A model of in vitro UDP-glucuronosyltransferase inhibition by bile acids predicts possible metabolic disorders

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Abstract

Increased levels of bile acids (BAs) due to the various hepatic diseases could interfere with the metabolism of xenobiotics, such as drugs, and endobiotics including steroid hormones. UDP-glucuronosyltransferases (UGTs) are involved in the conjugation and elimination of many xenobiotics and endogenous compounds. The present study sought to investigate the potential for inhibition of UGT enzymes by BAs. The results showed that tauroliotholic acid (TLCA) exhibited the strongest inhibition toward UGTs, followed by lithocholic acid.

Structure-UGT inhibition relationships of BAs were examined and in vitro-in vivo extrapolation performed by using in vitro inhibition kinetic parameters (K_i) in combination with calculated in vivo levels of TLCA. In conclusion, these studies suggest that disrupted homeostasis of BAs, notably tauroliotholic acid, found in various diseases such as cholestasis, could lead to altered metabolism of xenobiotics and endobiotics through inhibition of UGT enzymes.—Fang, Z-Z., R-R. He, Y-F. Cao, N. Tanaka, C. Jiang, K. W. Krausz, Y. Qi, P-P. Dong, C-Z. Ai, X-Y. Sun, M. Hong, G-B. Ge, F-J. Gonzalez, X-C. Ma, and H-Z. Sun. A model of in vitro UDP-glucuronosyltransferase inhibition by bile acids predicts possible metabolic disorders. J. Lipid Res. 2013. 54: 3334–3344.

Supplementary key words: cholestasis • endobiotics • structure-UDP-glucuronosyltransferase inhibition relationship • xenobiotics

Bile acids (BAs), the major end-products of cholesterol metabolism, play a key role in the solubilization, absorption, and transportation of dietary lipids in the intestine (1). In humans, the most abundant BAs contain cholic acid (CA), chenodeoxycholic acid, deoxycholic acid, lithocholic acid (LCA), and ursodeoxycholic acid (UDCA) (2). In the livers of mice and rats, chenodeoxycholic acid can be further converted into α- and β-muricholic acid (3). Under normal conditions, BAs exist at low concentrations in the peripheral circulation. Various hepatic and intestinal diseases can significantly affect the total BA levels and disrupt the BA homeostasis. For example, cholestasis can disturb the bile secretory process, leading to the accumulation of toxic BAs in the liver (4).

Abbreviations: AUC, area under the curve; AZT, azidothymidine; BA, bile acid; CA, cholic acid; CDCA, sodium chenodeoxycholate; DCA, sodium deoxycholate; DHCA, dehydrocholic acid; FXR, farnesoid X receptor; GCA, glycocholic acid hydrate; HDCA, hyodeoxycholic acid; HLM, human liver microsome; I.D., inner diameter; LCA, lithocholic acid; 4-MU, 4-methylumbelliferone; 4-MUG, 4-methylumbelliferone-β-d-glucuronide; PPAR, peroxisome proliferator-activated receptor; TCDCA, taurochenodeoxycholate; TDCA, sodium taurodeoxycholate hydrate; TFP, trifluoperazine; TLCA, taurilotholic acid; TUDCA, sodium tauroursodeoxycholate; UDCA, ursodeoxycholic acid; UDPGA, uridine-5′-diphosphoglucuronic acid trisodium salt; UGT, UDP-glucuronosyltransferase.
Human UDP-glucuronosyltransferases (UGTs), endogenous membrane proteins located in endoplasmic reticulum, can conjugate various endogenous and exogenous compounds (5). BAs are substrates of UGTs, and UGT-catalyzed glucuronidation of BAs facilitates their hydrophilicity, increase their elimination from the liver, and decrease their potential for hepatic toxicity (6–8). For example, UGT1A3 can catalyze C24-glucuronidation of chenodeoxycholic acid, LCA, and hyodeoxycholic acid (HDCA) through forming acyl glucuronides (9). UGT2B4 is involved in the 6-glucuronidation of BAs such as HDCA (10). UGT2B7 is the UGT isoform involved in the 3- and 6-glucuronidation of primary, secondary, and hydroxylated BA (9).

As substrates for UGT enzymes, BAs might also exhibit inhibitory effects toward various UGT forms. Previous studies revealed that some BA components exhibit inhibitory effects toward 4-methylumbelliferone (4-MU) glucuronidation, including LCA, dehydrocholic acid (DHCA), and sodium chenodeoxycholate (CDCA) (11). However, the complete inhibition profile of BAs toward different UGT forms remains unclear. Additionally, the BA structure-UGT inhibition relationships are important to determine what structural basis of BAs affects their inhibition potentials toward different UGTs. Therefore, the present study evaluated the inhibition potential of BAs toward UGT forms in liver and intestine. As previously described (12), recombinant UGT isoform-catalyzed 4-MU glucuronidation reactions were used to evaluate the inhibition potential of BAs toward UGTs except for UGT1A4, and where trifluoperazine (TFP) glucuronidation was performed as the standard reaction to evaluate the inhibition potential of BAs toward UGT1A4 activity.

![Image of Bile Acids](http://www.jlr.org/content/suppl/2013/10/10/jlr.M040519.DC1)

**Figure 1.** The structure of BAs used in this study.

**Table 1. Initial screening of BAs (100 μM) toward various important UGT forms**

<table>
<thead>
<tr>
<th>Bile Acids</th>
<th>UGT1A1</th>
<th>UGT1A3</th>
<th>UGT1A4</th>
<th>UGT1A6</th>
<th>UGT1A7</th>
<th>UGT1A8</th>
<th>UGT1A9</th>
<th>UGT1A10</th>
<th>UGT2B7</th>
<th>UGT2B15</th>
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<tbody>
<tr>
<td>HDCA</td>
<td>44.6 ± 0.4***</td>
<td>26.1 ± 6.8*</td>
<td>13.7 ± 0.5**</td>
<td>95.4 ± 2.1</td>
<td>47.8 ± 0.5***</td>
<td>84.0 ± 6.1*</td>
<td>50.4 ± 4.3*</td>
<td>52.5 ± 0.9***</td>
<td>9.5 ± 2.0**</td>
<td>34.6 ± 0.4***</td>
</tr>
<tr>
<td>LCA</td>
<td>21.1 ± 8.0***</td>
<td>16.4 ± 0.5*</td>
<td>0.0 ± 0.0***</td>
<td>19.9 ± 1.3**</td>
<td>30.0 ± 2.2**</td>
<td>64.3 ± 7.1**</td>
<td>65.2 ± 17.4</td>
<td>25.0 ± 0.5***</td>
<td>15.7 ± 1.9**</td>
<td>0.3 ± 0.0***</td>
</tr>
<tr>
<td>TCDCA</td>
<td>17.5 ± 6.7***</td>
<td>21.6 ± 1.3*</td>
<td>27.8 ± 2.1**</td>
<td>84.6 ± 1.6*</td>
<td>52.7 ± 3.5***</td>
<td>59.1 ± 20.0*</td>
<td>75.3 ± 1.1*</td>
<td>41.0 ± 8.2**</td>
<td>28.1 ± 2.0**</td>
<td>28.4 ± 1.1***</td>
</tr>
<tr>
<td>Taurocholic acid sodium</td>
<td>62.7 ± 0.1**</td>
<td>66.7 ± 17.3</td>
<td>73.8 ± 1.4</td>
<td>165.8 ± 0.4</td>
<td>94.1 ± 2.5</td>
<td>75.5 ± 8.1*</td>
<td>94.7 ± 6.2</td>
<td>71.9 ± 0.5**</td>
<td>69.3 ± 7.7*</td>
<td>70.2 ± 3.9*</td>
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<tr>
<td>salt hydrate</td>
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<tr>
<td>CDCA</td>
<td>37.7 ± 8.4***</td>
<td>47.2 ± 8.0</td>
<td>20.2 ± 1.4**</td>
<td>92.9 ± 2.6</td>
<td>47.4 ± 12.7**</td>
<td>29.2 ± 3.1***</td>
<td>74.0 ± 1.2*</td>
<td>39.9 ± 2.5**</td>
<td>15.4 ± 2.1**</td>
<td>19.8 ± 1.0***</td>
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<td>TLCA</td>
<td>5.4 ± 0.0***</td>
<td>3.4 ± 0.4*</td>
<td>0.0 ± 0.0***</td>
<td>52.4 ± 0.2**</td>
<td>9.1 ± 8.8**</td>
<td>24.1 ± 3.5***</td>
<td>54.4 ± 7.1*</td>
<td>14.8 ± 6.8**</td>
<td>1.7 ± 0.6**</td>
<td>5.3 ± 0.0***</td>
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<td>UDCA</td>
<td>27.4 ± 0.4*</td>
<td>92.8 ± 49.8</td>
<td>25.5 ± 0.8**</td>
<td>85.0 ± 0.9*</td>
<td>45.7 ± 9.6**</td>
<td>29.8 ± 3.8***</td>
<td>54.5 ± 5.8*</td>
<td>57.6 ± 1.4***</td>
<td>46.2 ± 4.9*</td>
<td>54.1 ± 1.2***</td>
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<tr>
<td>CA</td>
<td>61.9 ± 0.2**</td>
<td>135.0 ± 18.0</td>
<td>73.0 ± 1.1</td>
<td>100.7 ± 1.0</td>
<td>103.4 ± 2.8</td>
<td>86.1 ± 3.2**</td>
<td>89.0 ± 5.7</td>
<td>74.4 ± 0.5**</td>
<td>83.2 ± 7.9</td>
<td>72.1 ± 0.2**</td>
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<td>DHCA</td>
<td>76.4 ± 13.3</td>
<td>117.5 ± 30.2</td>
<td>80.7 ± 25.3</td>
<td>95.0 ± 0.7</td>
<td>80.9 ± 1.7**</td>
<td>87.1 ± 16.0</td>
<td>85.0 ± 3.5</td>
<td>90.5 ± 2.9*</td>
<td>81.0 ± 5.1</td>
<td>77.7 ± 1.1*</td>
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<td>DCA</td>
<td>33.9 ± 2.3***</td>
<td>57.8 ± 11.8</td>
<td>45.8 ± 21.8*</td>
<td>90.3 ± 2.5</td>
<td>68.0 ± 3.7**</td>
<td>88.7 ± 32.5</td>
<td>67.5 ± 2.6*</td>
<td>39.1 ± 1.5***</td>
<td>45.8 ± 2.2*</td>
<td>35.4 ± 1.0***</td>
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<tr>
<td>TUDCA</td>
<td>10.6 ± 0.1***</td>
<td>49.1 ± 8.5</td>
<td>30.8 ± 4.6**</td>
<td>76.0 ± 2.4*</td>
<td>48.8 ± 11.1**</td>
<td>59.3 ± 0.6***</td>
<td>82.9 ± 9.1</td>
<td>38.2 ± 13.2**</td>
<td>26.1 ± 2.3**</td>
<td>25.3 ± 0.7***</td>
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<tr>
<td>TDCA</td>
<td>34.1 ± 0.1**</td>
<td>61.1 ± 8.8</td>
<td>41.1 ± 13.3</td>
<td>93.4 ± 4.1</td>
<td>60.4 ± 2.5**</td>
<td>74.7 ± 11.3</td>
<td>85.0 ± 0.5</td>
<td>50.8 ± 1.0**</td>
<td>33.4 ± 2.6*</td>
<td>40.7 ± 1.9***</td>
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<td>GCA</td>
<td>65.4 ± 4.5**</td>
<td>143.2 ± 44.3</td>
<td>54.5 ± 10.3</td>
<td>91.6 ± 2.9</td>
<td>82.0 ± 15.0</td>
<td>80.9 ± 8.7</td>
<td>94.0 ± 4.8</td>
<td>75.7 ± 3.8**</td>
<td>48.4 ± 3.5*</td>
<td>65.4 ± 1.5***</td>
</tr>
</tbody>
</table>

The values shown are the residual activity, which was calculated using the following equation: % residual activity = (the activity at 100 μM BAs/the control activity at 0 μM BAs) × 100%. *P < 0.05, **P < 0.01, ***P < 0.001, compared with control activity.
Materials and Methods

Materials

4-MU, 4-methylumbelliferone-β-D-glucuronide (4-MUG), Tris-HCl, 7-hydroxycoumarin, azidothymidine (AZT) (purity ≥98%), TFP (purity ≥99%), estradiol (purity ≥98%), and uridine-5′-diphosphoglucuronic acid trisodium salt (UDPGA) were purchased from Sigma-Aldrich (St. Louis, MO). Recombinant human UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B7, and UGT2B15) expressed in baculovirus-infected insect cells were obtained from BD Gentest Corp. (Woburn, MA). HDCA, LCA, sodium taurochenodeoxycholate (TCDCA), taurocholic acid sodium salt hydrate, CDCA, tauroliothocholic acid (TLCA), UDCA, CA, DHCA, sodium deoxycholate (DCA), sodium taursodeoxycholate (TUDCA), sodium taurodeoxycholate hydrate (TDCA), and glycocholic acid hydrate (GCA) were obtained from Sigma-Aldrich. The purity of these compounds was above 95%. All other reagents were of HPLC grade or of the highest grade commercially available.

Initial screening of BAs’ inhibition toward the activity of recombinant UGTs

The inhibition capability of BAs toward all the UGT forms, except UGT1A4, was evaluated using recombinant UGT-catalyzed 4-MU glucuronidation as the probe reaction, as previously described (12, 13). The incubation system (total volume 200 μl) contained recombinant UGTs, 5 mM UDPGA, 5 mM MgCl2, 50 mM Tris-HCl (pH 7.4), and 4-MU in the absence or presence of different concentrations of various BAs. The incubation time used and protein concentration were previously determined to ensure the reaction rate within the linear range. The 4-MU concentration was equal to known Kₘ or S₅₀ values for each UGT form. The concentrations of 4-MU and recombinant UGTs, and incubation time are given in supplementary Table I. The incubation reaction was initiated through addition of UDPGA to the mixture after a 5 min preincubation at 37°C. The reactions were quenched by adding 100 μl acetonitrile with 7-hydroxycoumarin (100 μM) as internal standard. The mixture was centrifuged at 20,000 g for 10 min, and an aliquot of supernatant was transferred to an auto-injector vial for HPLC analysis. The HPLC
Inhibition of UGTs by bile acids

TLCA's inhibition toward hepatocyte UGT-catalyzed 4-MU glucuronidation

Primary hepatocytes were isolated from C57BL/6NCr mice and cultured as previously described (15). 4-MU (50 μM) and TLCA (50 nM) were added to the medium. After a 1 h incubation at 37°C, the medium and cells were isolated. Methanol (v/v) 1:1 and 5 μM chlorpropamide as an internal standard were added to the medium, and 1 ml methanol with 5 μM chlorpropamide as an internal standard were added to extract the compounds in the cells. After centrifugation at 20,000 g for 10 min, the aliquot of supernatant was determined to detect the formation of 4-MUG.

Due to the low catalytic activity of UGT1A4 toward 4-MU glucuronidation, the UGT1A4-catalyzed TFP glucuronidation was performed to evaluate the inhibition potential of BAs toward UGT1A4 activity. TFP (40 μM, near its \(K_m\) value), was incubated with recombinant UGT1A4 (0.1 mg/ml) at 37°C for 20 min in the absence or presence of BAs (14).

TLCA's inhibition toward hepatocyte UGT-catalyzed 4-MU glucuronidation

Primary hepatocytes were isolated from C57BL/6NCr mice and cultured as previously described (15). 4-MU (50 μM) and TLCA (50 nM) were added to the medium. After a 1 h incubation at 37°C, the medium and cells were isolated. Methanol (v/v) 1:1 and 5 μM chlorpropamide as an internal standard were added to the medium, and 1 ml methanol with 5 μM chlorpropamide as an internal standard were added to extract the compounds in the cells. After centrifugation at 20,000 g for 10 min, the aliquot of supernatant was determined to detect the formation of 4-MUG.

BAs' inhibition toward human liver microsome-catalyzed azidothymidine and estradiol glucuronidation

Twenty-five donor pooled human liver microsomes (HLMs) were purchased from Research Institute for Liver Diseases (RILD, Shanghai, China). For azidothymidine (AZT) glucuronidation, the typical incubation system (total volume 200 μl) contained 0.5 mg/ml HLMs, 5 mM UDPGA, 5 mM MgCl₂, 50 mM Tris-HCl (pH 7.4), 50 μg/mg protein alamethicin, and AZT (concentration...
is corresponding to the $K_i$ value. The incubation time was 30 min.

After centrifugation at 20,000 g for 10 min, aliquots of the supernatants were analyzed by HPLC (Shimadzu, Kyoto, Japan), equipped with a SCL-10A system controller, two LC-10AT pumps, a SIL-10A auto sampler, and a SPD-10AVP UV detector. A C-18 column (250 mm × 4.6 mm I.D., 5 μm, Kromasil) was used to separate AZT and its glucuronide. The mobile phase was acetonitrile (A) and 0.2% formic acid (B) at a flow rate of 1.0 ml/min, with an isocratic: 0–25 min 90% B. The detector wavelength was set at 260 nm. Because there was no standard for the AZT glucuronide, a standard curve of AZT was used to quantify glucuronide formation.

 Estradiol (10 μM) was incubated with HLMs for 20 min, with the final protein concentrations of 0.25 mg/ml. Estradiol glucuronidation samples were analyzed on the Ultra Fast Liquid Chromatography (UFLC) system. A Shim-pack XR-ODS (50.0 mm × 2.0 mm I.D., 2.2 μm, Shimadzu) analytical column with an ODS guard column (5 mm × 2.0 mm I.D., 2.2 μm, Shimadzu) was used and kept at 40°C. The mobile phase consisted of acetonitrile (A) and 0.2% formic acid (B) at a flow rate of 0.3 ml/min, with a gradient: 0–9 min, 90% B–30% B; 9–12.5 min, 5% B; 12.5–16 min, balance to 90% B. The detector wavelength was set at 250 nm. A standard curve of the estradiol-3-O-glucuronide was used to quantify glucuronide formation.

**In vitro-in vivo extrapolation**

The reaction velocity was determined at different concentrations of substrates and inhibitors. The durations in the inhibition kinetic study were the same as the initial screening study. Dixon and Lineweaver-Burk plots were used for determination of inhibition kinetic type. The second plot of slopes from the Lineweaver-Burk plot versus inhibitor concentrations was used to calculate the $K_i$ value. The in vivo inhibition magnitude is affected by both in vitro inhibition kinetic parameters ($K_i$) and in vivo concentrations of inhibitors. Therefore, the following equation was employed to predict in vivo situation.

$$\frac{AUC_{i}}{AUC} = 1 + \frac{[I]_{\text{m,o}}}{K_i}$$

The terms are defined as follows: $AUC_i/AUC$ is the predicted ratio of in vivo exposure of xenobiotics or endogenous compounds in control and disease situations, $K_i$ is the reversible inhibition constant, and $[I]_{\text{m,o}}$ is the in vivo concentration of BAs.

**RESULTS**

**Structure-inhibition relationship of BAs toward UGT isoforms**

The structures of tested BAs are listed in Fig. 1, and the inhibitory capabilities of these BAs toward different UGT forms is given in Table 1. Among the tested UGT enzymes, the activity of UGT1A6, UGT1A8, and UGT1A9 were not affected by various BAs. Among the tested BAs at 100 μM, TCDCA, taurocholic acid sodium salt hydrate, CDCA, UDCA, CA, DHCA, DCA, TUDCA, TDCA, and GCA exhibited
no or weak inhibition toward all the tested UGT forms, with inhibition magnitudes less than 90%. HDCA exerted 90.5% inhibition toward UGT2B7-catalyzed 4-MU glucuronidation, and low inhibition toward other UGTs. LCA exhibited the strongest inhibition (more than 90%) toward UGT1A4 and UGT2B15. TLCA showed strong inhibition toward most of UGT forms, including UGT1A1 (94.6%), UGT1A3 (96.6%), UGT1A4 (100%), UGT1A7 (90.9%), UGT2B7 (98.3%), and UGT2B15 (94.7%).

The structure-UGT inhibition relationship can be observed using the combination of the BAs’ structures (Fig. 1) and the UGT inhibition results (Table 1). The substitution of hydrogen with hydroxyl group at the R1, R3, R4, R5 sites significantly weakens the inhibition toward most UGT forms. In comparison with the inhibition magnitude of LCA and TLCA toward UGT2B7, a conclusion can be drawn that the taurine conjugation of LCA can strengthen the inhibition potential toward UGT2B7.

To demonstrate the physiological significance of these observations, TLCA (50 nM) was added to the medium to investigate whether it could inhibit hepatocyte UGT-catalyzed 4-MU glucuronidation activity. The results showed significant inhibition of TLCA toward hepatocyte UGT-catalyzed 4-MU glucuronidation (supplementary Fig. 1).

**Different inhibition type and kinetic parameters of TLCA toward UGT forms**

Due to the potent inhibition of TLCA toward many UGT forms, the inhibition kinetic type and parameters (Ki) were further investigated. Both Dixon and Lineweaver-Burk plots were employed to evaluate the inhibition type, and the second plot using the slopes obtained from Lineweaver-Burk plot versus the concentrations of TLCA was used to calculate the inhibition kinetic parameters (Ki). If the intersection point was located in the vertical axis and the second quadrant in the Lineweaver-Burk plot and Dixon plot respectively, the inhibition type was classified as competitive inhibition. If the intersection point was located in horizontal axis in both Dixon and Lineweaver-Burk plots, the inhibition type was classified as noncompetitive inhibition. The results show that TLCA competitively inhibited UGT1A1 (Fig. 2B, C),
Prediction of in vivo metabolic disorders in humans

Due to the strongest inhibition potential of TLCA toward different UGT enzymes, changes in in vivo metabolism could be predicted based on the concentration of TLCA. The concentration of TLCA in serum obtained from mice with intrahepatic cholestasis of pregnancy was reported to be 0.3 μM (16). Using this concentration in combination with the Kᵢ values for the inhibition of TLCA toward 4-MU glucuronidation, the AUCᵢ/AUC values were calculated to be 1.1, 2, 11, 1.02, 1.02, 4, and 1.1 for UGT1A1, UGT1A3, UGT1A4, UGT1A7, UGT1A10, UGT2B7, and UGT2B15, respectively. In humans, the TLCA concentration was reported to never exceed 25 nM (17). Using this maximum value, the AUCᵢ/AUC values were calculated to be 1.01, 1.08, 1.83, 1.002, 1.002, 1.25, and 1.008 for UGT1A1, UGT1A3, UGT1A4, UGT1A7, UGT1A10, UGT2B7, and UGT2B15, respectively.
DISCUSSION

The inhibitory capability of BAs toward UGTs is difficult to study using conventional animal models due to the complexity of factors influencing activity in vivo. For example, the role of BA in the activation of nuclear receptors might interfere with the evaluation of BA inhibition toward UGT enzymes. The pregnane X receptor was found to be activated by LCA (18, 19). LCA can also activate the vitamin D receptor (20). CDCA, CA, GCA, and GCDCA were reported to be agonists of farnesoid X receptor (FXR) (21–23). Tauro-β-muricholic acid in mice was reported to be a naturally occurring FXR antagonist (24). All affected nuclear receptors regulate the expression and activity of different genes encoding UGT enzymes. For example, BAs can induce the activity of UGT2B4 via activation of FXR (25). The UGT2B4 promoter also contains a peroxisome proliferator-activated receptor (PPAR)α response element, and can be activated by PPARα agonist fenofibrate (26). The activity of UGT2B7 can be inhibited by hydrophobic BAs via a negative FXR response element located in the UGT2B7 promoter (27). FXR activation can also regulate the expression of UGT1A3 (28). To avoid these complex factors, a relatively simple in vitro UGT enzyme incubation system was utilized in the present study.

The detailed metabolic inhibition profile of BAs toward important UGT isoforms was clarified in the present study, and TLCA was demonstrated to be the strongest inhibitor toward most of UGT isoforms, followed by LCA, which is consistent with the reports in which LCA and TLCA were reported to be able to significantly induce the liver damage (29, 30). LCA can be metabolized through UGT isoform-catalyzed glucuronidation elimination, so, the wide inhibition of LCA toward UGT isoforms was observed in the present study. TLCA has similar structure with LCA, and can enter the activity cavity. However, TLCA cannot be metabolized through glucuronidation by our present study (data not shown). Therefore, TLCA exhibited stronger inhibition than LCA. CA and GCA, at 100 μM, were found to increase the activity of UGT1A3 by 35 and 45%, respectively. Given that induced expression of UGT1A3 through nuclear receptors does not occur in the present in vitro system, the allosteric activation of CA and GCA toward UGT1A3 might be the potential reason. However, the detailed mechanism needs to be further investigated.

According to the predicted values of AUC_i/AUC, TLCA exhibited the strongest inhibition toward UGT1A4-catalyzed metabolic reaction. UGT1A4 is widely accepted to be
The accumulation of BAs was reported to potentiate colon carcinogenesis (32). Low activity of UGT1A7 and UGT1A10 was also strongly correlated with the risk of colon cancer (33, 34). Inhibition of UGT1A7 and UGT1A10 by TLCA could provide a possible link to the correlation of increased BAs and high risk of colon cancer. In the present study, two concentrations of TLCA were used to predict the in vivo situation due to the relatively few reports on the serum concentrations of TLCA. Information on the serum concentrations of TLCA in various diseases might reveal a better understanding of disease risk.

In conclusion, the present study details inhibition profiles of BAs toward UGT enzymes, and the BA structure-UGT inhibition relationships. In vivo risk was predicted based on the inhibition of TLCA toward UGTs due to the strongest inhibition of TLCA toward different forms of UGT. However, the influence of other BAs toward UGTs should not be neglected. It should also be noted that the present results should be considered in the context of the complex environment in vivo and in cells, such as the influence of BAs on nuclear receptors as summarized in Fig. 9.
The disrupted homeostasis of BAs due to diseases (e.g., cholestasis) could inhibit UGT-catalyzed metabolism resulting in altered metabolism of xenobiotics and endogenous compounds.

REFERENCES


