Organelle blockade: another mechanism for the cytoprotective effect of ursodeoxycholic acid conjugates in the hepatocyte


INTRODUCTION

Ursodeoxycholic acid (UDCA), a natural bile acid, has been shown to delay the progression of primary biliary cirrhosis in association with improvement in laboratory measurements of hepatocyte injury and cholestasis. There appear to be multiple mechanisms by which UDCA mediates these events. In this chapter we will propose yet another possible mechanism of action of UDCA which was suggested to us during our recent examination of the transport of fluorescent bile acids by the isolated perfused liver.

OVERVIEW OF UDCA PHYSICOCHEMICAL PROPERTIES AND METABOLISM

UDCA, as well as its glycine and taurine conjugates, is an extremely hydrophilic bile acid, based on its short retention time by reversed-phase HPLC in which the stationary phase has an octadecylsilane surface. The retention time is so short that it appears as if the 7β-hydroxy group prevents the binding of the hydrophobic face of the molecule to the octadecylsilane surface of the stationary phase. The hydrophilic behaviour of UDCA is also evidenced by its much weaker binding to phosphatidylcholine vesicles, when compared to that of chenodeoxycholic acid (CDCA). The lack of binding to membranes provides an explanation for the minimal cytotoxicity of conjugates of UDCA towards isolated cells, for example, hepatocytes, mast cells, and cholangiocytes.

Conjugates of UDCA aggregate to form micelles above a critical micellization concentration (CMC) that is only slightly higher than that of conjugates of
Bile Acids and Cholesterol: The Concentration of Cholesterol in the Extracellular Fluid is too low to destroy the cell membrane. Above the CMC, the concentration of monomers of deoxycholic acid (DCA) is at the CMC, the concentration of molecules enters a levelling off phase. On the other hand, when an untagged bile acid molecule enters a liposome, it is likely to rapidly interact with the bilayer forming the aqueous phase. The hydrophobic interaction is based on the behavior of the hydrophilic-philic model, the hydrophilic interaction is based on the behavior of the hydrophilic-philic model, and the hydrophilic interaction is based on the behavior of the hydrophilic-philic model.
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acid pool has two origins. The first is continuing endogenous bile acid biosynthesis; the second is the ingested UDCA. With the commonly prescribed doses the input of UDCA exceeds endogenous bile acid synthesis, explaining the well-known enrichment in UDCA that occurs in biliary bile acids in PBC patients ingesting UDCA\(^\text{19,20,32}\). There is likely to be an additional input of CDCA formed by bacterial epimerization of UDCA in the distal intestine\(^\text{33}\). To date, patients have not been clearly described whose bile became enriched in CDCA because of conversion of administered UDCA to CDCA, but it seems likely that such patients will eventually be described.

The above considerations make it highly probable that the actions of UDCA are mediated by its amidates, rather than by the unconjugated bile acid. The predominant amidate will be UDC-gly.

CYTOPROTECTION BY CONJUGATES OF UDCA

It has been known for some years that infusion of taurine-conjugated dihydroxy bile acids such as deoxycholytaurine (taurodeoxycholic acid, DC-tau) or chenodeoxycholytaurine (taurochenodeoxycholate, CDC-tau) at supraphysiological rates induces acute cholestasis\(^\text{34-36}\). Morphological changes can also be induced by increased intracellular concentrations of cholytaurine\(^\text{37}\). Superimposition of an equimolar load of UDC-taurine not only prevents the cholestasis, but also prevents storage of the cholestatic bile acid in the liver\(^\text{35,36}\). This 'cytoprotective' effect that is observed in such 'rescue' experiments is not specific for UDC-tau, because it can also be reproduced with unconjugated derivatives of UDCA such as its 6-fluoro-derivative\(^\text{38}\) or by other hydrophilic conjugated bile acids such as hyodeoxycholytaurine, as shown by Aldo Rada and his collaborators in Chapter 23. The cholestatic effect has generally been assumed to be mediated at the canaliculus, since this is the site of bile production, and loss of canalicular phospholipids into bile can be shown as cholestasis appears\(^\text{39}\).

No mechanism has yet been provided for the cytoprotective effect of UDC-taurine. One possibility is that UDC-taurine has an allosteric effect on the canalicular bile salt export pump, causing it to secrete the cholestatic bile acid. Such a mechanism has been proposed for the interaction of bile acids with sinusoidal oapt by the laboratory of Alan Wolko\(^\text{40}\).

A second possibility is that UDC-tau blocks the uptake of the cholestatic bile acid by cellular organelles, by competing for uptake. For example, uptake of cholestatic bile acids by pericanalicular vesicles might inhibit the canalicular bile salt expert pump (bsep) when these vesicles fuse with the canaliculus\(^\text{41,42}\). If uptake of cholestatic bile acids by pericanalicular vesicles could be prevented by UDC-tau uptake, such inhibition would not occur. UDC-tau might also induce the fusion with the canalicular membrane of pericanalicular vesicles containing the infused cholestatic bile acid, and thereby promote its excretion into bile. This would be consistent with the finding that UDC-taurine causes an elevation in the intracellular concentration of ionized Ca\(^{2+}\) and activates phosphokinase C\(^\text{43}\). A second organelle(s) that import and exports conjugated bile acids is the microsomal compartment. There are multiple examples of taurine-conjugated bile acids undergoing hydroxylation during hepatocyte transport\(^\text{44,45}\). Such
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Hydroxylation must involve uptake into and export from a microsomal compartment. Although it would be entirely reasonable for UDC-taurine to inhibit microsomal uptake of cholestatic bile acids by competing for the microsomal transporter, this has not been shown experimentally. Nor is any mechanism readily available whereby microsomal uptake of a cholestatic bile acid should cause cholestasis.

A third organelle that might be involved in uptake of bile acids that induce cholestasis is the mitochondrion. However, no transport systems for bile acids in mitochondria have been reported. Bile acids and advanced chronic cholestatic liver disease have been shown to impair mitochondrial function, but in these experiments uptake was considered to be passive, so that competition cannot occur. In principle, passive uptake of glycine dihydroxy-bile acids could occur in the mitochondrion in regions of low pH. Finally, bile acids could also be taken up by the Golgi apparatus, and immunohistochemical localization of bile acids in this organelle during hepatocyte transport has been observed.

UDC-taurine might also displace cholestatic bile acids bound to intracellular binding proteins. However, if binding were related to hydrophobicity, UDC-taurine should be a weak displacing agent, because of its hydrophilicity. If UDC-taurine were to dislodge cholestatic bile acids from simple binding proteins, it should increase the intracellular activity, and promote rather than inhibit uptake by organelles. Competition for binding proteins between indomethacin and conjugated bile acids has been shown; displacement of conjugated bile acids by indomethacin leads to regurgitation from the hepatocyte across the sinusoidal membrane.

Clearly, experiments that will confirm or refute these speculations are needed.

EVIDENCE FOR ORGANELLE BLOCKADE BY UDC-TAU

Our laboratory has recently characterized the hepatocyte transport of several natural conjugated bile acids using the single-pass isolated perfused rat liver (IPRL) under an ‘approach to steady-state conditions’. The perfusate was a buffered electrolyte solution that did not contain albumin, and a 15-min infusion at a rate well within the physiological range was used. Three natural bile acids were studied: cholyglycine, cholytaurine, and UDC-glycine. The two glycine-conjugated bile acids showed similar behaviour. Both had extremely high first-pass extractions. For cholyglycine, the value was 94%, and for UDC-glycine the value was 99.6%. Neither showed appreciable regurgitation (< 1%); both were excreted efficiently into bile and no sequestration occurred (Table 1). Cholytaurine behaved differently. Although its 1-min extraction (98%) was similar to that of the glycine-conjugated bile acids, its extraction decreased progressively with time, reaching a near-steady-state value at 15 min of 72%; much of the decrease in extraction could be explained by regurgitation into the perfusate. We expressed our data by relating bile acid excretion rate to the amount stored in the liver (Figures 1 and 2). A better approach would have been to determine the concentration of the bile acid in the hepatocyte and the cytosol (cf. ref. 51) and to relate biliary excretion to that value in order to obtain a more valid value for canalicular clearance.
Table 1  Transport of some natural conjugated bile acids and their lysyl-NBD derivatives by the isolated rat liver perfused in single-pass fashion (15 min perfusion of bile acid followed by 45 min perfusion of bile acid-free perfusate) (taken from Tables 1 and 2 in ref. 2)

<table>
<thead>
<tr>
<th>Compound</th>
<th>C-taurine</th>
<th>C-glycine</th>
<th>UDC-gly</th>
<th>C-L-NBD</th>
<th>UDC-L-NBD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Hepatic uptake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Net FE, 3 min</td>
<td>72.4 ± 0.4</td>
<td>94.6 ± 1.2</td>
<td>99.7 ± 0.1</td>
<td>97. ± 0.8</td>
<td>92.2 ± 3.5</td>
</tr>
<tr>
<td>Net FE, 15 min</td>
<td>71.1 ± 0.4</td>
<td>93.9 ± 1.4</td>
<td>99.6 ± 0.1</td>
<td>86.1 ± 1.8</td>
<td>83.8 ± 2.5</td>
</tr>
<tr>
<td>Cumulative uptake in 60 min, percentage infused</td>
<td>71.5 ± 0.2</td>
<td>94.0 ± 0.8</td>
<td>99.6 ± 0.1</td>
<td>89.3 ± 0.8</td>
<td>87.2 ± 3.0</td>
</tr>
<tr>
<td>Regurgitation, percentage of uptake</td>
<td>6.3 ± 1.3</td>
<td>0.8 ± 0.4</td>
<td>0.4 ± 0.4</td>
<td>4.1 ± 0.1</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>II. Biliary secretion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal rate* (nmol/g liver × min)</td>
<td>22.7 ± 1.2</td>
<td>27.7 ± 1.0</td>
<td>31.6 ± 1.3</td>
<td>13.3 ± 1.5</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Cumulative recovery, 60 min, percentage uptake</td>
<td>98.1 ± 0.6</td>
<td>98.4 ± 1.9</td>
<td>96.7 ± 3.8</td>
<td>81.9 ± 1.2</td>
<td>12.2 ± 3.7</td>
</tr>
<tr>
<td>Biliary secretion (max)/hepatic uptake (max)</td>
<td>0.91</td>
<td>0.91</td>
<td>0.91</td>
<td>0.45</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* The maximal rate is determined by the dose infused, which was 40 nmol/g liver × min for 15 min.

FB = fractional extraction
Figure 1 The left panel shows the kinetics of hepatic uptake (solid circles) and biliary recovery (open circles) of two natural conjugated bile acids (C-glycine and C-taurine) by the isolated rat liver perfused in single-pass fashion. Bile acids were present in the perfusate for the first 15 min of the experiment. In the right panel the recovery rate is plotted in relation to the amount present in the liver (the difference between uptake and biliary recovery). No correction has been made for biliary dead space; thus the amount of bile acids present in the hepatocyte is less than that of the liver. C-taurine has a lower fractional extraction, and shows regurgitation when the perfusate is stopped, as evidenced by negative transport (from ref. 2).

Using this preparation, hepatocyte transport of six bile acids containing a fluorophore in the side-chain (NBD or fluorescein) was characterized similarly. Among these fluorescent bile acids was the lysyl-NBD conjugate of UDC (UDC-L-NBD). This compound can be visualized as UDC-glycine with an n-butyramino tether to which an NBD group is attached. UDC-L-NBD was taken up rather efficiently by the isolated perfused liver, the first-pass extraction being 86% at 15 min (Table 1). In contrast to the natural conjugated bile acids, which were almost completely recovered in bile (recovery > 97%), the UDC was highly sequestered, with only 12% being recovered. In order to attempt to mobilize this bile acid, natural bile acids were either co-infused in equimolar proportions or introduced into the perfusate after sequestration had occurred. All
three natural bile acids that were co-infused (cholyl-taurine, UDC-taurine, DC-taurine) prevented sequestration (Figure 3). Infusion of UDC-taurine, after sequestration had occurred, also mobilized the sequestered UDC-L-NBD. We interpret these experiments to indicate that the co-infused bile acid prevented uptake by organelles by competing for transport. Displacement from binding proteins cannot be excluded, but, as noted, UDC-taurine is a hydrophilic bile acid that should bind weakly to binding proteins. The binding organelles appear to have a limited capacity for bile acid uptake as post-sequestration infusion of UDC-taurine also mobilized sequestered UDC-L-NBD. It is hoped to gain more insight into the sites of bile acid storage using confocal fluorescence microscopy. If the behavior of UDC-L-NBD were similar to that of a cholestatic bile acid, it would suggest that the cytoprotective effect of UDC-taurine could be explained by competition for transport into an as-yet-undefined organelle.
Figure 3  Mobilization of sequestered UDC-L-NBD by co-infusion of C-taurine or UDC-taurine. Addition of dibutyryl cAMP had no effect on the sequestered fluorescent bile acid, whereas co-infusion of UDC-taurine or C-taurine prevented sequestration of UDC-L-NBD. Sequestered UDC-L-NBD could also be mobilized if UDC-taurine was added to the perfusate after sequestration had occurred, that is, at 15 min (from ref. 2)

RELEVANCE OF CYTOPROTECTION EXPERIMENTS TO UDCA THERAPY OF CHOLESTATIC LIVER DISEASE

Whether the cytoprotection shown by UDC conjugates in animal models of acute cholestasis has any relevance to the pharmacodynamic action of UDC in
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cholestatic liver disease is uncertain, in our opinion. Cholestasis in primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) is attributable to pathology distal to the canalculus. Whether uptake of endogenous cytotoxic conjugated bile acids by organelles occurs in the cholestatic hepatocyte is not known. Improvement of hepatic histology in PBC has been reported in response to UDC in some studies, but not others, and the changes have been modest, rather than marked. Using acute cytoprotection experiments it is possible to identify other cytoprotective bile acids such as conjugates of hyodeoxycholic acid or 6-fluoro-UDC58, but whether such bile acids will be safe and efficacious in PBC and PSC is not known. Hyodeoxycholic acid is unlikely to be useful in humans because the compound is glucuronidated53,54 and not retained in the enterohepatic circulation55. This aberrant metabolism is likely to be explained by the hyodeoxycholic acid being a poor substrate for the bile acid amidation system, and thus resembling nor-dihydroxy bile acids in its metabolism56,57. As a result it partitions into the microsomes, is glucuronidated (at C-6) and promptly eliminated from the body.

Whether these experiments suggesting that UDC-taurine blocks uptake of endogenous cytotoxic bile acids by organelles bear on the anti-apoptotic effects of UDCA58,59 is also not known. A major challenge for the future is to define the fate of UDCA and its amidates in the hepatocyte and how an increased flux of UDCA-amidates alters the transport and physiological or pathological effects of endogenous bile acids in cholestatic liver disease. It is also desirable to find out how UDCA and its amidates interact with the multiple receptors, proteins, proteases, and organelles involved in the apoptosis cascade.

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References

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