# β Muricholic acid: a new hepatoprotective agent

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*The hepatoprotective effect of β muricholate against cholestasis induced by hydrophobic steroids was studied in rats. Acute cholestasis was caused by intravenous infusion of taurochenodeoxycholate at 3 μmol/min/Kg for 3 hr in the bile fistula rats. Bile flow and bile salt secretion decreased gradually, whereas lactico dehydrogenase biliary leakage increased. Simultaneous infusion of β muricholate, at a rate of 1.5 μmol/min/Kg, ameliorated cholestasis induced by taurochenodeoxycholate, but an increase in lactico dehydrogenase persisted. Infusion of 4 μmol/min/Kg of β muricholate resulted in the quasi-complete biliary elimination of taurochenodeoxycholate and prevented the cholestatic and cytotoxic effects of taurochenodeoxycholate infusion alone. β muricholate was as effective as tauroursodeoxycholate. Moreover, severe chronic cholestasis induced by ethinyl estradiol was partially prevented by hydrophilic bile acid feeding. When β muricholate was given at a 50 mg/Kg/day dose, bile flow and bile salt secretion were increased. Both bile salt dependent and bile salt independent bile flow were significantly improved. Lastly, the hydrophilic-hydrophobic balance of β muricholate was estimated by surface tension measurements. β muricholate appeared to have a weak affinity for a hydrophobic interface. It generated a lower surface pressure than ursodeoxycholate and much more lower than chenodeoxycholate. The low surface activity of β muricholate could account for its non-toxicity and protective action towards hepatocyte membranes.*

**Keywords:** bile salts; estrogen; cholestasis; hepatoprotection

## Introduction

The hydroxyl groups located on the steroid nucleus of bile salts can differ in number, position, and orientation. These polar groups define for each bile salt molecular species a hydrophilic-hydrophobic balance that determines its physicochemical properties and influences its biological properties.[1-7] The polarity of bile salts plays an essential role in lipid biliary secretion,[8] bile flow rate[9,10] and the maintenance of membrane integrity.[11-14] Thus, the most lipophilic bile salts, lithocholate and chenodeoxycholate (CDC), can induce cholestasis and hepatocellular necrosis.[15,16] In contrast, a hydrophilic bile salt, ursodeoxycholate (UDC), 3α 7β OH, has been recently used to treat patients with cholestatic disorders such as primary biliary cirrhosis,[17] chronic active hepatitis,[18] and liver disease associated with cystic fibrosis.[19-21] Similarly, we have shown that UDC also has a beneficial effect on ethinylestradiol (EE)-induced cholestasis in rats.[22]

During UDC chronic administration in humans, UDC represents no more than 45% of the bile acid pool. The remainder of the biliary bile salts are composed of CDC, cholate, and secondary species: deoxycholate and lithocholate. On the contrary, β muricholate (βMC), a trihydroxy bile salt, 3α 6β 7β OH, is only slightly transformed by human intestinal microflora,[23] thus precluding the formation of cytotoxic bile salts. Moreover, βMC is more hydrophilic than UDC as inferred by high pressure liquid chromatography[24] and its low lipid-micellar solubilizing capacities.[25,26] It has high choleretic properties[27,28] and induces low lecithin and cholesterol biliary secretion in the rat.[27] Thus, βMC could appear beneficial in humans considering its physicochemical and biological properties, together with the positive experience of UDC administration in liver disease.

In this study, we evaluated the hepatoprotective capacities of βMC in two cholestasis models, one induced by EE and the other by a hydrophobic bile salt,

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taurochenodeoxycholate (TCDC). Furthermore, to gain information on the relationship between bile salt hydrophobicity and cellular toxicity, we investigated the surface activity of βMC, UDC, and CDC at the air-water interface.

## Materials and methods

### Chemicals

EE (17 α-ethinyl-Δ-1,3,5-estratriene-3-17β-diol) was purchased from Sigma Chemical Co. (St. Louis, MO USA), and was 99% pure. UDC (3α,7β-dihydroxy-5β-cholanoic acid), CDC (3α,7α-dihydroxy-5β-cholanoic acid), and βMC, (3α,6β,7β-trihydroxy-5β-cholanoic acid) were a generous gift from Roussel-Uclaf Laboratories, Romainville, France. The purity of these compounds was > 99%. TCDC (> 98% pure), sodium tauroursodeoxycholate (TUDC) (>98 % pure), and taurine (>99%) were purchased from Calbiochem, Los Angeles, CA USA. Bovine serum albumine (99%) and cholyl glycine hydrolase were from Sigma Chemical Co. 3α-hydroxysteroid dehydrogenase was from Worthington Biochemical Corporation, Freehold, NJ USA. Neomycine sulfate was from Diamant Laboratories, Puteaux, France.

### Animal experiments

Male Sprague-Dawley rats weighing 200–250 g at the beginning of the experiment were obtained from IFFA-CREDO, L'Arbresle, France. They had free access to food and water (supplemented with 1% taurine). The daily diet, from U.A.R., Villemoisson, France, has been previously described.[22]

### Acute experiments

A polyethylene cannula (PE 10) was inserted into the proximal bile duct and a Venocath 18 catheter was placed into the femoral vein of ether-anesthetized rats. All animals were placed in restraining cages. An intravenous infusion of NaCl 0.9% was maintained at a constant rate (1 ml/hr) by means of an infusion pump. After 18 hr of biliary drainage, the infusion solution was changed to one containing NaCl 0.9%, albumine 3%, and TCDC 3 μmol/min/Kg.

Group 1, TCDC; group 2, TCDC + TUDC 1.5 μmol/min/Kg; group 3, TCDC + βMC 1.5 μmol/min/Kg; group 4, TCDC + TUDC 4 μmol/min/Kg; group 5, TCDC + βMC 4 μmol/min/Kg.

These solutions were infused for 3 hours. Bile was collected continuously in 30 min samples. Bile flow, total and individual bile salts, and biliary LDH were assayed.

Total bile salts were determined using the 3α-hydroxysteroid dehydrogenase method according to Turley and Dietschy.[29] The ratio between tauroconjugated bile salts and glycoconjugated bile salts was determined as previously described.[22] Individual bile salts were measured during the last 30 min of infusion. Biliary conjugated bile salts were hydrolyzed by cholyl glycine hydrolase. The rate of hydrolysis was dependent on the bile salt structure, the slowest rate being obtained with tauro β muricholate. After a 90-minute incubation at 37° C with a large excess of enzyme, TC, TCDC, and TUDC were completely deconjugated, whereas tauro βMC was 80% hydrolyzed into βMC. A corrective factor was therefore introduced to estimate the effective biliary βMC output. The resulting free bile acids were analyzed by gas liquid chromatography and thin-layer chromatography as previously described.[22] The activity of lactate dehydrogenase (LDH) in bile was determined immediately after bile collection using a Boehringer Mannheim kit (Mannheim, Germany).

### Chronic experiments

Rats were distributed into five groups and received a specific treatment for 6 days as follows: group A1, control rats; groups A2, B, C, and D were supplemented with neomycine sulfate 20 mg/Kg/day per os. Rats of groups B, C, and D were given daily subcutaneous injections of EE (2 mg/Kg per day) dissolved in propylene glycol. Rats of groups C and D were treated with both EE and bile salt; EE and βMC for group C, EE and UDC for group D. Each bile salt was given at 50 mg/Kg/day dose by gavage.

Rats were operated on 12 hr after the last dose. The bile duct and femoral vein were cannulated. While bile was being collected, normal saline was infused (1 mL/hr IV) to replenish body fluids. Bile was collected every 60 min for 3 hr. Bile flow and individual bile salts were measured. The total period of bile collection lasted 16 hr, the time necessary to wash out bile salts of enterohepatic circulation. The bile acid pool size was then determined.[30]

The relationship between bile acid secretion rate and bile flow was studied in all the animals to estimate the bile acid independent flow and the osmotic activity of the bile acids.

### Surface tension

Equilibrium surface tensions were measured with an automatic tensiometer Prolabo (Paris, France) employing a platinum Wilhelmy blade (2×1 cm). Surface tensions were recorded as a function of time until stable and reproducible values were obtained (15–90 min). Thirteen bile salt solutions (0.25 mmol/L–20 mmol/L) were studied in 0.15 M $Na^+$ aqueous solution (0.01 M carbonate-bicarbonate buffer plus NaCl) at pH 9.8 and at 24° C. The sharp break points in the curve provided estimates of the critical micellar concentration (CMC) of the bile salts, and the interfacial areas of the bile salt molecules were calculated from the simplified form of the Gibbs adsorption isotherm equation.[2,31]

## Statistics

The data are reported as mean ± SEM. The significance of differences among the various groups studied was calculated using analysis of variance followed by Newman-Keuls' procedure for multiple comparisons. To test bile flow dependency on bile salt outputs *(Figure 1)*, data were submitted to linear least-square regression analysis. One such analysis was performed for each experimental group, yielding four regression equations. These equations were then compared with each other by using the so-called "dummy" variables model in multiple regression and subsequent analysis of variance to test slopes and intercepts, i.e., parallelism and coincidence.[32]

## Results

### Acute experiments in the rat: effect of various bile salts on bile secretion

**Bile flow during bile salt infusion.** *Figure 2* shows the choleresis evolution in bile fistula rats to which various bile salts were infused. Rats receiving exclusively TCDC (panel A) had a bile flow rate that increased during the first hour and then tended toward a severe cho-

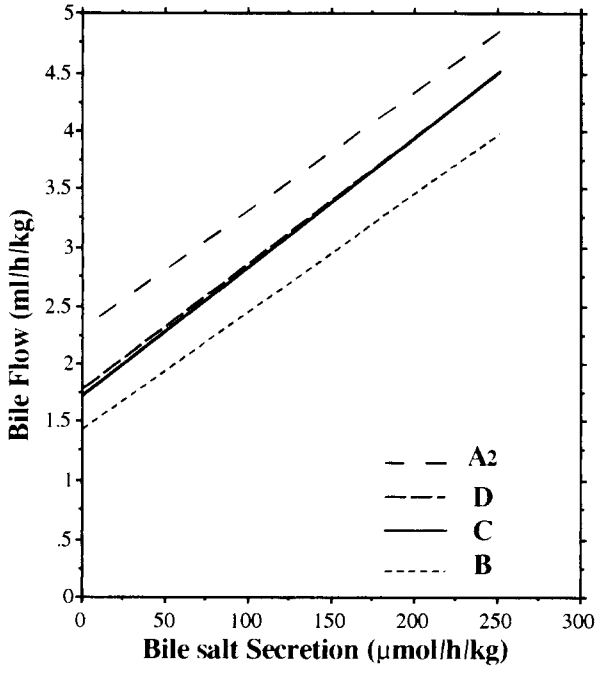


**Figure 1** Relationship between bile flow and bile acid secretion in control rats (group A2), in EE-treated rats (group B), in EE + βMC-treated rats (group C), and in EE + UDC-treated rats (group D). Linear regressions were calculated by the method of least squares. The four regression equations were: A2: $y = 0.01016 x + 2.30890$; $r = 0.73226$; $P < 0.0001$. B: $y = 0.00999 x + 1.46638$; $r = 0.66112$; $P < 0.0001$. C: $y = 0.01104 x + 1.69991$; $r = 0.83143$; $P < 0.0001$. D: $y = 0.01081 x + 1.77224$; $r = 0.89996$; $P < 0.0001$. No significant differences existed between the slopes of the different groups. Significant differences were found between the y-intercepts. See text for details.

lestasis. At the third hour of the perfusion, the bile flow was only 60% of the maximum bile flow value.

When βMC or TUDC were co-administered with TCDC, choleresis significantly increased compared with TCDC. The larger the bile salt infusion rate, the larger the increase (panels B,C). For 1.5 μmol $min^{-1}$ $Kg^{-1}$, however, a decrease in the bile flow was obtained after 3 hr of perfusion. In contrast, the infusion of 4 μmol $min^{-1}$ $Kg^{-1}$ of βMC or TUDC caused a sustained and quasi-constant choleresis. Choleresis was significantly larger with βMC than with TUDC.

**Biliary output of bile salts during bile salt infusion.** *Table 1* depicts the biliary bile salt output before and during the intravenous infusion of bile salts. TCDC was administered alone or co-infused with βMC or TUDC. During the hour preceding the perfusion, the biliary output of endogeneous bile salts was extremely low. After 60–90 minutes of perfusion, the bile salt secretion was maximal in all groups. The larger the perfusion rate, the larger the bile salt output. After 3 hr of perfusion, a large decrease (−34%) in the total bile salt output was observed in group 1. This diminution was significantly less in the other groups. It was moderate in groups 2 and 3 and very low in groups 4 and 5. The TCDC biliary secretion was largely modified by the co-infusion of βMC or TUDC. After the 3 hr perfusion, rats receiving only TCDC secreted 55% of the infused dose. This TCDC biliary output was significantly increased by the co-administration of βMC or TUDC at a dose of 1.5 μmol $min^{-1}$ $Kg^{-1}$. The biliary elimination of TCDC was again increased when βMC or TUDC were perfused at a rate of 4 μmol $min^{-1}$ $Kg^{-1}$ when compared with groups 2 and 3. The TCDC elimination then represented 89–91% of the infused dose. Lastly, there was no difference between the effects of βMC and TUDC, whatever the group. In all groups, bile acids were conjugated exclusively with taurine.

**Biliary lactate dehydrogenase leakage during bile salt infusion.** *Table 2* presents the biliary leakage of lactate dehydrogenase (LDH) during the first hour of drainage in the control group and during the second hour fol-

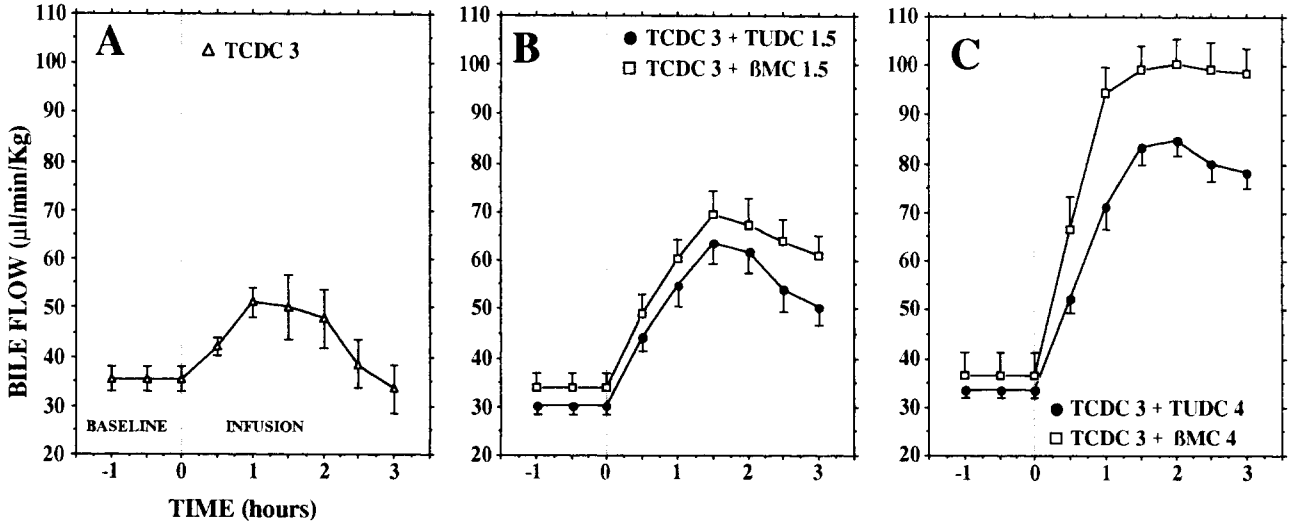


**Figure 2** Effect of bile salt infusions on bile flow. Infusion started after 18 hours of biliary drainage (t = 0) and continued for 3 hours. TCDC was perfused at 3 μmol/min/Kg TUDC and βMC were perfused at 1.5 or 4 μmol/min/Kg. Error bars represent mean ± SEM.

**Table 1** Biliary output of bile salts during bile salt infusions

| Groups | Perfused bile salts | n | Total bile salts: Before Infusion | Total bile salts: During Infusion, Maximum | Total bile salts: During Infusion, 6th half hour | Total bile salts: % Decrease | Individual bile salts at 6th half hour of infusion: CDC | UDC | βMC |
|---|---|---|---|---|---|---|---|---|---|
| | | | *(μmol/min/Kg)* | | | | | *(μmol/min/Kg)* | |
| 1 | TCDC 3 | 7 | 0.06 ± 0.01 | 2.63 ± 0.06 | 1.73 ± 0.31 | 34.12 ± 11.98 | 1.64 ± 0.29 | | |
| 2 | TCDC 3 + TUDC 1.5 | 6 | 0.10 ± 0.02 | 3.82 ± 0.15 | 3.41 ± 0.18 | 11.40 ± 3.30[a] | 2.05 ± 0.11[a] | 1.27 ± 0.06 | |
| 3 | TCDC 3 + βMC 1.5 | 7 | 0.10 ± 0.02 | 3.71 ± 0.25 | 3.43 ± 0.44 | 12.80 ± 4.54[a] | 2.13 ± 0.28[a] | | 1.24 ± 0.16 |
| 4 | TCDC 3 + TUDC 4 | 8 | 0.08 ± 0.01 | 6.51 ± 0.13 | 6.22 ± 0.16 | 4.48 ± 1.09[a] | 2.68 ± 0.07[abc] | 3.49 ± 0.09 | |
| 5 | TCDC 3 + βMC 4 | 7 | 0.08 ± 0.01 | 6.63 ± 0.16 | 6.40 ± 0.16 | 4.00 ± 0.90[a] | 2.73 ± 0.07[abc] | | 3.62 ± 0.11 |

Values represent mean ± SEM.
n, number of rats.
Bile salts were perfused at 1.5, 3, or 4 *μmol/min/Kg*.
Percent decrease expressed the decreases between the maximum outputs of bile salt and those of the 6th half hour.
a: Significantly different from group 1 ($P < 0.05$).
b: Significantly different from group 2 ($P < 0.05$).
c: Significantly different from group 3 ($P < 0.05$).

**Table 2** Biliary lactate dehydrogenase output

| | Groups | n | Biliary LDH output |
|---|---|---|---|
| | | | *(mU/Kg/h)* |
| | Control | 14 | 64 ± 11[a] |
| | βMC 3 | 5 | 41 ± 3[a] |
| | TUDC 3 | 5 | 47 ± 12[a] |
| 1 | TCDC 3 | 5 | 800 ± 60[b] |
| 2 | TCDC 3 + TUDC 1.5 | 5 | 222 ± 23[ab] |
| 3 | TCDC 3 + βMC 1.5 | 5 | 252 ± 35[ab] |
| 4 | TCDC 3 + TUDC 4 | 5 | 110 ± 5[a] |
| 5 | TCDC 3 + βMC 4 | 5 | 84 ± 12[a] |

Values represent mean ± SEM.
n, number of rats.
a: Significantly different from group 1 ($P < 0.05$).
b: Significantly different from control group ($P < 0.05$).

lowing the beginning of the bile salt infusion in the other groups. The LDH value in the control group corresponded to the leakage of LDH into the bile with a physiological transhepatic flux of bile salts. We can observe that: (1) an infusion of 3 μmol $min^{-1}$ $Kg^{-1}$ of βMC or TUDC does not modify the LDH output when compared to controls; (2) an infusion of 3 μmol $min^{-1}$ $Kg^{-1}$ of TCDC drastically increases the biliary leakage of LDH; (3) the co-infusion of TCDC (3 μmol $min^{-1}$ $Kg^{-1}$) with βMC or TUDC at 1.5 μmol $min^{-1}$ $Kg^{-1}$ significantly reduces the LDH leakage when compared with group 1; (4) the co-infusion of TCDC with βMC or TUDC (4 μmol $min^{-1}$ $Kg^{-1}$) restores the LDH output at a level comparable to that of the control group.

### *Estrogen-induced chronic cholestasis: effect of bile acid feeding*

*Table 3* describes the bile flow and the bile salt secretion rate of rats having received either ethinylestradiol (EE) for 6 days, EE plus βMC, or EE plus UDC. To

**Table 3** Bile flow and bile salt outputs in rats submitted to different treatments

| Groups | | n | Bile flow | Bile salt output | C | βMC |
|---|---|---|---|---|---|---|
| | | | *(mL/h/Kg)* | *(μmol/h/Kg)* | | |
| A1 | Control* | 10 | 3.36 ± 0.18 | 103.07 ± 9.21 | 62.16 ± 5.33 | 27.61 ± 4.10 |
| A2 | Control† | 10 | 3.40 ± 0.16 | 98.43 ± 7.49 | 61.78 ± 3.66 | 27.21 ± 2.71 |
| B | EE | 7 | 1.80 ± 0.07[a] | 51.58 ± 1.39[a] | 29.99 ± 1.52[a] | 15.70 ± 1.85[a] |
| C | EE + βMC | 10 | 3.05 ± 0.07[ab] | 103.96 ± 4.71[b] | 41.72 ± 2.64[ab] | 51.28 ± 4.61[ab] |
| D | EE + UDC | 10 | 2.99 ± 0.10[ab] | 101.78 ± 6.22[b] | 40.69 ± 4.49[ab] | 19.07 ± 2.37 |

Controls with (†) and without (*) neomycine.
n, number of rats.
Values represent mean ± SEM. Bile flow and bile salt outputs were obtained from the first 3-hour bile collection.
a: significantly different from group A2 ($P < 0.05$).
b: significantly different from group B ($P < 0.05$).

avoid the partial degradation of βMC into hyodeoxycholic acid by intestinal bacteria, all groups of rats were treated with neomycine 6 days before surgery. Addition of neomycine to rats (group A2) did not modify the bile flow nor the output of the major species of bile salts compared with untreated rats (group A1). Oral administration of taurine favored the exclusive tauroconjugation of biliary bile salts in all groups studied.

Treatment with EE resulted in a large decrease of bile flow. This EE cholestatic effect was significantly corrected by βMC or UDC, but the bile flow did not recover to a normal level. EE administration strongly depressed bile salt output. The output of each bile salt species was decreased by about two fold and the bile salt pool was significantly diminished. In EE-treated rats supplemented with βMC (group C), the biliary output of this bile salt was about two-fold larger than that of control rats. The cholic acid output was significantly increased when compared with group B, without recovering to a normal level. Minor bile salts, chenodeoxycholate, deoxycholate, and ursodeoxycholate were not significantly modified. It is noteworthy that in rats treated with EE plus βMC without addition of neomycine (data not shown on *Table 3*) bile composition was enriched in HDC (up to 12%) at the expense of βMC. In EE-treated rats supplemented with UDC (group D), the infused bile salt represented 35% of total bile salts. UDC feeding improved the cholic acid output but did not significantly modify the biliary secretion of βMC compared with group B. In the two groups treated with bile acids (groups C and D), the bile salt pool was not significantly different from that of control rats.

*Figure 2* shows the regression lines illustrating the relationship between bile flow and bile salt secretion in the four groups. The slopes of these lines were similar, indicating that the bile salt mixtures that flux the liver globally have a similar choleretic power. Extrapolation to zero of the regression lines gave distinct intercepts on the Y axis. The bile salt independent bile flow was significantly reduced by EE compared with controls. In rats treated with EE + βMC or EE + UDC, this fraction of bile water was significantly larger than that of rats receiving exclusively EE. The restoration of this fraction was not complete because it remained significantly lower than that of controls. There was no difference between βMC and UDC treatment.

### *Surface properties of bile salts*

The equilibrium surface tensions (γ) of βMC, UDC, and CDC in 0.15 M $Na^+$ solutions are plotted semilogarithmically against bile salt concentration in *Figure 3*.

For equimolar concentrations of the three bile salts, the γ values were higher for βMC and lower for CDC. The critical micellar concentration (CMC) of each bile salt was estimated from the break points in the surface tension plots. The CMC of CDC was 2 mmol/L, 5.7 mmol/L for UDC, and 7 mmol/L for βMC. As the solution contained a large excess of NaCl, the inter-

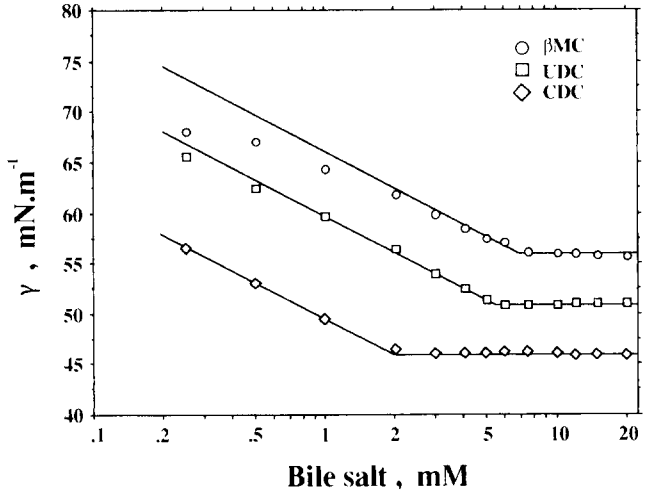


**Figure 3** Surface tension log concentration dependence of solutions of sodium CDC, sodium UDC, and sodium βMC at 24° C, 0.15 M $Na^+$, pH 9.8.

| Individual bile salt outputs | | | | |
|---|---|---|---|---|
| CDC | DC | UDC | HDC | Bile salt pool |
| *(μmol/h/Kg)* | | | | *(μmol/Kg)* |
| 4.14 ± 1.82 | 3.10 ± 0.20 | 1.05 ± 0.12 | 3.02 ± 0.10 | 640.74 ± 26.59 |
| 6.01 ± 2.35 | 2.31 ± 0.72 | 1.02 ± 0.74 | 0 | 645.10 ± 24.06 |
| 3.81 ± 0.45 | 1.38 ± 0.46 | 0.68 ± 0.49 | 0 | 544.12 ± 24.32[a] |
| 8.74 ± 1.08 | 0 | 2.22 ± 1.15 | 0 | 708.00 ± 28.58[b] |
| 6.38 ± 1.22 | 1.92 ± 0.58 | 34.48 ± 2.18[ab] | 0 | 697.86 ± 30.88[b] |

facial areas at surface saturation were calculated from the simplified form of the Gibbs adsorption isotherm equation.

The calculated molecular areas were contained between 80 and 83 Å$^2$ for all bile salts studied. These values indicate that the bile salt molecules are lying flat at the surface.

Above the CMC, the difference between the surface tension of the CDC solutions and the βMC solutions was about 10 *mN m-1*. This difference was still larger (16 *mN m-1*) for solutions whose concentrations were lower than the CMC. Between CDC and UDC solutions, surface tensions only differed by 5 *mN m*-1 above the CMC and by 10 *mN m*-1 below the CMC. So, βMC, which is the less surface active bile salt and which has the highest CMC, appears to be the most hydrophilic among the three bile salts studied.

## Discussion

These studies show that βMC is as efficient as UDC in precluding EE- or CDC-induced cholestasis in the rat. UDC has been shown to have a beneficial effect in various cholestatic liver diseases.[17–21,33] βMC could have a hepatoprotective activity, also.

In chronic cholestasis induced by EE, the decrease in bile flow was due to a large diminution of the endogenous bile salt secretion and a decrease in the bile acid independent flow. Studies by Davis et al.[34] have shown that EE treatment decreased both the rate of bile acid synthesis and the activity of the hepatic microsomal 7α hydroxylase. Triton restored the activity of this enzyme to control levels via a process that did not involve *de novo* protein synthesis. These results suggest that EE and Triton influence the expression of 7α hydroxylase via direct interactions with the microsomal membrane. Moreover, Rosario et al.[35] have clearly shown that EE reduced the activity of $Na^+$ $K^+$ ATPase of the liver sinusoidal membrane. This reduction may be due to the increased ordering and the decreased rotational rate observed in sinusoidal membranes.

Oral administration of βMC or UDC to EE-treated rats significantly improves the cholic acid biliary secretion together with the bile salt independent flow. It is reasonable to assume that βMC or UDC partly restores the activities of both of the membrane-bound enzymes 7 α hydroxylase and $Na^+$ $K^+$ ATPase, and that the reversal of EE effects by hydrophilic bile salts could occur via a modification of the physico-chemical properties of the membranes, such as fluidity.

In the cholestatic model induced by TCDC infusion, only 55% of the dose administered was recovered in the third hour of bile collection. Thus, TCDC accumulated in the liver and led to liver cell injury as witnessed by a large increase in biliary leakage of LDH and severe cholestasis. These disorders reflect an imbalance between hepatocyte uptake and canalicular secretion of TCDC. When βMC was simultaneously administered with TCDC, the quasi totality of both bile salts was recovered in the bile. βMC promoted TCDC secretion and restored the equilibrium between input and output of TCDC. Accordingly, there was neither cholestasis nor stimulation of biliary LDH secretion. Similar results were obtained with TUDC. These observations confirm and extend recent works[16,36,37] about the protective effect of 7β OH bile salts. Schubert and Schmidt[13] have described the disturbances in vesicle membranes induced by different bile salts and underscored the major influence of the binding strength between the bile salt and lecithin. Thus, the non-toxicity of βMC and UDC we observed could reflect their low affinity for lipids in cell membranes. Considering this, we estimated the compared affinity of βMC, UDC, and CDC for a hydrophobic interface from the measurements of surface tensions of bile salts.

In a previous paper,[2] we have shown that the orientation of the OH groups of the CDC and UDC epimers affected the bile salt hydrophilicity. The introduction of a third OH group (6β) leads to a less surface-active molecule. Thus, considering the estimated bile salt concentrations in the hepatocyte (500 μmol/L), we observe that CDC, UDC, and βMC induce surface tension values of 53, 63, and 67 mN $m^{-1}$, respectively; i.e., a surface pressure (decrease in surface tension) equal to 19, 9, and 5 mN $m^{-1}$. It has been shown by Quist et al.[38] that histamine release, from mast cells incubated with bile salts, was dependent on the nature and concentration of the bile salts. Moreover, histamine release occurred in every case when the surface pressure exceeded 15–17 *mN* $m^{-1}$. Surface tension plots in *Figure 3* clearly show that for physiological intrahepatic bile salt concentrations, CDC can generate a surface pressure larger than the 15–17 *mN* $m^{-1}$ threshold. For its part, UDC induces this critical pressure for a concentration of about 2 mmol/L and βMC for 5 mmol/L. From these data, it is conceivable that CDC, due to its larger surface activity, is more damaging to hepatocyte membranes than UDC, and much more so than βMC.

The hydrophilic characteristics of βMC explain its low affinity for lipids. We had previously shown that βMC poorly solubilized lecithin and cholesterol in micellar aggregates.[25,26] The solubility of hydrophilic bile salts in lipids is lower than that of hydrophobic ones. Indeed, the partition coefficient Kp of TUDC between monoolein and water is lower than that of TCDC.[39] On the other hand, Schubert and Schmidt[13] suggested that CDC may form dimers oriented perpendicular to the membrane surface with the hydroxyl groups facing each other. This self-aggregation is less probable for UDC whose hydrophilic and hydrophobic hemispheres are less defined. These two hemispheres are still less pronounced in the βMC molecule which renders its penetration as dimers into the membrane improbable. Therefore, hydrophilic bile salts have no damaging properties for membranes. In an attempt to explain the hepatoprotective effect of βMC and UDC, it may be assumed that these bile salts hampered the insertion of hydrophobic steroids into membranes between the fatty acid chains of phospholipids. Very hydrophilic

bile salts, such as βMC, may adsorb at the membrane/water interface, sterically impeding the penetration of CDC molecules.

In summary, we have demonstrated that oral supplementation with the very hydrophilic bile salt, βMC, could reverse cholestasis induced by hydrophobic steroids as efficiently as UDC. As opposed to UDC, βMC is resistant to human intestinal microflora and could constitute a larger part of the total bile acid pool. The very low surface activity of this hydrophilic steroid should guarantee its innocuity. Taken together, these data suggest that β muricholate should have a beneficial effect in chronic cholestatic liver disease.

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