST and ALT as well as cholesterol and immunoglobulin M levels, and ameliorated histological features in large doubleblind, placebo-controlled trials [121–125]. UDC delayed the histological progression of the disease in patients treated in an early stage [125,126]. However, a survival benefit was not shown in any of these trials possibly due to the limited number of patients included in these studies and the limited periods of follow-up too short for this slowly progressing disease. When a combined analysis of data from the French, Canadian and Mayo PBC patients was performed after a follow-up of 4 years UDC treatment was associated with a significant reduction in the likelihood of liver transplantation or death [127]. Interestingly, this benefit was disclosed during this limited observation period in patients with advanced disease but not yet in those with mild disease (serum bilirubin <1.4 mg/dL (24 μmol/L), histologic stage 1 or 2) [127].

Meta-analyses which included studies of 3 months' to 2 years' duration and trials using UDC doses which are today known to be ineffective were not able to confirm the beneficial effect of UDC on long-term survival [128,129]. However, trials with a short duration <2 years and inappropriate UDC dosage included in these meta-analyses may have diluted informations of long-term studies and might be regarded as inappropriately selected for a well-based survival analysis considering an estimated disease duration of one to two decades. Therefore, meta-analyses which included only studies of long duration (>2 years) and those that used an effective dose of UDC (13–15 mg/kg/day) showed that long-term UDC significantly improved transplant-free survival [130,131]. Recent reports confirmed favourable effects of UDC (13–15 mg/kg/day) on long-term survival in patients with PBC over 10–20 years [132–135]. Treatment with UDC led to a transplant-free survival similar to that of a healthy control population matched for age and gender in patients with early stage disease and to improved survival in comparison to the estimated survival at the start of treatment as calculated by the Mayo risk score for PBC [132–135].

A good biochemical response to UDC defined as a decrease in AP to less than 60% of pretreatment levels or normalization at 1 year ("Barcelona criteria") [133] or a serum bilirubin ≤ 1 mg/dL (17 μmol/L), AP $\leq 3 \times$ ULN, and AST $\leq 2 \times$ ULN at 1 year ("Paris criteria") [134] identified those patients with a good long-term prognosis. Prognostic superiority of the "Paris criteria" was observed in an independent cohort [135]. These data may allow the conclusion that UDC beneficially affects the long-term prognosis of PBC, but that additional therapeutic options for UDC non-responders are needed.

Primary sclerosing cholangitis (*PSC*): Pilot trials of UDC (10–15 mg/kg/day) in PSC in the early 1990s revealed improvement of serum liver tests [136–139]. In a small, placebo-controlled trial, the prognostic surrogate marker, serum bilirubin, and histological features as evaluated by a multiparametric score, improved [139]. In the first large-scale trial which analyzed 102 patients [140] after follow-up for a median of 2.2 years under 13–15 mg/kg/day of UDC or placebo, again, improvement in serum liver tests including bilirubin was observed, but no improvement in symptoms, histological disease stage, time to treatment failure or transplantation, and long-term prognosis was observed [140]. Higher doses of UDC (20–25 mg/kg/day) in order to maximally enrich UDC in bile [141] demonstrated significant improvements in serum liver tests, histological grade of fibrosis and cholangiographic features in a pilot trial when compared to placebo treatment [142]. In addition, an open label trial using high-dose UDC (25–30 mg/kg/day) showed a significant improvement in projected survival using the Mayo risk score as surrogate marker of survival [143]. These data were confirmed in a 2-year double-blind, randomized, placebo-controlled dose ranging pilot study of 31 patients in which the low dose (10 mg/kg/day) and the medium dose (20 mg/kg/day) tended to improve and the high dose (30 mg/kg/day) significantly improved projected survival calculated with the Mayo risk score albeit partly due to more potential for biochemical improvement in the high dose group [144]. A Scandinavian trial recruited the largest group of PSC patients ($n = 219$) for the longest treatment period (5 years) ever studied using a dose of 17–23 mg/kg/day and demonstrated a trend towards increased survival in the UDC-treated group when compared with placebo [145]. However, it was still insufficiently powered (*a priori* intended number of participants: $n = 369$) and the biochemical response was unexpectedly poor in this trial which prompted questions about adequate compliance in a part of the study population. Finally, a multicentre study using high doses of 28–30 mg/kg/day of UDC in 150 PSC patients over 5 years has been stopped because of an enhanced risk in the UDC treatment group to reach primary endpoints such as liver transplantation or development of varices in more advanced disease while biochemical features improved in the UDC group [146]. In this trial, more than 40% of patients were in an advanced histological stage 3 or 4 before starting 5-year treatment with high-dose UDC [146]. It remains a matter of speculation whether for patients with advanced stage disease [147], long-term high-dose UDC might have overturned the remaining hepatobiliary secretory function and, thereby, put additional risk to those patients. In conclusion, while UDC improves serum liver tests including prognostic surrogate markers of PSC at doses of 15–20 mg/kg/day, the role for UDC in slowing the progression of PSC is as yet unclear and high-dose UDC (30 mg/kg/day) may even be harmful particularly in late stage disease.

Patients with PSC have a markedly increased risk in comparison to the normal, age- and sex-matched population to develop intestinal and hepatobiliary malignancies [148]. A chemopreventive effect of UDC against intestinal and hepatobiliary malignancies in PSC is under discussion based on experimental studies *in vitro* and *in vivo* as well as clinical observations. A reduced risk of colonic dysplasia was described in a cross-sectional study of 59 PSC patients with ulcerative colitis (UC) undergoing colonoscopic surveillance for those under UDC while those in the control group showed an exceptionally high rate of dysplasia [149]. A trend towards reduced relative risk (RR) of colon dysplasia (RR 0.59, 95% CI 0.26–1.36, $p = 0.17$) and a lower mortality (RR 0.44, 95% CI 0.22–0.90, $p = 0.02$) was described in a study which compared 28 PSC patients with UC under UDC treatment to 92 PSC control patients with UC [150]. A marked risk reduction for developing colorectal dysplasia or carcinoma (RR 0.26; 95% CI 0.06–0.92, $p = 0.03$) was observed in a group of 52 patients with PSC and UC when they were treated with UDC as compared to placebo within the above mentioned trial [140] and followed endoscopically for 355 patient-years [151]. Thus, currently there is suggestive but limited evidence for the use of UDC for chemoprevention of colorectal cancer in PSC [120].

Effects of UDC on the risk to develop hepatobiliary malignancies in PSC are even less clear. Analysis of 198 and 150 PSC patients from Scandinavia and the U.S., respectively, during 5-year treatment did not reveal a difference between UDC- and placebo-treated patients regarding CCA development [145,147]. A cohort study including 150 patients followed for a median of 6.4 years under UDC and endoscopic treatment found CCA in 5 patients (3.3%) [152], about half the expected incidence of CCA of about 0.8–1.5% per year in PSC [153]. Lack of UDC treatment was identified as an independent risk factor for the development of hepatobiliary malignancy in a cohort of 255 PSC patients listed for liver transplantation within a period of 11 years [154].

Intrahepatic cholestasis of pregnancy (*ICP*): UDC improves pruritus and serum liver tests in up to 80% of ICP patients [155–161]. In recent trials, fetal complication rates and rates of premature delivery were markedly lower than those reported in the past and stillbirth was not observed in several of the trials [156–160]. Thus, no difference was found in fetal complication rates between UDC- and placebo-treated patients although the time to delivery was longer and closer to normal in UDC-treated than in placebo-treated ICP patients. Thus, UDC (10–20 mg/kg/day) is today regarded as the first-line treatment for ICP based on evidence obtained from randomized, controlled clinical trials.

Cystic fibrosis-associated liver disease (*CFALD*): UDC (20–30 mg/kg/day) improves serum liver tests [162,163], histological appearance (over 2 years) [164] and nutritional status in CFALD. The optimal dose of UDC and its impact on survival are unclear [120].

Progressive familial intrahepatic cholestasis type 1–3 (*PFIC*1, *PFIC*2, *PFIC*3): UDC can be considered as a first step in the medical treatment of all types of PFIC [165]. However, in PFIC1 and PFIC2, caused by mutations of the *ATP8B1*/*FIC* gene and *ABCB11*/*BSEP* gene, respectively, UDC appears to be ineffective in most cases. In PFIC3 characterized by impaired biliary phospholipid secretion due to *ABCB4*/*MDR3* mutations, UDC improves serum liver tests in almost half the patients [166].

Drug-induced cholestasis: Case series indicate that UDC may reverse prolonged drug-induced cholestasis in the majority of patients treated when it does not respond to drug withdrawal only [167].

Hepatic complications of allogeneic stem cell transplantation: Hepatic complications of allogeneic stem cell transplantation include development of sinusoidal obstruction syndrome (SOS, previously veno-occlusive disease (VOD)) and acute or chronic graft-versus-host disease (GvHD). UDC alone (without heparin) has been studied in three randomized trials for the prophylaxis of SOS (as defined by biochemical and clinical criteria) [168–170] and reduced the incidence of SOS in two of them [168,169] and in a meta-analysis of the three trials (relative risk (RR) 0.34; confidence interval (CI) 0.17–0.63) which also revealed a decrease in transplant-related mortality (RR 0.58; CI 0.35–0.95) [171]. These data have to be seen with some caution as patients with confounding liver diseases including GVHD or sepsis- and drug-induced liver disease were not excluded, and a decrease in SOS-induced mortality has not been shown. Although prophylactic use of UDC to prevent SOS after allogeneic stem cell transplantation is broadly prescribed worldwide today, UDC is not (yet) generally recommended as prophylaxis of SOS before allogeneic stem cell transplantation [172].

UDC is *not* recommended in actual guidelines for treatment of chronic hepatitis B [173] and chronic hepatitis C [174] and cannot be recommended for severe, icteric alcoholic cirrhosis and steatohepatitis (ASH) [175]. No convincing data are available for non-alcoholic steatohepatitis (NASH) [176].

6.2. Cholate

Taurocholate exerts anticholestatic and cytoprotective effects in hepatocytes and cholangiocytes in experimental cholestasis [177–179]. In comparison to UDC, cholate binds more effectively to the nuclear bile salt receptor FXR and may – in contrast to UDC – affect transcriptional activity of various genes including those involved in bile salt synthesis.

Inborn errors of bile salt metabolism: In patients with inborn errors of bile salt synthesis like 3β-hydroxysteroid Δ5-oxidoreductase deficiency or 5β-reductase deficiency, toxic bile salt intermediates are accumulating in the hepatocyte, leading to cholestasis and liver cell injury [180,181]. These patients often respond well to administration of the defective physiological bile salts [182]. Cholate appears to be both an effective and safe long-term treatment of at least the most common of these diseases [183].

6.3. Nor-ursodeoxycholate (norUDC)

NorUDC, the C23 analogue of UDC, is an effective anticholestatic, anti-inflammatory and antifibrotic agent in experimental sclerosing cholangitis [38,184]. In Mdr2−/− mice, norUDC improved cholangitis and fibrosis, while tauro-norUDC did not change these markers, and dinorUDC caused more fibrosis and cholangitis. NorUDC is a strong inducer of biliary HCO_3^- secretion and bile flow in experimental animals and humans, possibly by a cholehepatic cycling of its unconjugated form [185,186] as shown in mice by accumulation of norUDC in liver [184], which is not observed for UDC at therapeutic doses. NorUDC is only poorly amidated, which makes rapid cholehepatic shunting possible [184]. Unconjugated norUDC in bile could accept a proton from carbonic acid and could subsequently be taken up in the cholangiocyte and rerouted for cholehepatic shunting. NorUDC uptake would thus lead to addition of a HCO_3^- molecule to bile [187].

Patient studies with norUDC are not yet available, but are eagerly awaited, especially for treatment of primary sclerosing cholangitis [39].

6.4. 6-Ethyl-chenodeoxycholate (6-ECDC)

6-ECDC, a semi-synthetic bile salt homolog and selective farsenoid X receptor (FXR) agonist, is currently under investigation as a drug in the treatment of PBC, type 2 diabetes and non-alcoholic fatty liver disease (NAFLD) [188–190]. Phase II trials showed its efficacy in type 2 diabetes patients with NAFLD and in PBC, and studies in non-alcoholic steatohepatitis (NASH) patients are currently performed.

7. Conclusion

Fifty years ago bile salts were regarded as single-purposed detergents that were only produced to aid fat digestion. As for so many examples from the medical field new techniques unveiled numerous additional functions of bile salts, which enabled the development of clinical applications of these multifaceted molecules. Bile salt therapy is currently the pillar of treatment of several chronic cholestatic diseases, and while a better understanding of the potentially harmful properties of bile salts enables the design of protective strategies novel synthetic bile salts offer promising new options for the optimisation of patient care in cholestasis.

Conflict of interest statement
Ulrich Beuers has received lecture fees from the Falk Foundation, Gilead, Roche, Schering-Plough and Zambon. Lucas Maillette de Buy Wenniger has nothing to disclose.

List of abbreviations

ASBT, apical sodium-dependent bile salt cotransporter; BSEP, bile salt and conjugate export pump; C, cholate; CCA, cholangiocarcinoma; CDC , chenodeoxycholate; DC, deoxycholate; 6-ECDC, 6-ethylchenodeoxycholate; ER, endoplasmic reticulum; FGF19, fibroblast growth factor 19; FXR, farnesoid X receptor; MAPK, mitogen-activated protein kinase; NTCP, Na^+ taurocholate cotransporting protein; OATP, organic anion transporting polypeptide; PBC, primary biliary cirrhosis; PI3K, phosphatidylinositol-3 kinase; PKA, protein kinase A; PKC, protein kinase C; PSC, primary sclerosing cholangitis; PXR,

pregnane X receptor; RR, relative risk; SHP, small heterodimer partner; SOS, sinusoidal obstruction syndrome; UC, ulcerative colitis; UDC, ursodeoxycholate; VDR, vitamin D receptor.

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