

Hepatic and extrahepatic glucuronidation of bile acids in man. Characterization of bile acid uridine 5'-diphosphate-glucuronosyltransferase in hepatic, renal, and intestinal microsomes.

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Research Article

Microsomal UDP-glucuronosyltransferase activity toward the bile acids (chenodeoxycholic, deoxycholic, ursodeoxycholic, lithocholic, and glycolithocholic) has been detected in human specimens of liver, kidney, and intestinal mucosa. The characteristics of hepatic and extrahepatic UDP-glucuronosyltransferase activities toward these bile acids were compared with respect to kinetic parameters and other catalytic properties. Whereas no organ-specific differences in the affinities of individual bile acids to hepatic and extrahepatic UDP-glucuronosyltransferases were observed, the individual bile acids showed reaction rates in liver that were about twice the rates estimated in kidney and about twice to three times the rates observed in duodenal mucosa. In intestinal mucosa the rate of chenodeoxycholic acid glucuronidation exhibited a progressive decrease from duodenum to colon, where it was 30% of the duodenal level. Comparison of the glucuronidation rates that were estimated with different bile acids in hepatic or extrahepatic tissues showed that for each organ a bile acid structure-activity relationship existed, with highest activity observed for lithocholic and ursodeoxycholic acids, which was about twofold higher compared with chenodeoxycholic or deoxycholic acids. Lowest activity was estimated for glycolithocholic acid. UDP-glucuronosyltransferase activity toward chenodeoxycholic acid was studied in biopsy specimens of liver that were obtained from a large group of patients with the following liver diseases: liver cirrhosis, liver fibrosis, granulomatous hepatitis, fatty liver hepatitis, and fatty liver. A significant decrease in enzyme activity [...]

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Hepatic and Extrahepatic Glucuronidation of Bile Acids in Man

Characterization of Bile Acid Uridine 5'-Diphosphate-Glucuronosyltransferase in Hepatic, Renal, and Intestinal Microsomes

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Abstract. Microsomal UDP-glucuronosyltransferase activity toward the bile acids (chenodeoxycholic, deoxycholic, ursodeoxycholic, lithocholic, and glycolithocholic) has been detected in human specimens of liver, kidney, and intestinal mucosa. The characteristics of hepatic and extrahepatic UDP-glucuronosyltransferase activities toward these bile acids were compared with respect to kinetic parameters and other catalytic properties. Whereas no organ-specific differences in the affinities of individual bile acids to hepatic and extrahepatic UDP-glucuronosyltransferases were observed, the individual bile acids showed reaction rates in liver that were about twice the rates estimated in kidney and about twice to three times the rates observed in duodenal mucosa. In intestinal mucosa the rate of chenodeoxycholic acid glucuronidation exhibited a progressive decrease from duodenum to colon, where it was 30% of the duodenal level.

Comparison of the glucuronidation rates that were estimated with different bile acids in hepatic or extrahepatic tissues showed that for each organ a bile acid structure-activity relationship existed, with highest activity observed for lithocholic and ursodeoxycholic acids, which was about twofold higher compared with chenodeoxycholic or deoxycholic acids. Lowest activity was estimated for glycolithocholic acid. UDP-glucuronosyltransferase activity toward chenodeoxycholic acid was studied in biopsy specimens of liver that were obtained from a large group of patients with the following liver diseases: liver

cirrhosis, liver fibrosis, granulomatous hepatitis, fatty liver hepatitis, and fatty liver. A significant decrease in enzyme activity was observed in patients with liver cirrhosis and in patients with granulomatous hepatitis compared with patients without liver disease.

Introduction

Bile acid glucuronides have been shown to be present not only in urine of patients with hepatobiliary disease (1-3), but also in urine of healthy subjects amounting to 12-36% of the total bile acid excretion in urine (4). Therefore, in addition to sulphate esterification (5), conjugation of bile acids with glucuronic acid may serve as a protective mechanism against accumulation of potentially toxic bile acids (6-9). However, little is known about the UDP-glucuronosyltransferase(s) involved in the glucuronidation of bile acids in man. Only some properties of the enzyme(s) from human liver have been described in previous reports (10, 11). Enzyme activity in liver disease has not been studied. Several extrahepatic tissues such as adrenal glands (12), intestine (12-14), or kidney (12, 13, 15) of various species or human cultured lymphocytes (16) have been shown to be capable of carrying out the glucuronidation of various foreign and endogenous compounds. However, whether or not glucuronidation of bile acids also occurs in extrahepatic tissues of man is not known.

The present paper shows that in addition to the liver (10, 11), extrahepatic tissues such as the kidney and small intestinal mucosa are capable of glucuronidation of bile acids in man. Properties of hepatic and extrahepatic UDP-glucuronosyltransferase(s) that catalyze the glucuronidation of bile acids are compared. Furthermore, results on hepatic bile acid conjugating activity, which were estimated from a large group of patients with a variety of liver diseases, are described.

Methods

Human tissue specimens. Liver tissue was obtained either at laparotomy or laparoscopy or after percutaneous needle biopsy of the liver with a Menghini needle. On the basis of histological findings of liver biopsy specimens and clinical assessment, the patients were classified into those

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with liver cirrhosis, liver fibrosis, granulomatous hepatitis, fatty liver hepatitis, fatty liver, and normal liver. Patients that comprised the group with normal liver histology were either undergoing laparotomy or laparoscopy for staging of Hodgkin's disease or were investigated for possible liver disease. Intestinal biopsy specimens were obtained from surgical patients who had undergone either Billroth II gastrectomy due to gastric ulcer (four cases) or gastric carcinoma (five cases), intestinal resection due to Crohn's disease (nine cases) or resection of the ileum after traumatic damage of the small intestine due to a traffic accident (one case), or adenocarcinoma of the colon (five cases). Intestinal samples which exhibited macroscopic abnormality were excluded. Kidney samples were obtained from five males who were nephrectomized for renal cancer.

Only apparently normal tissue was used. Adrenal tissue was obtained either from a 40-yr-old female operated on for adrenal hyperplasia or from a 20-yr-old male with fatal brain injury of alcoholic origin in connection with removal of kidneys for transplantation. Because of the known enzyme-inducing properties of barbiturates and of other drugs (17), patients in whom there was a history of recent drug ingestion have been excluded. Sufficient material of all human specimens was ensured for histologic examination. Gut segments were rinsed in ice-cold saline solution immediately after the resection and were stepwise frozen first at -20°C for 24 h and then at -70°C for at least 24 h, as described by Hoensch et al. (18). Liver and kidney specimens were immediately frozen at -20°C . The activity of UDP-glucuronosyltransferase toward bile acids was found to be stable for at least 4 wk under these conditions.

Materials. Nonlabeled bile acids were obtained from Steraloids, Inc., Wilton, NH. $[24\text{-}^{14}\text{C}]$ Chenodeoxycholic acid (50 mCi/mmol), $[^3\text{H}(\text{G})]$ -glycolithocholic acid (17 Ci/mmol), and $[11,12\text{-}^3\text{H}(\text{N})]$ ursodeoxycholic acid (37 Ci/mmol) were purchased from New England Nuclear, Dreieich, Federal Republic of Germany. $[24\text{-}^{14}\text{C}]$ Deoxycholic acid (52 mCi/mmol) and $[24\text{-}^{14}\text{C}]$ lithocholic acid (55 mCi/mmol) were obtained from Amersham Buchler GmbH & Co. KG, Braunschweig, Federal Republic of Germany. The purity of the radioactive bile acids was checked by thin-layer chromatography in two different systems and was found to be $>99\%$, except for glycolithocholic acid, which was purified with silica gel 60 thin-layer plates using trimethylpentane/ethyl acetate/acetic acid (10:10:2, v/v) as solvent (19). 3α -hydroxysteroid dehydrogenase (Sterognost- 3α Flu) was obtained from Nyegaard & Co. A/S, Oslo, Norway.

Preparation of tissue homogenates and microsomes. Human liver and kidney homogenates were prepared as described in a previous report (10). Homogenates of intestinal mucosa were prepared after stepwise thawing of frozen tissues (-70°C), first at -20°C for 12 h and then at 4°C for 5 min, as described by Hoensch et al. (18). As shown by Hoensch et al. (18), this procedure resulted in thawing only of the villous cell region of the mucosa, which was removed from the tissue by gentle scraping with a metal spatula. After homogenization, as described for liver tissues (10), the intestinal homogenates were sonicated according to Hoensch et al. (18) for 25 s at 35 W (Branson B-12 sonifier, Branson Sonic Power Co., Stamford, CT). Microsomes were prepared from liver, kidney, and intestinal homogenates, as described for rat liver microsomes in previous reports (20, 21), with the modification that the microsomes were washed once with 0.25 M sucrose and suspended in 0.25 M sucrose.

Analytical procedures. For the determination of UDP-glucuronosyltransferase activity toward bile acids, $\sim 100\ \mu\text{g}$ of hepatic, renal, or small intestinal microsomal protein were incubated for 30 min at 37°C in a reaction mixture which contained 9 mM UDP-glucuronic acid, 5 mM MgCl_2 , 5 mM saccharolactone, 0.1 M imidazole/HCl (pH 6.7) which contained one of the following labeled bile acids: 0.5 mM $[^{14}\text{C}]$ chenodeoxycholic acid (0.8 $\mu\text{Ci}/\mu\text{mol}$), 0.2 mM $[^{14}\text{C}]$ deoxycholic acid (2 $\mu\text{Ci}/\mu\text{mol}$), 0.05 mM $[^{14}\text{C}]$ lithocholic acid (8 $\mu\text{Ci}/\mu\text{mol}$), 0.1

mM $[^3\text{H}]$ glycolithocholic acid (3.5 $\mu\text{Ci}/\mu\text{mol}$), or 0.5 mM $[^3\text{H}]$ ursodeoxycholic acid (0.7 $\mu\text{Ci}/\mu\text{mol}$). In the presence of lithocholic acid, the reaction mixtures contained 1% (v/v) dimethyl sulphoxide and 2% (v/v) propylene glycol, used as described elsewhere (22), for dissolution of nonlabeled lithocholic acid and $[^{14}\text{C}]$ lithocholic acid, respectively. For the assay of bile acid conjugating activity from renal or small intestinal microsomes, the assay mixtures additionally contained 5 mM cytidine 5'-monophosphate, which was added to inhibit nucleotide pyrophosphatase activity (23) in these tissues. Bile acid glucuronidation was not affected by the presence of 5 mM cytidine 5'-monophosphate. Details of the assays and identification of bile acid glucuronides using thin-layer chromatography with *n*-butanol/acetic acid/water (50:5:10, v/v) as solvent system before and after incubation with β -glucuronidase in the presence and absence of the β -glucuronidase inhibitor saccharolactone are described in another report (22). Areas of radioactivity were located by using a type LB 2723 scanner (Laboratorium Berthold, Wildbad, Federal Republic of Germany). Spots of nonlabeled bile acids were visualized by spraying the plates with a solution of 1.5 g molybdatophosphoric acid in 10 ml acetone and heating the plates at 100°C for 2 min.

Serum bilirubin was estimated by the method of Michaëlsson et al. (24). The upper limit of normal for the total serum bilirubin with this method was 0.8 mg/100 ml (25). DNA was determined according to Burton (26) after extraction from precipitated protein by heating with 0.5 N perchloric acid for 15 min at 95°C . Calf thymus DNA was used as standard. Protein was estimated by the method of Lowry et al. (27) from the trichloroacetic acid-precipitable material, using crystalline bovine serum albumin as standard.

Statistical analysis. Values are expressed as mean \pm SD. Means of groups were compared by unpaired *t* test (28). *P* values < 0.05 were considered significant. Linear regression analysis were performed by the least squares method.

Results

Identification of bile acid UDP-glucuronosyltransferase activity in different organs of man

Incubation of the radioactively labeled bile acids (chenodeoxycholic, deoxycholic, ursodeoxycholic, or lithocholic) with microsomes prepared from human liver, kidney, or duodenal mucosa in the mixtures described for the assay of bile acid glucuronidation resulted in the formation of one radioactive product for each bile acid that had the same retardation factor (R_f) value in thin-layer chromatography as the respective reference bile acid glucuronide that was obtained by enzymatic synthesis using rat liver microsomes as enzyme source (22). The identity of the bile acid glucuronides was further demonstrated by incubation of the isolated reaction products with β -glucuronidase, which yielded the corresponding unconjugated bile acids only in the absence of the β -glucuronidase inhibitor saccharolactone. The bile acid glucuronides that were isolated after incubation of chenodeoxycholic, deoxycholic, ursodeoxycholic, or lithocholic acids with hepatic, renal, or duodenal microsomes did not react with 3α -hydroxysteroid dehydrogenase, which suggested that the glucuronyl moiety was attached at C-3 of these bile acids. This observation is in accordance with findings of Almé and Sjövall (4), who identified the glucuronides of chenodeoxycholic and cholic acids that were isolated from urine

of man as 3-glucuronides. The product of the enzymatic reaction with deoxycholic acid and UDP-glucuronic acid exhibited the same R_f -value in thin-layer chromatography as the commercially available deoxycholic acid-3 β -glucuronide, which suggested that only the monoglucuronide of deoxycholic acid was formed. Since, furthermore, for the dihydroxylated bile acids, only one reaction product was identified in thin-layer chromatography after incubation of the radioactively labeled bile acids with UDP-glucuronic acid and microsomes of human liver, kidney, or duodenal mucosa, and since all of these reaction products exhibited similar R_f -values (22) that reflected similar polarity of these molecules, these results suggest that only monoglucuronides are formed from the dihydroxylated bile acids that were studied. This is in agreement with studies of Almé and Sjövall (4) on bile acid glucuronides from urine of man, which indicated the existence of only bile acid monoglucuronides.

Due to the low sensitivity of the assay method available (22) for determination of UDP-glucuronosyltransferase activity toward the trihydroxylated bile acid cholic or toward glycine- and taurine-conjugated bile acids, only glycolithocholic acid was studied as substrate of UDP-glucuronosyltransferase(s) from human tissues. Since the rate of glycolithocholic acid glucuronidation with renal or duodenal microsomes was estimated to be <0.01 nmol/min per mg of protein, identification of glycolithocholic acid glucuronide as product of the enzymatic reaction was only performed for hepatic microsomes as enzyme source exhibiting a reaction rate of ~0.05 nmol/min per mg of protein. As described above for the unconjugated bile acids, only one radioactive reaction product was obtained with labeled glycolithocholic acid as substrate of human hepatic microsomal UDP-glucuronosyltransferase. This reaction product exhibited the same R_f -value in thin-layer chromatography as reference glycolithocholic acid glucuronide enzymatically synthesized with rat liver microsomes, (22) and yielded glycolithocholic acid after incubation with β -glucuronidase if the β -glucuronidase inhibitor saccharolactone was absent. UDP-glucuronosyltransferase activity toward the unconjugated bile acids, chenodeoxycholic acid, deoxycholic acid, ursodeoxycholic acid, and lithocholic acid could thus be found in microsomes of liver, kidney, and duodenum of man in addition to enzyme activity toward glycolithocholic acid that was detectable in microsomes of human liver.

In order to investigate whether or not UDP-glucuronosyltransferase activity toward bile acids is equally distributed throughout the whole human intestine, different segments of intestinal mucosa were assayed for UDP-glucuronosyltransferase activity toward chenodeoxycholic acid. As shown in Table I, a progressive decrease in microsomal UDP-glucuronosyltransferase activity toward chenodeoxycholic acid from the oral to the aboral end of intestinal mucosa was observed with the highest activity estimated in the duodenum and the lowest activity found in the colon.

In contrast to microsomes from human liver, kidney, or small intestinal mucosa, crude homogenate or microsomes that

Table I. UDP-Glucuronosyltransferase Activity toward Chenodeoxycholic Acid from Microsomes of Different Segments of Human Intestinal Mucosa

Gut segment	No. of patients	Enzyme activity nmol/min/mg of protein
Duodenum	9	0.10±0.02
Jejunum	4	0.085±0.02
Ileum	6	0.06±0.02
Colon	5	0.03±0.01

For the sources of human intestinal specimens and assay of UDP-glucuronosyltransferase activity toward chenodeoxycholic acid, see Methods.

were prepared from human adrenal obtained from two patients, as described in Methods, did not exhibit UDP-glucuronosyltransferase activity toward the bile acids chenodeoxycholic acid, deoxycholic acid, ursodeoxycholic acid, and lithocholic acid.

Catalytic properties

Kinetics. The kinetic parameters of microsomal hepatic and extrahepatic UDP-glucuronosyltransferase activities toward the bile acids (chenodeoxycholic, deoxycholic, ursodeoxycholic, and lithocholic) are shown in Table II. Since, in the presence of glycolithocholic acid as substrate, the reaction rate with renal or duodenal microsomes was too low to perform correct determination of enzyme activity (<0.01 nmol/min per mg of protein), the kinetic parameters for glycolithocholic acid were only determined with liver microsomes as enzyme source. For all bile acids estimated as the variable substrates of UDP-glucuronosyltransferase from microsomes of liver, kidney, and duodenal mucosa, initial rate data yielded straight lines when graphed as double reciprocal plots according to Lineweaver-Burk. Note in Table II that the apparent Michaelis constant (K_m)-values for the individual bile acids did not exhibit considerable organ-specific differences. However, marked differences were observed between the K_m -values for the individual bile acids that were independent of the enzyme source, with the lowest K_m -value estimated for the monohydroxylated bile acid lithocholic acid and the highest K_m -value obtained for the dihydroxylated bile acid ursodeoxycholic acid.

In contrast to the K_m -values, the maximum velocity (V_{max})-values for the individual bile acids exhibited organ-specific differences (Table II). The highest V_{max} -values for the individual bile acids were observed with liver microsomes as enzyme source amounting to about twice the values estimated with renal microsomes and about twice to three times the values estimated with microsomes from duodenal mucosa. Among the individual bile acids, the reaction rate with chenodeoxycholic acid approximately equaled the reaction rate with deoxycholic acid and, both rates were about half to one-third the reaction rates

Table II. Kinetic Parameters of Microsomal UDP-Glucuronosyltransferase Activities toward Bile Acids in Different Organs of Man

Bile acid substrates	Liver		Kidney		Duodenum	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
	mM		mM		mM	
Lithocholic acid (3 α -ol)	0.019	0.54	0.017	0.27	0.015	0.18
Glycolithocholic acid (3 α -ol)	0.11	0.083	—	—	—	—
Chenodeoxycholic acid (3 α ,7 α -ol)	0.083	0.23	0.081	0.11	0.10	0.11
Deoxycholic acid (3 α ,12 α -ol)	0.11	0.24	0.17	0.11	0.11	0.08
Ursodeoxycholic acid (3 α ,7 β -ol)	0.29	0.42	0.26	0.25	0.21	0.23

The kinetic parameters were calculated from double reciprocal plots of the initial rates of enzyme activities as a function of varying concentrations of the individual bile acids, while the concentration of UDP-glucuronic acid was kept constant at 9 mM. The bile acid concentrations in the assay mixtures varied between 0.002 and 0.05 mM for lithocholic and glycolithocholic acids, between 0.01 and 0.5 mM for chenodeoxycholic and ursodeoxycholic acids, and between 0.01 and 0.2 mM for deoxycholic acid. The assay mixtures contained 100 μ g of microsomal protein from normal liver, kidney, or duodenal mucosa, and 0.7 mg of Triton X-100 per milligram of protein. The position and configuration of hydroxyl groups in the bile acid skeleton are given in parentheses. V_{max} , nanomole of bile acid conjugated per minute per milligram of microsomal protein.

observed in the presence of ursodeoxycholic acid or lithocholic acid (Table II) when either microsomes from liver, kidney, or duodenal mucosa were used as enzyme source. A comparison of the apparent K_m - and V_{max} -values estimated for glycolithocholic acid or for lithocholic acid as substrates of hepatic microsomal UDP-glucuronosyltransferase indicate a decrease in the reaction rate as well as in the affinity to the hepatic microsomal enzyme by a factor of ~ 6 when the glycine conjugate of lithocholic acid instead of unconjugated lithocholic acid is used as substrate.

pH Dependence. The dependence of conjugating activity toward bile acids on the pH of the reaction mixtures was similar for the enzymes from human liver, kidney, or duodenal mucosa. Enzyme activities were optimal at about pH 6.7 and showed a steep decline of activity toward pH 6.0 and 8.0.

Effect of metal ions. The effect of divalent metal ions on the activity of hepatic and extrahepatic microsomal UDP-glucuronosyltransferase toward chenodeoxycholic acid is shown in Table III. Marked differences between hepatic, renal, and small intestinal bile acid conjugating activities were observed with regard to their response to Mg^{2+} , Mn^{2+} , or Ca^{2+} , as well as to Co^{2+} , Ni^{2+} , or Fe^{2+} . Whereas the enzyme from human liver was stimulated to $\sim 140\%$ by the addition of 5 mM Mg^{2+} , Mn^{2+} , or Ca^{2+} , these ions did not exhibit a significant effect on the enzyme activities from human kidney and duodenal mucosa.

Table III. Effect of Divalent Metal Ions on Hepatic and Extrahepatic UDP-Glucuronosyltransferase Activity toward Chenodeoxycholic Acid

Addition to assay	Enzyme activity in microsomes of		
	Liver	Duodenal mucosa	Kidney
	% of control*		
None	100	100	100
10 mM EDTA	97	109	98
5 mM Mg^{2+}	138	108	100
5 mM Mn^{2+}	134	90	91
5 mM Ca^{2+}	135	88	89
5 mM Ba^{2+}	101	104	89
5 mM Co^{2+}	35	89	64
5 mM Ni^{2+}	44	101	74
5 mM Zn^{2+}	6	40	14
5 mM Fe^{2+}	13	64	92
5 mM Pb^{2+}	16	38	30

* Control, enzyme activity without addition of EDTA and metal ions. For the determination of enzyme activity, see Methods.

No further increase or a decrease in enzyme activities from liver, kidney, or duodenal mucosa was observed when the concentration of Mg^{2+} was raised from 5 to 15 mM. Whereas the enzymes from liver and kidney were inhibited by Co^{2+} and Ni^{2+} , enzyme activity from duodenal mucosa was not decreased by Ni^{2+} and not significantly influenced by the addition of Co^{2+} . Fe^{2+} did not exhibit a significant effect on chenodeoxycholic acid glucuronidation in renal microsomes, whereas bile acid conjugating activity from liver and duodenal mucosa was decreased by Fe^{2+} . Whether or not these differences between hepatic, renal, and small intestinal microsomes with regard to the effect of divalent metal ions on enzyme activities indicate the existence of different organ-specific UDP-glucuronosyltransferase activities toward bile acids can only be clarified when the purified enzymes become available (29, 30).

Effect of detergents

Since microsomal UDP-glucuronosyltransferase activities toward various acceptor substrates have been reported to be activated in *in vitro* incubations by the addition of detergents (31), the effect of the nonionic detergents Brij 58 and Triton X-100 on the activity of hepatic and extrahepatic microsomal UDP-glucuronosyltransferase toward chenodeoxycholic acid was investigated. At a low concentration of the substrate chenodeoxycholic acid in the reaction mixtures (0.01 mM), bile acid glucuronidation was slightly enhanced to $\sim 130\%$ in renal microsomes and to $\sim 160\%$ in hepatic microsomes at a concentration of 0.7 mg of Triton X-100 per milligram of protein. Concentrations of Triton X-100 > 0.7 mg per mg of renal or hepatic microsomal protein produced a progressive inhibition of chenodeoxycholic

acid glucuronidation in contrast to Brij 58, which was inhibitory to the enzymes from hepatic and renal microsomes even at the lowest concentration tested (0.3 mg per mg of protein). At saturating concentration of the substrate chenodeoxycholic acid (0.5 mM), Triton X-100 did not activate conjugating activity toward the bile acid in renal or hepatic microsomes, which might be due to the detergent effect of the bile acid itself (32). In small intestinal microsomes, Triton X-100 or Brij 58, at concentrations up to ~2 mg per mg of protein, did not activate chenodeoxycholic acid glucuronidation, even at low substrate concentration (0.01 mM chenodeoxycholic acid). At concentrations higher than 2 mg per mg of protein, Triton X-100 or Brij 58 inhibited the conjugating activity toward chenodeoxycholic acid in small intestinal microsomes.

In the presence of a low concentration of the substrate chenodeoxycholic acid (0.01 mM) and 50 μ g of egg yolk phosphatidylcholine or egg yolk phosphatidylethanolamine, which were added after formation of mixed micelles with the substrate chenodeoxycholic acid to the assay mixtures, no significant activation of UDP-glucuronosyltransferase from renal or hepatic microsomes was observed. Bilirubin glucuronide formation by rat liver microsomes has recently been shown to be increased by phospholipids (33).

Bile acid UDP-glucuronosyltransferase activity in liver disease

Since up to now no studies were available on UDP-glucuronosyltransferase activity toward bile acids in liver disease, a survey of glucuronidating activity toward chenodeoxycholic acid was carried out in liver biopsy specimens from patients with a variety of liver diseases ($n = 75$) or with normal liver histology ($n = 23$) (Fig. 1). Patients with normal liver exhibited a mean value of UDP-glucuronosyltransferase activity toward chenodeoxycholic acid of 201 ± 77.6 (SD) nmol/h per mg of DNA (0.063 ± 0.024 (SD) nmol/min per mg of protein), which amounted to about twice the mean value observed in patients with liver cirrhosis (Fig. 1). The mean value of bile acid UDP-glucuronosyltransferase activity in patients with liver cirrhosis was not only significantly lower, compared to the mean value of enzyme activity estimated for the group with normal liver ($P < 0.001$), but differed also significantly from the mean values of bile acid UDP-glucuronosyltransferase activity from patients with fatty liver hepatitis ($P < 0.02$), fatty liver ($P < 0.003$), and fibrosis of the liver ($P < 0.003$). The mean value of bile acid UDP-glucuronosyltransferase activity from patients with granulomatous hepatitis differed only significantly from the mean value of enzyme activity observed for the group with normal liver ($P < 0.03$) (Fig. 1). No significant differences were observed for the mean values of UDP-glucuronosyltransferase activity toward chenodeoxycholic acid between all other groups tested (Fig. 1).

Since human hepatic UDP-glucuronosyltransferase activity toward chenodeoxycholic acid has been shown to be competitively inhibited by bilirubin, as described in previous reports (10, 11), the values of UDP-glucuronosyltransferase activity to-

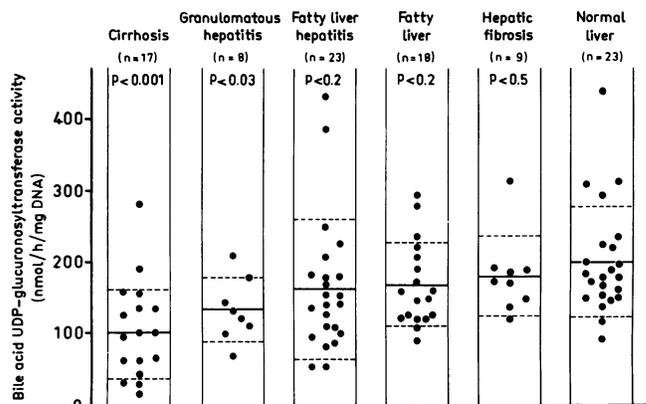


Figure 1. UDP-glucuronosyltransferase activity toward chenodeoxycholic acid in liver biopsy specimens from patients with normal liver and various liver disease. Enzyme activity and DNA were determined as described in Methods from 10% liver homogenates (wet weight/volume) that was prepared with 0.25 M sucrose, which contained 5 mM Tris/HCl, pH 7.4. Horizontal lines represent means \pm SD. P values < 0.05 were considered significant when compared with the normal liver. n , number of liver biopsy specimens.

ward chenodeoxycholic acid obtained from human liver biopsy specimens shown in Fig. 1 were plotted against total serum bilirubin values. As shown in Fig. 2, UDP-glucuronosyltransferase activity toward chenodeoxycholic acid exhibited an inverse correlation with the concentration of total serum bilirubin, i.e., low bile acid UDP-glucuronosyltransferase activity was observed in patients with high total serum bilirubin values. Whether or not the observed inverse correlation between total serum bilirubin and bile acid UDP-glucuronosyltransferase activity may be due to bilirubin acting as competitive inhibitor of chenodeoxycholic acid glucuronidation, as previously observed in *in vitro* studies (10, 11), or due to an unspecific inhibitory effect of bilirubin on UDP-glucuronosyltransferase because of its toxicity (34), can only be clarified when the purified UDP-glucuronosyltransferase becomes available.

Discussion

Previous studies have shown that glucuronidation is an important end step in bile acid metabolism, both in health (4, 35) as well as in liver disease (1-4, 36). However, up to now only the liver has been known as a site of bile acid glucuronidation in man (10, 11). The present study shows that in man, conjugation of bile acids with glucuronic acid does not only occur in the liver but also in the kidney and small intestinal mucosa. No enzyme activity could be detected in homogenates or microsomes of human adrenal.

The V_{max} -values for the individual bile acids show that microsomal UDP-glucuronosyltransferase activity toward bile acids in liver is about twice that found in kidney and about twice to three times that observed in duodenal mucosa (Table II). The

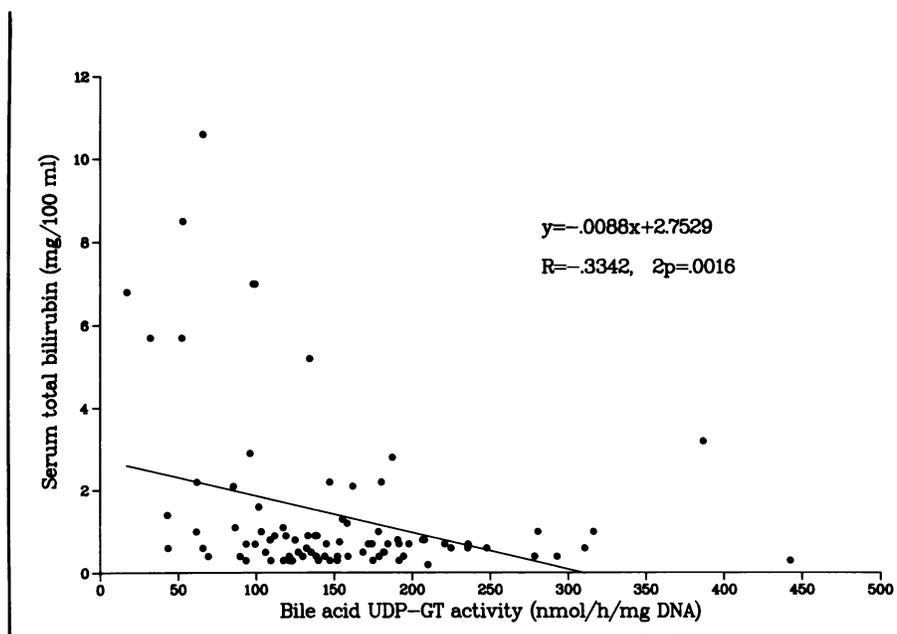


Figure 2. Correlation of human hepatic UDP-glucuronosyltransferase (UDP-GT) activity toward chenodeoxycholic acid with total serum bilirubin concentrations. Enzyme activities were obtained from liver biopsy specimens of patients with and without liver disease (values of Fig. 1). Total serum bilirubin in these patients was determined as described in Methods.

relative glucuronidation rates with chenodeoxycholic acid in different segments of human intestinal mucosa indicate a decrease in the activity of UDP-glucuronosyltransferase toward bile acids when going from the oral end of the small intestine to the colon, where it is 30% of the duodenal level (Table I). A similar decrease in rat intestinal enzyme activity from duodenum to ileum was observed for UDP-glucuronosyltransferase activity toward xenobiotics such as *o*-aminophenol and *p*-nitrophenol (14), and for the mixed function monooxygenase system which catalyzes biotransformation of xenobiotics by oxidative reactions (37). Therefore, enzymes involved in the biotransformation of foreign or endogenous compounds either by glucuronidation or by oxidative reactions appear to be concentrated in the upper small intestine, which is also the region primarily exposed to bile acids of the enterohepatic circulating bile acid pool or to xenobiotics in the diet. With regard to bile acid metabolism, the upper small intestine, such as the jejunum, is mainly the site of passive absorption of bile acids, in contrast to the ileum, where bile acids are predominantly taken up by an active transport system (38, 39). Since, according to the present study, the upper small intestine, such as the duodenum and the jejunum, is the site of high UDP-glucuronosyltransferase activity toward bile acids, passive absorption of these compounds may be associated with metabolism of these molecules, as already suggested by Rachmilewitz and Saunders (40) from studies on bile acid metabolism by rat intestinal mucosa. These authors observed that metabolism of bile acids during their absorption by rat intestinal segments is higher in the jejunum than in the ileum and colon, leading to the formation of polar bile acid metabolites that had not been further identified (40).

The apparent K_m -values for the various bile acids as substrates

indicate a similar affinity of the individual bile acids to the enzymes from liver, kidney, or small intestine (Table II). Among the individual bile acids, differences in the affinities to the respective enzymes were observed independent of the source of enzyme. The monohydroxylated bile acid lithocholic acid exhibited an ~4–15-fold higher affinity to the enzymes from liver, kidney, or small intestinal mucosa compared with the dihydroxylated bile acids chenodeoxycholic acid, deoxycholic acid, or ursodeoxycholic acid (Table II). The dihydroxylated bile acids themselves showed differences in the affinities to the enzymes independent of the enzyme source, which suggested that the affinity of the bile acids to hepatic, renal, or small intestinal UDP-glucuronosyltransferase does not only depend on the number but also on the position and configuration of hydroxyl groups in the bile acid skeleton. Since within the dihydroxy bile acids, lowest affinity to the enzymes was observed for ursodeoxycholic acid, which has been shown to be significantly more hydrophilic than its 7α epimer chenodeoxycholic acid, and more hydrophilic than deoxycholic acid (41), these results suggest that the affinity of bile acids to hepatic, renal, and small intestinal UDP-glucuronosyltransferase appears to increase with increasing hydrophobicity of bile acids. Furthermore, conjugation of bile acids with amino acids appears to decrease the affinity of bile acids to UDP-glucuronosyltransferase, since in the presence of glycolithocholic acid a sixfold lower affinity to hepatic microsomal UDP-glucuronosyltransferase was observed, compared with unconjugated lithocholic acid (Table II).

Since, according to the present study in man, unconjugated bile acids appear to be substrates of higher affinity to hepatic and extrahepatic bile acid UDP-glucuronosyltransferase(s) (Table II) than amino acid-conjugated bile acids, the question arises

about the physiological significance of glucuronidation of unconjugated bile acids in man.

Unconjugated primary, secondary, and tertiary bile acids were recently found in serum samples of healthy subjects (7, 42), and were shown to increase to concentrations between two and three micromole per liter after breakfast (43). The existence of unconjugated bile acids in serum of healthy subjects and the recent finding of more than 20 individual bile acid glucuronides in urine of healthy subjects, most of which were otherwise unconjugated (4), indicate that glucuronidation of unconjugated bile acids plays a physiological role in bile acid metabolism in health.

However, not only in health but also in patients with small bowel overgrowth or ileal resection, in patients with cholesterol cholelithiasis that was treated with chenodeoxycholic acid (44) or ursodeoxycholic acid (45) for gallstone dissolution, as well as in patients with hepatobiliary disease, glucuronidation of unconjugated bile acids may be of biological significance.

In patients with small bowel overgrowth or ileal resection, increased concentrations of serum unconjugated bile acids have been found (42, 46) which may serve as substrates for hepatic and extrahepatic bile acid UDP-glucuronosyltransferase(s). This assumption is supported by the description of a patient with chronic malabsorption, who excreted glucuronides of otherwise unconjugated mainly 6 α -hydroxylated bile acids in urine, which amounted to 20% of the total urinary bile acid excretion (47).

In patients with cholesterol cholelithiasis, which was treated orally with chenodeoxycholic acid or ursodeoxycholic acid for gallstone dissolution, high concentrations of these unconjugated bile acids were found in serum immediately after oral application of these drugs (42, 48). These serum unconjugated bile acids may serve as substrates for hepatic and extrahepatic bile acid UDP-glucuronosyltransferase(s), since glucuronides of chenodeoxycholic acid and ursodeoxycholic acid have been identified in serum (35) and urine of healthy subjects (4). Since chenodeoxycholic acid and ursodeoxycholic acid are both metabolized to the toxic lithocholic acid (49, 50), elevated concentrations of sulfated lithocholic acid as well as of nonsulfated lithocholic acid were found in serum and bile during treatment of patients with chenodeoxycholic acid or ursodeoxycholic acid for gallstone dissolution (50). Due to the high affinity observed for lithocholic acid toward hepatic and extrahepatic UDP-glucuronosyltransferase(s) (Table II), glucuronidation of lithocholic acid may serve as a further detoxication mechanism in man in addition to the well-known sulfation (5, 51), which is supported by the identification of lithocholic acid glucuronide in serum (35) and urine (4) of healthy subjects.

In patients with hepatobiliary disease, glucuronidation of unconjugated bile acids may be of biological significance, since analysis of serum bile acid profiles in patients with liver cirrhosis indicated the existence of unconjugated bile acids in serum which amounted to 40% of total serum bile acids (52). Furthermore, bile acid glucuronides, most of which were otherwise unconjugated, have been found in urine and bile of patients with liver disease (1–4).

UDP-glucuronosyltransferase activity toward chenodeoxycholic acid in liver biopsy specimens from patients with various liver diseases was found to be decreased in patients with cirrhosis or granulomatous hepatitis (Fig. 1), compared with enzyme activity estimated in normal liver, whereas liver biopsy specimens from patients with fatty liver hepatitis, fatty liver, or fibrotic liver exhibited UDP-glucuronosyltransferase activity toward chenodeoxycholic acid that did not differ significantly from the enzyme activity estimated in normal liver. The reduced hepatic bile acid UDP-glucuronosyltransferase activity observed in patients with liver cirrhosis (Fig. 1), as well as in patients with liver disease in connection with high total serum bilirubin values (Fig. 2), is in agreement with levels of bile acid glucuronides that were recently estimated in urine and serum of healthy subjects (4, 35) and in patients with liver cirrhosis or patients with other intrahepatic or extrahepatic cholestasis (2–4, 35, 36). These levels of bile acid glucuronides were recently determined using highly specific analysis such as lipophilic anion exchange (2–4) or mass fragmentography (35, 36).

Takikawa et al. (36) recently found diminished serum bile acid glucuronides in patients with compensated liver cirrhosis (2.7% of total serum bile acids), in patients with jaundiced liver cirrhosis (0.9% of total serum bile acids), and in patients with obstructive jaundice (5.1% of total serum bile acids), whereas in healthy subjects, 8.6% of the total serum bile acids were glucuronidated (36). Almé and Sjövall (4) showed that in healthy subjects bile acid glucuronides represented 12–36% of the total bile acids excreted in urine, whereas in mild cholestasis, 8.4% of the total urinary bile acids were bile acid glucuronides. Levels of urinary bile acid glucuronides in patients with liver cirrhosis were determined recently to be 6.8 (2) or 2.2% (3) of total urinary bile acids, and were therefore lower than those estimated for healthy subjects (12–36%) (4).

According to the results of the present study, the reduced relative amounts of serum (36) or urinary bile acid glucuronides (2, 3) that were observed in patients with liver cirrhosis (2, 3, 36) or in patients with other intrahepatic (4) or extrahepatic cholestasis (36), compared with the relative amounts of bile acid glucuronides in serum (35, 36) or urine (4) of healthy subjects, may be explained by a decrease in hepatic bile acid UDP-glucuronosyltransferase activity in liver cirrhosis as well as in liver disease in connection with high serum bilirubin values. The decreased hepatic UDP-glucuronosyltransferase activity toward chenodeoxycholic acid that was observed in patients with liver cirrhosis or granulomatous hepatitis (Fig. 1) may be explained by the reduced amounts of hepatocytes per milligram of DNA, by hepatocellular dysfunction and/or by an inhibitory effect of bilirubin on bile acid UDP-glucuronosyltransferase activity (10, 11), since low enzyme activities in liver biopsy specimens of patients with cirrhosis or with granulomatous hepatitis were found when serum bilirubin values were in the normal or elevated range (Figs. 1 and 2).

The presented results are the first report which indicates that not only the liver but also the intestinal mucosa and the kidney are able to glucuronidate bile acids in man. However,

the relative contribution of hepatic and extrahepatic sites to the formation of bile acid glucuronides in man still has to be determined.

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