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

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### 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<sup>1</sup>. 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<sup>2</sup>.

### 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<sup>3</sup>. The retention time is so short that it appears as if the 7 $\beta$ -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)<sup>4</sup>. The lack of binding to membranes provides an explanation for the minimal cytotoxicity of conjugates of UDCA towards isolated cells, for example, hepatocytes<sup>5-8</sup>, mast cells<sup>9</sup>, and cholangiocytes<sup>10</sup>.

Conjugates of UDCA aggregate to form micelles above a critical micellization concentration (CMC) that is only slightly higher than that of conjugates of

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chenodeoxycholic acid (CDCA)<sup>11</sup>. At the CMC the concentration of monomers and the surface activity is too low to destroy the cell membrane. Above the CMC the concentration of UDCA monomers remains relatively constant. As a result UDCA is not cytotoxic, either below or above its CMC.

On the other hand, when an unconjugated UDCA molecule enters a lipid bilayer, it is likely to rapidly flip across the bilayer passively after accepting a proton from the aqueous phase. This prediction is based on the behaviour of chenodeoxycholic acid (CDCA), which has been shown to rapidly flip flop across lipid bilayers<sup>12</sup>, as well as the rapid passive absorption of UDCA from the perfused rat small intestine<sup>13</sup>. The high passive membrane permeability can also explain the hypercholeretic activity of UDCA. When UDCA is presented to the hepatocyte at a rate exceeding its conjugation capacity, UDCA is secreted in unconjugated form, undergoes cholehepatic shunting, and induces hypercholeresis<sup>14-16</sup>.

The metabolism of UDCA in humans is not complex. The compound does not undergo appreciable biotransformation during its transport through the enterocyte. Hepatic uptake is less efficient than for other common natural conjugated and unconjugated bile acids, averaging 50%<sup>17,18</sup>; as a result, plasma is enriched in unconjugated UDCA. In the hepatocyte, UDCA is first converted to its coenzyme A thioester, and then transferred to glycine or taurine, forming UDC-glycine and UDC-aurine. Because this process is highly efficient, UDCA is secreted predominantly in amidated form in human PBC patients<sup>19,20</sup>. Nonetheless, the efficiency of bile acid conjugation may decline in severe cholestatic disease<sup>21</sup>. Hypercholeresis, because of biliary secretion of unconjugated UDCA, has yet to be documented in patients receiving UDCA.

The secreted conjugates of UDCA undergo carrier-mediated absorption from the small intestine. If the pH is sufficiently acidic in the duodenum, passive absorption of ursodeoxycholyglycine (UDC-glycine) is likely to occur<sup>22</sup>. In some animals a sodium-independent transporter that prefers dihydroxy-amidates is present in the jejunum<sup>22</sup>. Although there is a considerable volume of experimental evidence favouring absorption of dihydroxy-amidates in the proximal small intestine in humans<sup>23-25</sup>, the carrier(s) involved in such transport has (have) not been identified. In the distal small intestine, UDC-amidates are transported by the ileal apical bile salt transporter (*abs1*)<sup>26</sup>. An undefined fraction undergoes deconjugation. Some of the resultant unconjugated UDCA that is formed is absorbed passively; the remainder remains bound to bacteria and/or dietary residues, and passes into the large intestine.

In the large intestine UDCA may be absorbed passively, altered by bacterial enzymes, or excreted from the body. The major bacterial biotransformations of UDCA are 7-epimerization to form CDCA and 7-dehydroxylation to form lithocholic acid. The CDCA may be absorbed, may be metabolized back to UDCA or may also undergo 7-dehydroxylation to form lithocholic acid. Lithocholic acid is in part absorbed, returns to the liver where it undergoes amidation and sulphation and is promptly eliminated from the body.<sup>27-29</sup>

UDCA, in contrast to the common natural bile acids (cholic acid, CDCA, deoxycholic acid) does not suppress primary bile acid synthesis in PBC patients, based on very limited experimental evidence<sup>30,31</sup>. As a result, in PBC patients ingesting UDCA, the input of newly formed bile acids into the circulating bile

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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 UDC that occurs in biliary bile acids in PBC patients ingesting UDCA<sup>19,20,32</sup>. There is likely to be an additional input of CDCA formed by bacterial epimerization of UDCA in the distal intestine<sup>33</sup>. 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<sup>34-36</sup>. Morphological changes can also be induced by increased intracellular concentrations of cholytaurine<sup>37</sup>. Superimposition of an equimolar load of UDC-aurine not only prevents the cholestasis, but also prevents storage of the cholestatic bile acid in the liver<sup>35,36</sup>. 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<sup>38</sup> or by other hydrophilic conjugated bile acids such as hyodeoxycholytaurine, as shown by Aldo Roda 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<sup>39</sup>.

No mechanism has yet been provided for the cytoprotective effect of UDC-aurine. One possibility is that UDC-aurine 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 *oatp* by the laboratory of Alan Wolkoff<sup>40</sup>.

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 export pump (*bsep*) when these vesicles fuse with the canaliculus<sup>41,42</sup>. 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-aurine causes an elevation in the intracellular concentration of ionized  $Ca^{2+}$  and activates phosphokinase  $C^{43}$ . A second organelle(s) that imports and exports conjugated bile acids is the microsomal compartment. There are multiple examples of taurine-conjugated bile acids undergoing hydroxylation during hepatocyte transport<sup>44,45</sup>. Such

