Bile Changes after Liver Surgery: Experimental and Clinical Lessons for Future Applications

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\textbf{Introduction}

During periods of fasting, bile, which is secreted by the liver, is concentrated and stored in the gallbladder. The composition of bile is complex, but bile acids are the most abundant constituents of human bile. Over 95\% of the bile acids entering the duodenum are conserved and returned to the liver after reabsorption in the terminal ileum (enterohepatic circulation). Their rate of absorption exerts a regulatory effect upon both bile flow and the concentrations of other biliary lipids (phospholipids and cholesterol). Bile acid-dependent flow is related to bile acid secretion and accounts for the greatest proportion (70–85\%) of canalicular flow \cite{1}. Bile acid-independent bile flow is thought to be driven by inorganic ion transport \cite{2} and is both canalicular and ductal \cite{1}.

Disruption of the normal physiological production of bile leads to pathological conditions of varying severity. Some degree of liver failure is inevitable following major liver surgery and particularly formal resections, ablation of liver tissue and tumours and major biliary reconstructions, but unless resolution is rapid and uncomplicated, potentially serious complications are possible. This type of post-operative liver failure is characterized by a markedly elevated serum bilirubin, hepatic encephalopathy and the myriad sequelae of an impaired ability to synthe-
size proteins [3]. Its incidence following hepatobiliary (HPB) surgery is estimated at 10–20% [4–6], and it is associated with a high mortality rate. A simple and reliable laboratory test would be invaluable and allow clinicians to identify patients whose post-operative outcome is likely to be complicated. Serum biochemical tests are available but have been shown to have a relatively low specificity in comparison to the direct analysis of bile [7]. Using both experimental models and clinical studies, different groups have identified a number of changes that occur in bile secretion and composition, and have also shown them to be early predictors of liver function following surgery. The purpose of this review is to summarize the available evidence in respect of these changes and determine whether they could be used as a potential screen in the clinical setting to indicate patients at high risk of developing significant post-operative complications.

**Materials and Methods**

A literature search was undertaken for all studies focusing on modifications of bile volume or composition as a consequence of liver surgery. Articles were selected from MEDLINE, Embase and the Cochrane Central Register of Controlled Trials databases up to May 2009.

The search strategy was conducted using three different sets of key words, one for HPB surgery, one for bile characteristics and one for outcome measures. Key words used for HPB surgery were: HPB resection, liver resection, hepatectomy, partially hepatectomized rats, biliary tract surgical procedures, obstructive jaundice, transplantation, orthotopic transplantation, and liver transplantation. Key words used for bile characteristics were bile, bile composition, bile flow, bile acids and salts, biliary lipids, cholesterol saturation, bile bilirubin, bilirubin subfractions, hyperbilirubinaemia, hormones, hepatocyte growth factor (HGF), inflammatory cytokines, and interleukin-6 (IL-6). Key words used for outcome measures were liver regeneration, post-operative liver failure, prognosis, and allograft rejection. All types of studies were included in the search strategy. No language restrictions were employed.

Potentially relevant articles were identified by the title and the abstract, and full papers were obtained and assessed in detail by two of the authors (T.K. and G.G.) prior to their inclusion in the review. The reference list for each article (including copies of previously published reviews on the topic) was also screened to identify further relevant publications which were obtained and assessed. Finally, the Current Controlled Trials (www.controlled-trials.com) database was also screened for randomised trials currently ongoing.

Data collection and analysis was carried out independently by two researchers (T.K. and G.G.). Studies were classified into two groups which investigated either pre-clinical or clinical investigations and/or outcomes.

**Results**

After the initial search, ninety-nine potentially relevant articles were retrieved and assessed in detail and eighteen of them were discarded. One was the original description of the surgical technique of orthotopic liver transplantation (OLTx) [8], one focused on biliary tract histology [9] and nine only on serum factors [10–16] or other factors but not including bile samples [17, 18]. One study examined the effects of taurocholic acid administration [19], one HGH uptake in the liver [20], one post-operative biliary obstructions only [21], one liver regeneration only [22], one the influence of bile salts on cyclosporine absorption [23] and two were letters to the editor [24, 25]. Eighty-one articles were suitable for the analysis including twenty experimental studies and sixty-one clinical studies (tables 1–3).

**Liver Transplantation**

Changes in hepatic secretory function in relation to all aspects of bile flow and composition have been investigated extensively (table 1). Clearly distinct stages following liver transplantation have been identified, and during these stages various factors influence the physiology and production of bile. Immediately after transplantation, the most influential factor is the recovery from cold ischaemia (1–3 h), and acute rejection or immunosuppressive drugs exert their influence later (7–14 days and 3–4 weeks, respectively). The first study to analyse bile composition in an animal model of OLTx found that during episodes bile volume and bile acid output were significantly decreased [26]. Modifications of bile composition and volume were attributed to the restitution of the liver blood flow, while the duration of the cold ischaemia was a significant exacerbating factor [27], and these results suggested that post-operative bile changes were representative of the early graft function [27]. Later changes in bile volume and bile salt synthesis and secretion at 2 weeks and one month following an uneventful OLTx show changes which are significantly different from those seen immediately after recovery (table 1). Such modifications in bile composition are probably due to the influence of immunosuppressive drugs [28, 29]. Both experimental [26, 29–31] and clinical studies [32–34] have confirmed that various immunosuppressive drugs exert different effects on bile flow and composition (table 1).

Different factors have been investigated in clinical studies of OLTx as potential predictors of post-operative complications and graft function. Sub-optimal donor livers demonstrate a low choleretic activity and an altered
Table 1. Studies on orthotopic liver transplantation and liver ischaemia

<table>
<thead>
<tr>
<th>Topic investigated</th>
<th>Author</th>
<th>Subjects</th>
<th>Model</th>
<th>Results</th>
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<tbody>
<tr>
<td>OLTx</td>
<td>McMaster [26], Chan et al. [28], Chan et al. [29], Tono et al. [78], Daloze et al. [79], Chan et al. [80]</td>
<td>Rhesus monkeys, rats, dogs</td>
<td>OLTx</td>
<td>↑ or normal bile volume&lt;br&gt;Normal bile salt output, secretion, synthesis and pool size, phospholipids secretion&lt;br&gt;No differences if the allograft only receives blood from the portal vein&lt;br&gt;Immunology: ↑ bile IL-6, decreasing after 48 h and maintaining low levels afterwards</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>McMaster [26], Chan et al. [29], Le Thai et al. [30], Chan et al. [31], Bell et al. [81], Le Thai et al. [82], Stone et al. [83], Stone et al. [84]</td>
<td>Rhesus monkeys, rats</td>
<td>OLTx + different immunosuppressive drugs</td>
<td>Azathioprine and prednisolone: normal or ↑ bile volume&lt;br&gt;Normal bile salt output, concentration, synthesis, secretion and pool size, phospholipids secretion, cholesterol secretion and concentration&lt;br&gt;Cyclosporine: ↑ bile flow and volume, bile salt output, concentration, synthesis, secretion and pool size&lt;br&gt;↓ phospholipids secretion. Normal cholesterol secretion and concentration</td>
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<tr>
<td>Rejection</td>
<td>McMaster [26], Tono et al. [78]</td>
<td>Rhesus monkeys, rats</td>
<td>OLTx with rejection</td>
<td>↑ bile volume, bile salt output and concentration&lt;br&gt;Normal cholesterol concentration&lt;br&gt;Immunology: ↑ bile IL-6, correlated with the histological severity of rejection</td>
</tr>
<tr>
<td>Ischaemia time</td>
<td>Kamiike et al. [43], Xu et al. [27], Bowers et al. [85]</td>
<td>Rats</td>
<td>In vivo: induced ischaemia (clamping) L-Ethionine (= 1 ATP)&lt;br&gt;Ex vivo: reperfusion with bicarbonate/albumin solution</td>
<td>Ischaemia &gt; 1 ATP &gt; 1 or stop of bile volume and flow, ↑ bile salt concentration, secretion, synthesis and pool size, phospholipids concentration and secretion. Normal or ↑ cholesterol concentration and secretion&lt;br&gt;More prolonged ischaemia = further ↑ bile volume and flow, recovery more depressed</td>
</tr>
<tr>
<td>OLTx</td>
<td>Javitt et al. [86], Ericzon et al. [38], Baiocchi et al. [39], Tisone et al. [40], Sanchez-Bueno et al. [42], Umesita et al. [48], Carrasco et al. [51], Baiocchi et al. [87], Baumgartner et al. [41], Goesky et al. [88], Melendez et al. [35], Kubota et al. [52], Geuken et al. [36], Haagsma et al. [89], McCashland et al. [32], Ko et al. [33], Shiffman et al. [90], Waldram et al. [91], Lenzen et al. [92], Bowers et al. [93], Umesita et al. [94], Ericzon et al. [95], Roberti et al. [53], Theilmann et al. [96], Carrasco et al. [97], Oldhafer et al. [98], Kubota et al. [99], Kubota et al. [100], McMaster et al. [101], McMaster et al. [102]</td>
<td>Patients</td>
<td>OLTx</td>
<td>Steady increase in bile flow, in bile salt output, synthesis, concentration and pool size, of cholesterol and phospholipids secretion and concentration after OLTx&lt;br&gt;Bilirubin subfractions ↑ BDG, ↑ BMG&lt;br&gt;Electrolyte, glucose, urea and creatinine concentrations similar to plasma&lt;br&gt;T-tube open (first p.o. days) = bile supersaturated with cholesterol = ‘early’ biliary sludge&lt;br&gt;T-tube clamped = re-establishment of enterohepatic circulation = bile salt = bile unsaturated with cholesterol&lt;br&gt;Immunology: ↑ bile IL-6, decreasing after 48 h and maintaining low levels afterwards&lt;br&gt;Cytology: cells for the first 5 days, with the highest cell density on p.o. day 1 (PMN, ductal and ghost cells), then decrease afterwards&lt;br&gt;Polarizing and electron microscopy: increased proportion of vesicles and reduction of their size, presence of lamellae</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>McCashland et al. [32], Ko et al. [33], Ericzon et al. [34], Söderdahl et al. [103], Sauer et al. [104]</td>
<td>Patients</td>
<td>OLTx + CyA&lt;br&gt;OLTx + FK 506&lt;br&gt;OLTx + Cya versus FK 506</td>
<td>CyA: steadily increase in bile flow, bile salt output and secretion&lt;br&gt;Normal bile salt concentration, ↑ deoxycholic and chenodeoxycholic acid synthesis, ↑ relative concentration&lt;br&gt;Cholic acid. Bile salt, cholesterol and phospholipid concentration correlated with serum CyA levels&lt;br&gt;FK506: steadily increase of bile flow, bile salt output and secretion, cholesterol and phospholipid secretion, more marked than with CyA&lt;br&gt;Normal bile salt concentration, ↑ deoxycholic acid synthesis</td>
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Table 1 (continued)

<table>
<thead>
<tr>
<th>Topic investigated</th>
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<th>Model</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PNF</strong></td>
<td>Ericzon et al. [38], Tisone et al. [40], Sanchez-Bueno et al. [42], Carrasco et al. [51], Baumgartner et al. [41], Goresky et al. [88], Kubota et al. [52], Haagsma et al. [89], Adams et al. [49], Adams et al. [45], Hathaway et al. [46], Hathaway et al. [105], Ko et al. [33], Akamatsu et al. [106], Lenzen et al. [92], Adams et al. [50], Umeshita et al. [94], Roberti et al. [53], Adams et al. [107], Carrasco et al. [97], Hathaway et al. [108], Oldhafer et al. [98], Kubota et al. [99], Kubota et al. [100]</td>
<td>Patients</td>
<td>OLTx with PNF</td>
<td>bile volume and flow, bile salt output and secretion, extremely low or undetectable concentrations of bile salt, cholesterol and phospholipids until recovery Immunology: normal IL-6 Cytology: high cell density since OLTx until retransplantation.</td>
</tr>
<tr>
<td><strong>Rejection</strong></td>
<td>Ericzon et al. [38], Sanchez-Bueno et al. [42], Umeshita et al. [48], Carrasco et al. [51], Baumgartner et al. [41], Goresky et al. [88], Kubota et al. [52], Haagsma et al. [89], Adams et al. [49], Adams et al. [45], Hathaway et al. [46], Hathaway et al. [105], Ko et al. [33], Akamatsu et al. [106], Lenzen et al. [92], Adams et al. [50], Umeshita et al. [94], Roberti et al. [53], Adams et al. [107], Carrasco et al. [97], Hathaway et al. [108], Oldhafer et al. [98], Kubota et al. [99], Kubota et al. [100]</td>
<td>Patients</td>
<td>OLTx with rejection</td>
<td>bile volume, bile salt, cholesterol and phospholipids concentration No differences according to histological severity of rejection Bilirubin subfractions: † BDG, †† BMG Immunology: † in bile chemotactic activity for CD3+ and CD8+ lymphocytes, monocytes and neutrophils. † bile β2-microglobulin, † IL-2 receptor, † IL-6. † IgG and IgM No differences according to histological severity of rejection Cytology: high cell density (PMN, MN, macrophages, lymphocytes, blasts, ghost cells). Contrasting results for correlation of cell density and blasts with histological grades</td>
</tr>
<tr>
<td><strong>IPF</strong></td>
<td>Tisone et al. [40]</td>
<td>Patients</td>
<td>OLTx with IPF</td>
<td>Delayed bile salt output, synthesis, concentration and secretion</td>
</tr>
<tr>
<td><strong>Vascular thrombosis</strong></td>
<td>Ericzon et al. [38], Akamatsu et al. [106], Bowers et al. [93], Roberti et al. [53], Oldhafer et al. [98], Kubota et al. [100]</td>
<td>Patients</td>
<td>OLTx with HA or vena cava thrombosis</td>
<td>Sharp decline of bile salt output, synthesis, concentration and secretion until extremely low concentration Bilirubin subfractions: † BDG, †† BMG Cytology: high cell density (PMN, MN, lymphocytes, ductal cells)</td>
</tr>
<tr>
<td><strong>Sepsis</strong></td>
<td>Carrasco et al. [51], Akamatsu et al. [106], Adams et al. [50], Umeshita et al. [94], Roberti et al. [53], Carrasco et al. [97], Kubota et al. [99], Kubota et al. [100], McMaster et al. [102]</td>
<td>Patients</td>
<td>OLTx with HPB sepsis (e.g. cholangitis)</td>
<td>Bilirubin subfractions: † BDG, †† BMG Obstruction &gt; cholangitis &gt; unconjugation of bilirubin &gt; 'late' biliary sludge Immunology: † IL-2 receptor, † IL-6 Cytology: high cell density during the episode (PMN)</td>
</tr>
<tr>
<td><strong>Biliary strictures</strong></td>
<td>Geuken et al. [36], Buis et al. [37]</td>
<td>Patients</td>
<td>OLTx with p.o. biliary strictures</td>
<td>bile salt, cholesterol and phospholipids secretion † bile acid/phospholipid ratio (increased cytotoxicity for bile duct cells)</td>
</tr>
<tr>
<td><strong>Sub-optimal donors</strong></td>
<td>Melendez et al. [35]</td>
<td>Patients</td>
<td>OLTx from sub-optimal donors</td>
<td>† bile flow</td>
</tr>
<tr>
<td><strong>Ischaemia time</strong></td>
<td>Carrasco et al. [109]</td>
<td>Patients</td>
<td>Correlation with ischaemia time</td>
<td>Inverse relationship with ATP recovery &gt; † graft function &gt; † bile production Cytology: increased cell density and ductal cells with longer ischaemia time</td>
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</table>

HA = Hepatic artery; p.o. = post-operative; PMN = neutrophil polymorphonuclear leucocytes; MN = mononuclear leukocytes; CyA = cyclosporine.

Bile acid composition when compared to normal grafts [35]. Bile cytotoxicity (measured by an increase in the bile salt/phospholipid ratio) appears to be involved in the pathogenesis of non-anastomotic biliary strictures [36, 37]. In transplant recipients, a progressive increase in the bile salt pool and concentration is seen in functioning grafts as opposed to those with initial poor function (IPF) or primary non-function (PNF) [38–41]. In grafts with IPF, there is an initial, transient incapacity to synthesize and secrete bile salts, while in PNF this is permanently impaired [38, 40]. These differences mean that sequential monitoring of biliary bile salt secretion is able to reliably discriminate between the two conditions [40]. A reduction in bile flow and bile lipids is also found with both
PNF and acute rejection, but was more marked in the former [41, 42]. Hepatic energy status (and hence organ viability) influences the recovery of normal liver function after ischaemia and liver transplantation. Higher levels of hepatic ATP result in a higher rate of bile secretion after controlled ischaemia [43] and a reduced rate of post-operative rejection in the transplant recipients [44].

Potentially, the most interesting findings relate to the early diagnosis of rejection. Bile samples taken 2–3 days before clinical symptoms become apparent are chemo-tactic for lymphocytes, monocytes and neutrophils to an even greater extent than those collected during the episode itself [45, 46]. IL-6, an important component of the signalling pathway for hepatocyte proliferation and liver regeneration [47] may explain this chemotactic activity and has been shown to be elevated in the bile of patients experiencing rejection episodes [48]. Similar results are found for β2-microglobulin, a molecule associated with particular HLA class 1 antigens [49], and for IL-2 receptor [50]. It is likely that CD8+ T lymphocytes are recruited in response to IL-6 (and other chemotactic factors) secreted by CD4+ cells significantly before the onset of the symptoms of rejection [46]. Following this recruitment, the bile cellularity starts to increase, usually 24 h before the clinical symptoms [51–53]. In this setting, the analysis of bile may provide a useful screening tool for patients who are experiencing leukocyte activation and recruitment but are still in the asymptomatic phase of the rejection.

Liver Resection

The analysis of bile flow and composition has also been used to assess changes in hepatic secretory function following HPB surgery (table 2). In experimental studies, liver resection had a greater effect on the rate of secretion of bile salts, cholesterol and phospholipids than their final concentration in the bile [54, 55]. Liver regeneration affected the total volume of bile and its rate of secretion following liver resection. The maximum reduction occurs between 12 and 48 h post-operatively, and from days 3 and 9 recovers to normal levels as liver regeneration proceeds [55, 56].

There are also a number of clinical studies which have investigated the ability of modifications in bile composition to predict the incidence of liver failure after surgical resection. Pre-operative and post-operative bile salt concentrations on day 2 have the highest predictive power for post-operative liver failure, suggesting that these measurements could indicate patients at high risk of developing complications [7], and these results also confirmed those obtained in experimental studies [56]. Furthermore, they are also supported by subsequent clinical studies that demonstrated a normalization of bile composition after the second post-operative day which coincides with liver regeneration [57].

The proportions of the biliary bilirubin subfractions are usually altered in HPB diseases [58]. Pre-operative changes in jaundiced patients consist of lower levels of pre-operative bilirubin diglucuronide (BDG) and higher levels of bilirubin monoglucuronide (BMG), corresponding to a lower BDG:BMG ratio and less efficient bile processing. Such modifications have been shown to be predictive of post-operative liver function after hepatic resections [3], and furthermore liver resection per se exerts an additional influence on the normal production of biliary bilirubin subfractions, further decreasing BDG and increasing BMG from the 5th to the 21st post-operative day [59, 60]. A significant correlation was also found with the energy state of the remnant liver [60], confirming the importance of the hepatic ATP on the post-operative outcomes [43].

Finally, after hepatic parenchymal loss, regeneration is stimulated by a number of different factors, including HGF – a potent hepatocyte mitogen. Biliary HGF correlates with the remnant liver volume after hepatectomy and shows an increased specificity for the detection of post-operative liver failure as opposed to serum HGF [61, 62]. Similar results are also obtained for IL-6 [63].

Biliary Drainage

Pre-operative biliary drainage and the particular method used have also been shown to influence the amount of liver regeneration occurring after surgery (table 3). Internal biliary drainage is superior to external biliary drainage stimulating more liver regeneration demonstrated by an increased rate of hepatic DNA synthesis, liver function, liver weight, bile flow, bile salt and phospholipid secretion in the internally drained group [64–66]. External biliary drainage markedly suppresses regenerative capacity [67], while with internal biliary drainage it is preserved [64]. This suggested that the bile itself contains important factors that are involved in hepatocyte regeneration, and that these factors are irreversibly removed by external drainage with adverse effects on liver regeneration. HGF is physiologically reabsorbed from the gastrointestinal tract into the portal venous circulation and is likely to be one of these factors [68, 69]. The continuous loss of bile resulting from external drainage may reduce this reabsorption, stimulating additional HGF secretion in the bile [69]. As a con-
Table 2. Studies on the effects of liver resection and regeneration

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Subject</th>
<th>Model</th>
<th>Results</th>
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<tbody>
<tr>
<td>Fukano et al. [54]</td>
<td>1985</td>
<td>Rats</td>
<td>(1) 66% HT</td>
<td>↑ bile flow and bile salt, cholesterol, phospholipid secretion until p.o. day 4, ↑ cholic acid, ↓ chenodeoxycholic acid until p.o. day 7, ↑ pool size until p.o. day 14</td>
</tr>
<tr>
<td>Perez-Barriocanal et al. [55]</td>
<td>1987</td>
<td>Rats</td>
<td>(1) 66% HT, (2) controls</td>
<td>Concentration: normal for bile salt and phospholipid from 12 h to 16 days p.o., ↑ for cholesterol secretion: ↑ for bile salt, cholesterol and phospholipid at 12 to 24 h p.o., normal thereafter</td>
</tr>
<tr>
<td>Xu et al. [56]</td>
<td>1993</td>
<td>Rats</td>
<td>(1) 75% HT (n = 8), (2) 50% HT (n = 8), (3) controls (n = 8)</td>
<td>Liver size approached controls on (1) day 9 or (2) day 7 p.o. ↑ Bile flow and volume on day 1 and 3 for (1) and (2), normal on day 5 p.o. ✶ bile salt secretion, synthesis and pool size, cholesterol and phospholipid secretion on day 1–5 p.o. for (1) and (2)</td>
</tr>
<tr>
<td>Igarashi et al. [59]</td>
<td>1999</td>
<td>Rats</td>
<td>(1) 80% HT, (2) 70% HT, (3) controls</td>
<td>↓ BDG and UDP-GA from day 5 to 21 p.o. for (1) and (2) vs. (3) ↓ UDP-GT activity at 12 hours for (1) and (2) vs. (3) ↑ BMG and other subfractions from day 3 to 21 p.o. for (1) and (2) vs. (3)</td>
</tr>
<tr>
<td>Suto et al. [60]</td>
<td>2002</td>
<td>Rats</td>
<td>(1) 33% HT with sepsis, (2) 33% HT, (3) sepsis, (4) controls</td>
<td>↓ BDG, UDP-GA, UDP-GT and NAD+ on p.o. day 3 and 4 for (1) and (3) vs. (2) and (4) Normal UDP-glucose, UDP-GDH activity, and cytosolic free (NAD+)/ (NADH) ratio</td>
</tr>
<tr>
<td>Takeuchi et al. [61]</td>
<td>1997</td>
<td>Patients</td>
<td>(1) 1EBD &gt; HT or extended HT without liver failure (n = 20), (2) EBD &gt; HT or extended HT with liver failure (n = 4)</td>
<td>1. ↑ bile HGF from p.o. day 1 to 7 2. Normal bile HGF after surgery (no increase)</td>
</tr>
<tr>
<td>Ishiyama et al. [3]</td>
<td>1998</td>
<td>Patients</td>
<td>(1) EBD &gt; HT without liver failure (n = 10), (2) EBD &gt; HT with liver failure (n = 5), (3) EBD &gt; controls (PD, n = 8)</td>
<td>↓ BDG and ↑ BMG on p.o. day 1 for (1), (2) and (3). On p.o. day 14 normal for (3) and (1), ↑ for (2)</td>
</tr>
<tr>
<td>Kurumiya et al. [62]</td>
<td>1999</td>
<td>HT + bile duct resection (n = 21), HT + PD (n = 10), PD (n = 15), bile duct resection (n = 4)</td>
<td>↑ bile HGF from p.o. day 1 to 7 ↑ bile HGF after bile duct resection as well as after HT</td>
<td></td>
</tr>
<tr>
<td>Maeda et al. [63]</td>
<td>1999</td>
<td>Patients</td>
<td>(1) EBD &gt; HT or extended HT without liver failure (n = 18), (2) EBD &gt; HT or extended HT with liver failure (n = 6)</td>
<td>↑ bile IL-6 from p.o. day 1 to 6 for (1) and (2), but lower values for (2)</td>
</tr>
<tr>
<td>Kurumiya et al. [7]</td>
<td>2003</td>
<td>Patients</td>
<td>(1) EBD &gt; HT without liver failure (n = 29), (2) EBD &gt; HT with liver failure (n = 7), (3) EBD &gt; controls (PD or bile duct resection; n = 15)</td>
<td>↓ bile flow, bile salt secretion and concentration, cholesterol and phospholipid concentration on p.o. day 1 and thereafter increased in (1) and (3) but not in (2) Correlation of bile salt concentration and secretion with remnant liver clearance of indocyanine Bile salt concentration predicts p.o. liver failure: 81% accuracy when dosed preoperatively, 88% post-operatively</td>
</tr>
<tr>
<td>Hotta et al. [57]</td>
<td>2005</td>
<td>Patients</td>
<td>HT + EBD</td>
<td>↓ bile salt concentration on p.o. day 2, normal on day 7 Cirrhosis and patients with poor remnant liver clearance of indocyanine maintained ↓ bile salt concentration on day 7</td>
</tr>
</tbody>
</table>

HT = Hepatectomy; PD = pancreateoduodenectomy; UDP-GA = uridine diphosphoglucuronic acid; UDP-GT = UDP-glucuronyltransferase; UDP-GDH = UDP-glucose dehydrogenase; NAD+ or NADH = nicotinamide adenine dinucleotide.
sequence, due to the compensatory mechanism for the continuous loss, peak concentrations of bile HGF are higher when external drainage is used, compared to internal drainage [69]. Following external biliary decompression, bile acid output decreases drastically and gradually increases again to reach a plateau in most patients [70]. In some cases, bile acid output remains low due to a delayed recovery of liver function [70]. The increase in bile acid output after the positioning of an external biliary drainage is dependent on the hepatic ATP content [71]. For these reasons, as for transplant surgery [43, 44], the hepatic energy status and hence organ viability influences the recovery of normal liver functions after relief of obstructive jaundice.

Ex vivo Liver Perfusion

Bile analysis has also been used as a measure of hepatic viability and function in ex vivo perfused porcine livers. These experimental models have been used to study liver physiology [72], metabolism [73] and the toxic effects of a wide range of inorganic and biological compounds [72, 73]. Foley et al. [74] used bile flow and phospholipid and cholesterol concentrations to examine differences between a single and a dual vessel porcine perfusion model. The dual vessel perfusion model (hepatic artery and portal vein) produced significantly more bile than single vessel perfusion (portal vein only) with more aqueous bile and a greater cholesterol output. However, the livers perfused from a single vessel did produce a greater output of phospholipid [74]. The same author used the composition of bile to investigate the potential of the ex vivo perfused liver to support 4 patients with fulminant hepatic failure [75]. Samples after 1 h of perfusion demonstrated the predominance of pig bile acids (65%), but after 3 h human bile acids made up the largest fraction (85%) [75].

<table>
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<th>Results</th>
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<tbody>
<tr>
<td>Iyomasa et al.</td>
<td>1992</td>
<td>Rats 70% HT and EBD 70% HT and obstructive jaundice</td>
<td>DNA polymerase-α activities, mitotic index and (3H) thymidine incorporation higher for (2) vs. (1)</td>
</tr>
<tr>
<td>Suzuki et al.</td>
<td>1994</td>
<td>Rats 70% HT and IBD 70% HT and EBD 70% HT and obstructive jaundice</td>
<td>DNA polymerase-α activities and mitotic index at p.o. day 1 and 5 days higher for (1) and (3) vs. (2)</td>
</tr>
<tr>
<td>Hayata et al.</td>
<td>1999</td>
<td>Rats 70% HT and EBD (n = 54) 30% HT and external biliary drainage (n = 54) EBD (n = 54) 70% HT (n = 54) 30% HT (n = 54) Controls (n = 54)</td>
<td>Liver weight was greater for (4) vs. (1) and (5) vs. (2) HGF concentrations were lower for (4) vs. (1) and (5) vs. (2)</td>
</tr>
<tr>
<td>Saiki et al.</td>
<td>1999</td>
<td>Rats 70% HT and IBD 70% HT and EBD 70% HT and obstructive jaundice</td>
<td>Liver weight and hepatic DNA synthesis rate at p.o. day 1 and 2 greater for (1) vs. (2) and for both vs. (3)</td>
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<tr>
<td>Mizuta et al.</td>
<td>2002</td>
<td>Rats 70% HT and IBD 70% HT and EBD 70% HT and obstructive jaundice</td>
<td>Liver weight, hepatic DNA synthesis, bile flow, bile salt secretion and phospholipid secretion at p.o. day 1 and 2 greater for (1) vs. (2) and for both vs. (3) Similar cholesterol secretion among groups Differences in biliary bile acid composition between (1) vs. (2) and (3)</td>
</tr>
<tr>
<td>Eklund et al.</td>
<td>1980</td>
<td>Patients with obstructive jaundice EBD</td>
<td>Cholic, chenodeoxycholic and hyocholic bile excretion immediately increase following drainage</td>
</tr>
<tr>
<td>Chijiiwa et al.</td>
<td>2002</td>
<td>Patients with obstructive jaundice EBD &gt; PD (n = 4) EBD pylorus-preserving PD (n = 4) EBD &gt; extended right HT (n = 1) EBD &gt; extended left HT (n = 1)</td>
<td>Normal bile flow and bile salt secretion rates after EBD Hepatic ATP: correlation with bile salt secretion, not with bile flow</td>
</tr>
</tbody>
</table>

**IBD** = Internal biliary drainage; **EBD** = external biliary drainage.
Discussion

It is relatively straightforward to collect bile following liver surgery, and this has stimulated interest in its value as a tool to monitor and predict liver function in both clinical and research studies. Initial studies focused mainly on bile volume and composition, although other aspects were also examined including bile cytology, immunology and the influence of specific drugs on its production. All of these studies provided new insights in respect of the liver’s response to various physiological and pathological conditions, and had important clinical applications in both liver transplantation and following resectional surgery.

Early studies where bile was analysed in detail greatly contributed to the understanding of the various aspects of OLTx per se as well as those which were influenced by immunosuppressive drugs/regimens, rejection and post-operative complications. At the end of the period of cold ischaemia and immediately following transplantation, the liver undergoes a period of ‘stunning’ or ‘hibernation’ that is related to the intracellular ATP content. The longer the ischaemia time, the more ATP is consumed within the cells and the longer the organ needs to recover its normal biosynthetic activities, including the synthesis and secretion of bile components. Additionally, the presence of an open T-tube (and consequent bile drainage) in the first few post-operative days also contributes to the observed alterations in bile physiology. Disruption of the enterohepatic circulation decreases the secretion of bile, which is directly dependent on the acid bile synthesis (‘choleretic effect’) and limits the synthesis of bile salts to those derived only from the primary acids. This increases the overall lithogenic index and the likelihood of calculi formation. Siphoning off the bile is responsible for those changes, and these are usually reversed by the closure of the external drainage and the restoration of the internal bile flow. The fact that both the cold ischaemia and the T-tube contribute to changes in the early post-operative days after OLTx makes it difficult to use bile production and analysis as a direct measure of the organ viability and functionality in this period. However, bile analysis could be especially useful immediately following the closure/removal of the T-tube as a diagnostic tool for primary organ non-functions or delayed organ recovery.

Other important findings in the field of liver transplantation correlate with the use of different immunosuppressive drugs and their influence on the bile production. Changes are often related to specific pharmacologic mechanisms of action of the drug (e.g. the cholestatic effect of cyclosporine). Particular bile components (e.g. IL-6, HGF, β2-microglobulin, number and types of cells) are also useful in predicting post-operative rejection as they are altered days before the appearance of symptoms. This makes them a useful and non-invasive tool for monitoring rejection in the early post-operative period. The same constituents are also reliable markers of the liver’s response to anti-rejection therapy as they remained persistently elevated in cases that are resistant.

The importance of bile analysis in HPB surgery lies in its ability to predict post-operative liver failure. Monitoring of liver function includes the assessment of standard biochemical parameters, radiological estimates of residual functional volumes and assessments of the hepatocyte uptake and elimination (indocyanine green clearance) or metabolism (lignocaine or galactose) [76, 77]. A number of studies have shown that changes in the composition of bile provide a useful tool for the pre-operative screening and the early post-operative monitoring of patients at high risk of liver failure following HPB surgery. Pre-operative bile analysis might also be helpful in guiding the extent of resection or the need for an alternative pre-operative approach such as portal vein embolization. In the post-operative setting, it could provide an additional reliable method of monitoring the patient’s clinical condition and possibly predict the need for additional treatment or a change of management. Bile analysis is likely to be most valuable as a complementary investigation augmenting those currently used, and would increase the overall sensitivity for the early and reliable detection of post-operative liver failure. Prospective studies should examine the predictive value and the sensitivity and specificity of each biliary parameter (bile volume, bile salt concentrations, bilirubin subfractions, biliary HGF of IL-6) at different time points after surgery and in sufficient detail to allow their integration into clinical practice. A direct comparison with the other methods already used in clinical practice, or with combinations of them, could possibly generate new scoring systems to increase the overall accuracy.

The findings in respect of biliary drainage (external and T-tubes) are similar for liver transplantation and major HPB surgery and both significantly affect the physiology of bile production. The differences observed between external and internal drainages demonstrate the importance of the enterohepatic circulation of bile salts and confirm that internal drainage results in less disturbance of normal physiologic conditions. The importance of the enterohepatic circulation is not limited to the bile salt content (which could potentially be replaced by the post-
operative administration of taurocholic acid), but also involves specific molecules that are directly related to liver regeneration (e.g. HGF, IL-6) and that cannot be easily substituted. The loss of these substances with external drainages theoretically increases the risk of post-operative liver failure through a resultant decrease in hepatocyte regeneration after liver resection [76]. This requires confirmation, and prospective studies should evaluate the advantages of internal drains over the external to prevent liver failure in patients who require biliary drainage in the perioperative period.

Conclusions

Results of experimental and clinical studies confirm that changes in bile composition may be useful for monitoring the outcome of liver surgery. Future comparisons with the other methods are required and may demonstrate that its assessment is more reliable and could provide a valuable tool to be employed in a number of clinical situations, from the screening of organ rejection to the identification of patients at high risk of developing post-operative complications. Future studies should further examine the predictive value of alterations in bile composition to allow their definitive integration into clinical practice.

References

Bile Changes after Liver Surgery


