Bile Acid Sulfonates: Are They Useful?

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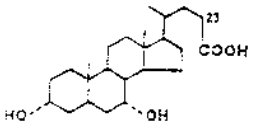
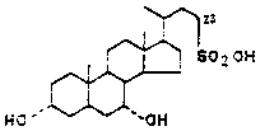
Introduction

Bile acid sulfonates are bile acid analogues in which the carboxylic acid group of a cholanoic acid has been replaced by a sulfonic acid or sulfonate group (R-COOH → R-SO_3H or R-SO_3Na). For example, the sulfonic acid analogue of chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholan-24-oic acid [CDCA]) has the structure 3α,7α-dihydroxy-24-nor-5β-cholane-23-sulfonic acid [CDC-sul] (Fig. 1).

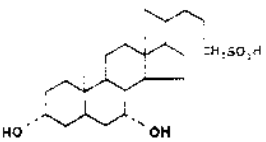


CDCA

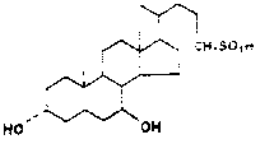


CDC-SUL

In the initial studies of the bile acid sulfonates, the compounds synthesized were 3α,7α-dihydroxy-5β-cholane-24-sulfonate (homoCDC-sul) and 3α,7β-dihydroxy-5β-cholane-24-sulfonate (homoUDC-sul) (Fig. 2) (1). The abbreviations, homo-



homo CDC-SUL



homo UDC-SUL

CDC-sul and homoUDC-sul, were chosen because these compounds are, strictly speaking, analogues of homoCDCA and homoUDCA.
In 1975, during a conference entitled "Frontiers in Inflammatory Bowel Disease", Hofmann discussed ileal function tests (2). He pointed out that tests specifically measuring ileal absorption would require a compound that was totally resistant to bacterial degradation. He suggested the use of bile acid sulfonates but pointed out that such compounds had not been synthesized. In 1982, Mosbach suggested that bile acid sulfonates (for example, "bishomochenodeoxycholic sulfonate" [3α,7α-dihydroxy-25,26-ethyl-homo-5β-cholane-26-sulfonic acid]) should be resistant to degradation by the intestinal flora and might be useful as cholelitholytic drugs (3).

Synthesis of bile acid sulfonates

The chemical synthesis of the sulfonate analogues of homoCDCA and homoUDCA employs the natural bile acids CDCA and UDCA as starting materials (1). The synthesis proceeds in four steps and is described here for the conversion of UDCA to homoUDC-sul:

1) UDCA is reduced to the bile alcohol 5β-cholane-3α,7β,24-triol with aqueous $NaBH_4$ at room temperature (yield 48 %).
2) The triol is converted selectively to the tosyl (24-p-toluenesulfoxy) ester in the presence of p-toluenesulfonyl chloride at 4 °C for 3–7 days (yield 72 %).
3) The 24-tosylate is then treated with sodium iodide in acetone at 40 °C, resulting in the formation of 24-iodo-5β-cholane-3α,7β-diol (yield 93 %).
4) The iodide is treated with sodium sulfite in aqueous ethanol under reflux giving the sulfonate (homoUDC-sul) in 72 % yield.

The overall yield of the four steps listed above was 23 % (30 % for the reaction sequence CDCA → homoCDC-sul [4]). The reaction products were identified by fast atom-bombardment high resolution mass spectrometry, infrared spectrometry (bands at 1050 cm^{-1} and 1190 cm^{-1}) and PMR (the hydrogen atoms of the C-24 methylene group gave a multiplet at 2.74 ppm, indicating the presence of the sulfonate group at the terminal end of the bile acid side chain). Upon TLC with the solvent system, n-butanol-acetic acid-water, 85:10:5, the sulfonic acid analogues, homoCDC-sul and homoUDC-sul, were slightly less polar than CDC-tau and UDC-tau (1).
The synthesis of sulfonate analogues derived from norCA, norCDCA, norUDCA, norDCA, norHDCA, CA, DCA, HDCA and LCA (4) and homoCDCA (5) has been reported.
The metabolism and some biological properties of bile acid sulfonates have been studied in male

golden Syrian hamsters. These investigations have been carried out at the Institute of Pharmaceutical Sciences, University of Hiroshima School of Medicine, in collaboration with the Lipid Research Laboratory, Department of Surgery, Beth Israel Medical Center, New York, NY.

Metabolism of bile acid sulfonates

The first bile acid sulfonate studied was the homoCDCA analogue, homoCDC-sul (3α,7α-dihydroxy-5β-cholane-24-sulfonate) (Fig. 2) (6). In these experiments, emphasis was placed on the site of intestinal absorption. Tritium-labeled homoCDC-sul was injected into either jejunal or ileal loops of bile fistula hamsters and the recovery of radioactivity in bile was measured. ^{14}C-labeled CDCA was injected simultaneously for comparison. When ^{3}H-homoCDC-sul was injected into the ileal loop, 75 % of the administered radioactivity was recovered in the bile as unchanged homoCDC-sul within 4 hr. The recovery of CDCA was slightly greater, about 80 %, and this natural bile acid was secreted into the bile conjugated with glycine and taurine.
Feeding experiments with homoCDC-sul and CDCA showed that about 50 % of the dose was recovered in the feces during a 7-day period. While CDCA was almost entirely converted to LCA by the intestinal bacterial flora, the sulfonate was recovered unchanged. No LCA or homoLC-sul was formed. Intravenous infusion studies were not carried out in these pilot experiments.

In a similar, more comprehensive study, the urso analogues, UDC-sul and homoUDC-sul, were tested in male hamsters (7). After instillation into an ileal loop, the recovery of the sulfonates in fistula bile was 100 % after 3 hr. Following injection into a jejunal loop, the recovery of homoCDC-sul was 50 %, that of CDC-sul 35 %, and that of CDCA 80 % at the end of 3 hr. The two urso sulfonates were not biotransformed during absorption and hepatic transport. CDCA was conjugated quantitatively with glycine and taurine. Upon TLC, homoUDC-sul and UDC-sul have about the same polarity as CDC-tau, and it is not clear at present why homoCDC-sul was absorbed more readily from the jejunum than CDC-sul. More studies on the physical-chemical properties of the sulfonic acid analogues are needed.

Feeding experiments with 10 mg of sulfonates per hamster per day for one week disclosed that there was no weight loss ascribable to administration of the analogues. As expected, CDCA, fed simultaneously, was largely 7-dehydroxylated to LCA during passage through the intestinal tract. In contrast, UDC-sul and homoUDC-sul were recovered unchanged from the feces. Clearly, the bile acid sulfonates studied so far are very resistant to bacterial 7-dehydroxylation. This resistance is presumably ascribable to the lack of a terminal carboxyl group which is a requirement for bacterial 7-dehydroxylation (9).

Additional short-term infusion experiments were carried out with CDC-sul and bishomoCDC-sul. After intravenous infusion, both of these compounds appeared rapidly in the bile (90 % of the infused dose was recovered from fistula bile within 1 hr, a rate similar to that of the natural bile acid CDC-tau). Following intraduodenal injection, about 20 % of the administered radioactivity appeared in bile after about 1 hr; following intraileal administration, 85–90 % of the sulfonates were recovered in the bile within 1 hr. There was no appreciable biotransformation of the sulfonates in liver or intestine; this finding is ascribed to the high polarity of the sulfonate analogues. Like the natural taurine conjugates, the sulfonates are poorly absorbed by passive diffusion in the jejunum, but they exhibit rapid transport across the ileum by virtue of the single negative charge on the highly polar sulfonate group (10).
To evaluate the effect of CDC-sul and bishomoCDC-sul on bile flow, a constant dose (3.0 μmol/min/kg) or increasing doses were infused intravenously into biliary fistula animals (5). Both CDC-sul and bishomoCDC-sul were cholestatic in the hamster: CDC-sul at an infusion rate of 2.0 μmol/min/kg, bishomoCDC-sul at a rate of 0.75 μmol/min/kg. In contrast, withCDC-tau, bile flow increased as long as the bile acid was administered even at an infusion rate of 3.0 μmol/min/kg. HomoCDC-sul was not studied under these conditions.
The reasons for the cholestatic effect of the synthetic sulfonates remains to be elucidated. CDC-tau, which possesses both a sulfonic acid moiety and a peptide bond in the side chain, was not cholestatic at the doses employed. We can speculate that the absence of the peptide bond plays a role as yet unknown in the etiology of the cholestasis. The relatively high hydrophobicity of bishomoCDC-sul (as determined by reversed-phase HPLC) must also be considered. Its relative retention time was quite similar to that of taurolithocholate which is highly cholestatic in the hamster. It will be interesting to determine whether the cholestatic effect of the bile acid sulfonates is related to the length of the side chain with the terminal sulfonate group.

Effect of bile acid sulfonates (homoUDC-sul and UDC-sul) on cholesterol metabolism

The effect of homoUDC-sul and UDC-sul (0.1 % of the diet) on the intestinal absorption of cholesterol was examined in hamsters on diets supplemented with 0.1 % cholesterol (7). As expected, the high cholesterol diet alone produced an increase in serum cholesterol concentration, amounting to about 50 %. Neither the sulfonates nor UDC-tau or UDCA significantly affected cholesterol absorption which was about 60–65 %. Perhaps, as a consequence, serum cholesterol levels were not affected significantly by the administered bile acids.

Effect of bile acid sulfonates on biliary bile acid composition

UDC-sul, homoUDC-sul, UDC-tau and UDCA (0.1 % of the diet) were fed to groups of hamsters for a period of 2 wk (7). UDC-tau and UDCA produced a large increase in the proportion of CDCA in bile (about 60 % of total bile acids); very little biliary UDCA was detected. This phenomenon (the bacterial/hepatic conversion of UDCA to CDCA in the hamster) has been reported previously; its extent seems to depend in part on the source of the hamsters and the diets studied. In the groups fed 0.1 % homoUDC-sul and UDC-sul, these analogues accounted for 24 % and 17 % of total biliary bile acids, respectively. The percentage composition and the G/T ratio of the natural bile acids in gallbladder bile were not affected by feeding the sulfonate analogues. It will be noted in the gallstone prevention experiments described below, that even with a 6-wk feeding period, the percentage of administered bile acid sulfonates did not exceed 33 % (homoCDC-sul), 12 % (homoUDC-sul) or 17 % (homoHDC-sul).

Litholytic potential of bile acid sulfonates

The "cholelitholytic potential" of three bile acid sulfonates, homoCDC-sul, homoUDC-sul and homoHDC-sul, was examined in our hamster model of cholesterol cholelithiasis (8, 11). The corresponding taurine-conjugated bile acids (CDC-tau, UDC-tau and HDC-tau) were tested simultaneously for comparison. The bile acids (0.1 %) were incorporated into the semipurified, nutritionally adequate, lithogenic diet which contained 4 % butterfat and 0.3 % cholesterol. Gallstone prevention was evaluated in a 6-wk feeding experiment. CDC-tau and UDC-tau were relatively ineffective inhibitors of gallstone formation (stone incidence was 14/15 in the lithogenic control group, 10/15 with CDC-tau, and 12/15 with UDC-tau). HomoCDC-sul (8/14), homoUDC-sul (2/12) and homoHDC-sul (4/15) were more effective. HDC-tau (2/13) was the best gallstone inhibitor of the three taurine conjugates studied. HDC-tau, but not homoHDC-sul, inhibited the accumulation of cholesterol in liver and serum to a significant extent (8). At the end of the 6-wk feeding period, the proportion of the sulfonates in biliary bile acids was relatively low (12–32 %); the lithogenic indices of the bile were close to or greater than unity. These results do not provide a clear-cut explanation concerning the mechanism whereby certain bile acid sulfonates prevent the formation of gallstones. One may speculate that large multilamellar vesicles of high cholesterol content can form and remain stable in the presence of the hydrophilic bile acid sulfonates. It is also possible that some or all of the analogues promote the formation of antinucleating agents.

Histopathologic examination revealed that liver damage due to the bile acid analogues was relatively mild; however, portal tract injury in the homoCDC-sul group was manifested by moderate ductular proliferation, mild inflammatory infiltration and fibrosis.

Summary

The recent successful synthesis of bile acid sulfonates has made these compounds available for biological studies. The work completed so far has been done exclusively in rodents (hamster, rat), and it will have to be established whether these compounds can be shown to be safe and effective in higher vertebrates and in man. At the present time, it cannot be decided whether the favorable properties of the bile acid sulfonates (lack of bacterial degradation, participation in the enterohepatic circulation, cholelitholytic properties) outweigh the potential toxic effects (liver damage, cholestasis).

References

1. Kihira, K., Yoshii, M., Okamoto, A., Ikawa, S., Ishii, H., Hoshita, T.:
   Synthesis of new bile salt analogues, sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate and sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate.
   J. Lipid Res. 1990; **31:** 1323–1326.

2. Shorter, R. G., Shepherd, D. A.:
   Frontiers in inflammatory bowel disease.
   Am. J. Dig. Dis. 1975; **20:** 639–670.

3. Mosbach, E. H.:
   Approaches to the development of new cholelitholytic agents.
   In: Bile Acids and Cholesterol in Health and Disease (G. Paumgartner, A. Stiehl and W. Gerok, eds.), pp. 11–20.
   MTP Press, Lancaster, England, 1983.

4. Kihira, K., Mikami, T., Ikawa, S., Okamoto, A., Yoshii, M., Miki, S., Mosbach, E. H., Hoshita, T.:
   Synthesis of sulfonate analogues of bile acids.
   Steroids 1992; **57:** 193–198.

5. Miki, S., Mosbach, E. H., Cohen, B. I., Yoshii, M., Ayyad, N., McSherry, C. K.:
   Sulfonate analogues of chenodeoxycholic acid: metabolism of sodium 3α,7α-dihydroxy-24-homo-5β-cholane-25-sulfonate and sodium 3α,7α-dihydroxy-24-nor-5β-cholane-23-sulfonate in the hamster.
   J. Lipid Res. 1992; **33:** 1629–1637.

6. Kihira, K., Okamoto, A., Ikawa, S., Mikami, T., Yoshii, M., Mosbach, E. H., Hoshita, T.:
Metabolism of sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate in hamsters.
J. Biochem. 1991; **109:** 879–881.

7. Mikami, T., Kihira, K., Ikawa, S., Yoshii, M., Miki, S., Mosbach, E. H., Hoshita, T.:
Metabolism of sulfonate analogues of ursodeoxycholic acid and their effects on biliary bile acid composition in hamsters.
J. Lipid Res. 1993; **34:** 429–435.

8. Cohen, B. I., Miki, S., Mosbach, E. H., Ayyad, N., Stenger, R. J., Mikami, T., Yoshii, M., Kihira, K., Hoshita, T.:
Bile acid sulfonates alter cholesterol gallstone incidence in Sasco hamsters.
Hepatology 1993; **17:** 103–110.

9. Coleman, J. P., White, W. B., Egestad, B., Sjövall, J., Hylemon, P.B.:
Biosynthesis of a novel bile acid nucleotide and mechanism of 7α-dehydroxylation by an intestinal *Eubacterium* species.
J. Biol. Chem. 1987; **262:** 4701–4707.

10. Carey, M. C.:
The enterohepatic circulation.
In: The Liver: Biology and Pathology (I. Arias, H. Popper and D. A. Shafritz, eds.), pp. 429–465.
Raven Press, New York. 1982.

11. Cohen, B. I., Matoba, N., Mosbach, E. H., McSherry, C. K.:
Dietary induction of cholesterol gallstones in hamsters from three different sources.
Lipids 1989; **24:** 151–156.

Abbreviations

TLC	= thin-layer chromatography
PMR	= proton magnetic resonance
CDCA	= chenodeoxycholic acid
UDCA	= ursodeoxycholic acid
HDCA	= hyodeoxycholic acid
CA	= cholic acid
LCA	= lithocholic acid
DCA	= deoxycholic acid
CDC-tau	= chenodeoxycholyl taurine
UDC-tau	= ursodeoxycholyl taurine
HDC-tau	= hyodeoxycholyl taurine
CDC-sul	= 3α,7α-dihydroxy-24-nor-5β-cholane-23-sulfonate
UDC-sul	= 3α,7β-dihydroxy-24-nor-5β-cholane-23-sulfonate
homoCDC-sul	= 3α,7α-dihydroxy-5β-cholane-24-sulfonate
homoUDC-sul	= 3α,7β-dihydroxy-5β-cholane-24-sulfonate
homoHDC-sul	= 3α,6α-dihydroxy-5β-cholane-24-sulfonate
homoLC-sul	= 3α-hydroxy-5β-cholane-24-sulfonate
bishomoCDC-sul	= 3α,7α-dihydroxy-25-homo-5β-cholane-25-sulfonate