Tauro-β-muricholate Preserves Choleresis and Prevents Taurocholate-Induced Cholestasis in Colchicine-Treated Rat Liver

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In recent clinical and animal experimental studies, ursodeoxycholic acid (UDCA) has been noted to have marked choleretic and cytoprotective actions. To define the mechanism and determine whether such favorable influence is specific to UDCA, the choleretic action of β-muricholic acid (β-MCA), which has a similar chemical structure, was studied using an isolated rat-liver-perfusion system. As a result, β-MCA and taurine-conjugated β-MCA (Tβ-MCA) stimulated bile flow accompanied by elevation of bile acid output and phospholipid output, and β-MCA caused an elevation in biliary HCO₃⁻ concentration in normal rat livers. After colchicine treatment, taurocholic acid (TCA) administration was associated with marked cholestasis while both β-MCA and Tβ-MCA still increased bile flow under the same conditions. Furthermore, simultaneous administration of β-MCA or, more markedly, Tβ-MCA reversed the effects of TCA alone in colchicine-treated rat liver; significant preventive effects against the cholestasis could be shown. These data suggest that β-MCA and especially Tβ-MCA can support choleresis even under conditions of colchicine-dependent microtubule dysfunction. The effects of Tβ-MCA on organelle lipids and their intracellular transport may differ from those of TCA, presumably because of the anticholestatic and cytoprotective effects of Tβ-MCA.

Bile acids form an important driving force for biliary secretion, which may, however, be accompanied by toxic effects causing cholestasis. Each bile acid thus has both primary choleretic and secondary cholestatic effects, and the relative extent of these influences varies with the individual species. Furthermore, although ursodeoxycholic acid (UDCA) is only an isomer of chenodeoxycholic acid (CDCA), the manner of gallstone resolution by the two compounds is not exactly the same⁴ and the accompanying incidence of diarrhea or liver function disorder also differs.⁵

Recently, UDCA has attracted interest for treatment of primary biliary cirrhosis (PBC)⁶ and other chronic liver diseases.⁷ In animal experiments, administration of UDCA has been reported to result in hypercholeresis and elevation of biliary HCO₃⁻ concentration.⁸,⁹ Preventive action against cholestasis or so-called cytoprotective effects have been noted especially for the taurine-conjugated UDCA form.¹⁰,¹¹ Thus, there are a number of interesting findings regarding clinical application of UDCA for liver diseases and the underlying mechanisms of influence. However, whether these favorable actions for liver cells are specific just for UDCA is not clear, although similar action has recently been reported for taurine-conjugated β-muricholic acid (β-MCA).¹²,¹³ In the present study, in view of the common 7-hydroxy bond chemical structure between UDCA and β-MCA, the choleretic actions of the latter were evaluated in terms of possible mechanisms underlying the cytoprotective action of bile acids.

**Materials and Methods**

**Animals**

Male Sprague-Dawley rats weighing 250–280 g were used for all studies. The animals had free access to water, were fed on conventional diets, and were housed under standard conditions with a normal lighting cycle. The experiments were begun after overnight fasting; the responses of groups of rats injected intraperitoneally with 0.2 mg of colchicine were compared with those of control, nontreated animals.

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Drugs

Taurocholic acid (TCA), \( \beta \)-MCA, and \( \text{T\( \beta \)} \)-MCA were generously donated by Tokyo Tanabe Co., Ltd. (Tokyo, Japan). Colchicine was purchased from Sigma (St. Louis, MO).

Liver Perfusion

Perfusion of isolated livers was performed by the standard recirculating method of Miller using a Krebs'–Ringer bicarbonate buffer perfusate containing 240 mg/L glucose and 0.83 g/L bovine serum albumin. After 30 minutes, single 36-\( \mu \)mol doses of bile acid, individually or in combination (total dose, 72 \( \mu \)mol), were administered and perfusion was continued for another 40 minutes. Bile samples were collected every 5 minutes and perfusate every 10 minutes.

Analytical Methods

Total bile acid content was enzymatically measured using a commercial kit from Daiichi Chemicals (Tokyo, Japan). Phospholipid content was determined by an enzymatic method using a commercial kit from Wako Chemicals (Tokyo, Japan). The concentration of bicarbonate ions in bile was measured by a back-titration method using ABU13 with Titrator TTT2 from Radiometer Co., Ltd., Copenhagen, Denmark).

Statistics

Data are expressed as mean \( \pm \) SD. Comparisons between groups were made by analysis of variance followed by the Student's t test (paired or unpaired).

Results

Changes in Bile Flow, Phospholipid Output, and Bile Acid Output After \( \text{T\( \beta \)} \)-MCA Administration to Control Rats

Bile flow increased significantly 5 minutes after administration of 36 \( \mu \)mol of \( \text{T\( \beta \)} \)-MCA, with a peak of 182 \( \pm \) 32.8 \( \mu \)l/h-g liver\(^{-1}\), about three times the basal value, at 10 minutes, followed by reduction, although significantly elevated levels continued to be maintained until 25 minutes. Bile acid and phospholipid outputs also started to increase 5 and 10 minutes after \( \text{T\( \beta \)} \)-MCA administration; maximal values were observed in the 15- and 20-minute fractions, respectively (Figure 1).

\( \beta \)-MCA also induced an elevation of bicarbonate ion concentration in bile; a significant increase was observed 10 minutes after its addition, and a peak value was evident 15 minutes after treatment. Administration of \( \text{T\( \beta \)} \)-MCA showed no elevation of the biliary bicarbonate concentration (Table 1). The choleresis efficiency of \( \beta \)-MCA did not differ markedly from that of \( \text{T\( \beta \)} \)-MCA (Figure 2).

Changes in Bile Flow, Phospholipid Output, and Bile Acid Output After \( \text{T\( \beta \)} \)-MCA Administration to Control Rats

\( \text{T\( \beta \)} \)-MCA administration also induced choleresis including increments of both phospholipid and bile acid outputs. Bile flow increased significantly 5 minutes after the addition, becoming maximal at 10 minutes then beginning to decrease. Maximal outputs of phospholipid and bile acid were also evident in the 10-minute fractions. The elevation of bile secretion by \( \text{T\( \beta \)} \)-MCA and the return toward basal levels were more rapid than with \( \beta \)-MCA (Figure 3).

Cholestasis Induced by TCA in Colchicine-Treated Rat Livers

Whereas the addition of 36 \( \mu \)mol of TCA into the perfusate produced significant elevations of bile flow, phospholipid output, and bile acid output in control rat livers (Figure 4), in colchicine-treated rat livers the same TCA treatment resulted in marked cholestasis. In the latter case, transient and slight increases in bile flow and bile acid output were seen 5 minutes after administration and a reduction to \( \leq 50\% \) of the basal value was evident thereafter. Cholestatic status was conspicuous in the 10-minute and 40-minute fractions, and bile acid and phospholipid outputs were also clearly decreased compared with those of nontreated rats (Figure 5).

Choleresis Induced by \( \beta \)-MCA in Colchicine-Treated Rat Livers

After addition of \( \beta \)-MCA to the perfusate in the colchicine-treated rat liver, no depletion of bile
Table 1. Change in Biliary Bicarbonate Concentration After Administration of \( \beta \)-MCA and T\( \beta \)-MCA

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>B</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta )-MCA</td>
<td>19.5 ± 2.6</td>
<td>24.4 ± 1.3</td>
<td>31.3 ± 2.3(^b)</td>
<td>34.9 ± 3.3(^b)</td>
<td>33.2 ± 4.2(^b)</td>
<td>27.7 ± 4.2(^a)</td>
<td>22.0 ± 1.4</td>
</tr>
<tr>
<td>T( \beta )-MCA</td>
<td>22.6 ± 1.5</td>
<td>22.4 ± 2.3</td>
<td>19.3 ± 2.8</td>
<td>23.5 ± 3.7</td>
<td>24.5 ± 1.4</td>
<td>22.7 ± 2.5</td>
<td>23.4 ± 2.2</td>
</tr>
</tbody>
</table>

After 30 minutes of pretreatment with buffer alone, a single 36-\( \mu \)mol aliquot of \( \beta \)-MCA or T\( \beta \)-MCA was added and perfusion was continued for another 30 minutes. Bile samples were collected every 5 minutes. Concentration (mmol/L) of bicarbonate ion in bile was measured by a back-titration method. Results are mean ± SD.

\(^{a}P < 0.05, \(^{b}P < 0.01\) compared with basal value taken 5 minutes before administration.

flow from basal values was seen in any fraction. On the contrary, significant choleretic action was evident 5–25 minutes after administration, although only to about 50% the extent of the effects in control rats (Figure 6). Bile acid output showed a change similar to that of bile flow. Relatively lower but more prolonged elevation of phospholipid output was also seen after \( \beta \)-MCA administration (Figure 7).

Choleresis Induced by T\( \beta \)-MCA in Colchicine-Treated Rat Livers

T\( \beta \)-MCA treatment resulted in prominent choleresis even in colchicine-treated rat livers; the repression ratios of choleretic actions compared with control livers were only 25% and 39% at 5 and 10 minutes, respectively. Similar levels of choleresis were seen from 15 minutes onward in both colchicine-treated and nontreated groups (Figure 8). Bile acid and phospholipid outputs were apparently stimulated by T\( \beta \)-MCA administration even in colchicine-treated rats. Relatively repressed elevation was observed in the 10- and 15-minute fractions after the stimulation, but the ratios were not significantly different from control values (Figure 9). Prolongation of bile acid and phospholipid outputs was conspicuous in colchicine-treated rats.

Prevention by \( \beta \)-MCA or T\( \beta \)-MCA of Cholestasis Induced by TCA in Colchicine-Treated Rat Livers

The influence of combined \( \beta \)-MCA administration on the cholestasis induced by TCA in colchicine-treated rat livers was examined. As illustrated in Figure 10, except for the 20-minute fraction, slightly but not significantly decreased bile flows compared with basal levels were maintained after administration of both bile acids, in clear contrast to the significant reduction observed with TCA alone. The preventive effects of T\( \beta \)-MCA were even clearer. About a 160% choleresis compared with basal flow was evident in every fraction after simultaneous administration of TCA and T\( \beta \)-MCA to colchicine-treated rats (Figure 11). Significant increases in...
bile acid and phospholipid outputs were observed in the 5-minute to the final fractions, these changes being more prolonged than after simultaneous administration of β-MCA (Figure 12).

Discussion

This study clearly shows that both β-MCA and Tα-MCA bring about marked increases in bile flow accompanied by elevated phospholipid and bile acid outputs in nontreated rat livers. The peak time of bile acid and lipid output after administration of Tβ-MCA was relatively early compared with the results.

Figure 5. Effects of TCA perfusion on bile flow, phospholipid output, and bile acid output in isolated colchicine-treated rat livers. Thirty-six micromoles of TCA was administered into the perfusate after 30 minutes of preperfusion with buffer alone. The basal value for bile flow is represented by the B fraction taken 5 minutes before administration. Results are expressed as mean ± SD for five experiments.

Figure 6. Effects of β-MCA perfusion on bile flow in isolated colchicine-treated or nontreated rat livers. Thirty-six micromoles of β-MCA was administered into the perfusate after 30 minutes of preperfusion with buffer alone. The basal value for bile flow is represented by the B fraction taken 5 minutes before administration. Results are expressed as mean ± SD for five experiments. *Significantly different (P < 0.05) from nontreated; †P < 0.01 compared with nontreated.

Figure 7. Changes in bile acid and phospholipid output after β-MCA administration. *Significantly different (P < 0.05) from nontreated.
for β-MCA and approximately equal to those for TCA. However, the total amounts of lipid released were more similar between Tβ-MCA and β-MCA (Figures 1, 3, and 4). The chemical structure of bile acids often influences the speed and strength of bile flow and lipid output. The present results show that both β-MCA and Tβ-MCA surpass TCA in affecting secretion of biliary lipid, thus showing characteristics of the so-called micelle-forming bile acid group.

Although β-MCA was also found to cause elevation of the bicarbonate ion concentration in the bile, this was not the case for Tβ-MCA, as also described in a recent paper agreeing with our preliminary report. This is of interest given the similar choleretic efficiency for both β-MCA and Tβ-MCA in the present experiment (Figure 2). With regard to the link between increased concentration of bicarbonate in bile and enhanced choleresis, two representative theories by Kitani and Kanai and Hofmann et al. have been proposed, but the exact mechanistic significance remains unclear. Further experiments using separately isolated hepatocytes and intrahepatic choledochocytes would be required to resolve the mechanism(s) and site(s) of action not only of UDCA but also of β-MCA.

The choleretic efficiencies of β-MCA and Tβ-MCA were relatively high compared with that of TCA, and also higher than the values generated for these bile acids using an in vivo perfusion system. The questions of whether methodological differences are responsible for this discrepancy and whether this method of isolated liver perfusion with a bolus addi-
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Figure 11. Effects of simultaneous administration of Tß-MCA with TCA on isolated colchicine-treated rat livers. Thirty-six micromoles of Tß-MCA was simultaneously administered with the same dose of TCA into the perfusate after 30 minutes of preperfusion with buffer alone. The basal value for bile flow is represented by the B fraction taken 5 minutes before administration. Results are expressed as mean ± SD for five experiments. *Significantly different (P < 0.01) from TCA alone.

Figure 12. Changes in bile acid and phospholipid output after simultaneous administration of TCA and ß-MCA or Tß-MCA. *Significantly different (P < 0.05) from TCA alone; †P < 0.01 compared with TCA alone.

In the present isolated liver-perfusion experiment, a 36-μmol dose of TCA proved choleretic for normal rat livers, whereas the same dose induced marked cholestasis in colchicine-treated rats. Colchicine inhibits polymerization of microtubules, thereby interfering with intracellular transport of secretory proteins,20 exogenous substances such as horseradish peroxidase,21 and probably biliary lipid precursors.22 Although colchicine alone does not exert significant influence on bile flow, bile lipid output, or bile acid output, earlier work showed that a distinct cholestasis occurs after additional loading with TCA or taurochenodeoxycholic acid (TCDCA) but not with taurodeoxycholate (TDHCA). From these findings it has been concluded that cholestasis in colchicine-treated rat livers is caused only by the infusion of micelle-forming bile acids,15,22 a conclusion not supported by the finding of preserved choleresis in the β-MCA and especially Tß-MCA cases. The present results indicate that administration of TCA may somehow exacerbate the effects of microtubule dysfunction, resulting in the observed marked cholestasis. In contrast, β-MCA and especially Tß-MCA showed almost normal choleretic action even after colchicine treatment, suggesting that the biliary lipid precursor formed in these cases can be transported using a microtubule-independent or –partly independent manner. However, because the exact reason for the differences in action between TCA and Tß-MCA in colchicine-treated liver remain unclear, conclusions regarding the possible association between microtubule function and intracellular transport of lipids stimulated by bile acids must remain speculative. With both fusion-budding and exocytosis models, canalicular transport of biliary-type lipids involves vesicles, and therefore the functional integrity of the cytoskeleton, including the microtubule system, would be required.22–24 However, the physicochemical properties of bile acids may influence the quality of the lipid secretion.25 Thus, the strength of TCA and Tß-MCA detergent binding to organelles differs with influence on resultant formation of biliary lipid precursors. However, further experiments are needed to determine whether bile acid and associated lipids themselves can directly affect microtubular function.

The findings that simultaneous addition of TCA and ß-MCA generally maintained bile flow comparable to basal levels and that an approximately 60% increase over basal bile flow was gained by combined use of TCA and Tß-MCA are of particular interest. These findings are in clear contrast with the find
ing that a double-dose administration of TCA resulted in an almost complete cessation of bile secretion (data not shown). Prolonged and delayed elevation of bile acid and phospholipid output was observed; the degree of elevation of lipid and bile acid output was linked to the preventive effects on cholestasis (Figures 10-12).

In the previously described experimental case of excess toxic bile acid–induced cholestasis and its alleviation by simultaneous administration of tauroursodeoxycholic acid (TUDCA) or Tβ-MCA, the main reason for the bile salt–induced cholestasis was thought to be a reduction in toxic detergent action, particularly at the level of the canalicular membranes. Thus TUDCA and Tβ-MCA were proposed as inhibiting TCA- or TCDCA-induced cholestasis by reducing the accumulation of cytotoxic bile acids in hepatocytes. Moreover, it was recently speculated that the coexistence of cytoprotective and cytotoxic bile acids in the liver and their coexistence may be important for the preventive effect. However, also in this model, how and at which site in the hepatocyte the cytoprotective bile acids act to reduce the toxicity of TCA or TCDCA remain unclear.

With the present colchicine-treated model, the preventive action of Tβ-MCA may operate by a different mechanism. One of the possibilities is that Tβ-MCA competes with the interaction between TCA and lipids, such as in the organelle-binding step or at the stage of biliary lipid precursor formation. The other possibility is direct or physicochemical interaction between both agents resulting in a change in the strength of detergent action of TCA and in altered biliary lipid formation. Whichever is the case, simultaneously administered Tβ-MCA clearly reduces the toxic influence of TCA on hepatocytes under microtubule dysfunctional conditions, resulting in improved bile secretion.

In conclusion, the present findings lead to the important suggestion that although TCA and TCDCA show choleretic action under normal conditions, after loading with other risks such as immunological factors in PBC these bile acids might show toxicity and thereby promote cholestasis. However, the simultaneous presence of TUDCA or Tβ-MCA may reduce the toxicity and help hepatocytes recover from damage.

References


