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HEPATIC EXTRACTION OF BILE ACIDS IN RATS*

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Abstract—The hepatic extraction ratio (E_h) of five common bile acids was determined by comparison of the area under the plasma concentration time curve (AUC) after administration into the femoral and portal veins of rats. Taurocholic acid (TCA), glycocholic acid (GCA), cholic acid (CA), deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) were studied. Using equimolar doses of the bile acids (9.3 μmoles), the effects of both conjugation and the number and position of hydroxyl groups on E_h were compared. TCA had the highest E_h (about 80 per cent) followed in order by GCA (65 per cent), CA (55 per cent) and CDCA (55 per cent), with DCA exhibiting the lowest E_h (40 per cent). This suggests that conjugation has a more important effect on hepatic extraction of bile acids than the number of hydroxyl groups.

Bile acids are biologically important compounds that are extracted by the liver from the portal blood stream, excreted into the bile, and then reabsorbed by the distal half of the ileum to complete the enterohepatic circulation. Hepatic extraction of bile acids from the portal circulation plays an important role in this enterohepatic circulation of bile acids. Investigators have used whole animals, isolated perfused livers, and isolated hepatocytes to study the hepatic extraction of bile acids and have concluded that a carrier-mediated transport mechanism is responsible for the hepatic extraction of bile acids [1–10]. Recently, Hoffman *et al.* [11] examined the effect of conjugation and position of hydroxyl groups on hepatic bile acid transport by comparing their biliary excretion rates. Taurocholic acid was transported most efficiently, followed in order by glycocholic acid, cholic acid, deoxycholic acid, and chenodeoxycholic acid. They suggested that the differing rates of biliary excretion were, at least in part, determined by the efficiency of hepatic uptake.

The purpose of the present study was to determine the hepatic extraction of a series of bile acids, which differ slightly in chemical structure and physical properties, by use of the area under the plasma concentration time curve (AUC) after portal and femoral vein administration in an attempt to determine the effect of conjugation and position of hydroxyl groups on the hepatic extraction of bile acids. The bile acids employed were taurocholic acid (TCA), glycocholic acid (GCA), cholic acid (CA), deoxycholic acid (DCA), and chenodeoxycholic acid (CDCA). TCA and GCA are excreted exclusively into bile without prior biotransformation, while CA, DCA, and CDCA are excreted into bile mainly as conjugates [12–14]. The biliary excretion of these bile acids after femoral and portal vein administration was also compared.

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MATERIALS AND METHODS

Adult Sprague–Dawley (Bio-Laboratories, White Bear, MN) male rats (250–280 g) were used. The rats were anesthetized with ethyl carbamate (urethane, 1000 mg/kg, i.p.), and the femoral vein and artery were cannulated with polyethylene tubing (PE-50) for drug administration and blood sampling respectively. The total sampling volume was 2.5 ml and replaced by the same volume of saline. The common bile duct was cannulated with PE-10 tubing and bile was sampled at 5-min intervals. The rectal temperature of the rats was maintained at 37° with a heat lamp. The injection of bile acids and saline into the portal and femoral vein was accomplished with PE-10 polyethylene tubing attached to a No. 30 gauge needle. To minimize the effect of cannulation on hepatic blood flow, the cannulae were fixed with surgical glue (Aron Alpha, Sankyo Co. Ltd., Japan) eliminating the need for suture. The injections were made over a 30-sec interval with a 250 μl syringe.

The bile acids used in this study were [^{3}H]-taurocholic acid (3.39 Ci/mmole), [^{3}H]-glycocholic acid (3.08 Ci/mmole), [^{3}H]-cholic acid (14 Ci/mmole), [^{3}H]-deoxycholic acid (4 Ci/mmole), and [^{14}C]-chenodeoxycholic acid (50.0 mCi/mmole). All these bile acids were purchased from the New England Nuclear Corp., Boston, MA, and found to be at least 98–99 per cent pure by thin-layer chromotography. Sodium taurocholic acid, sodium glycocholic acid, sodium cholic acid, sodium deoxycholic acid and sodium chenodeoxycholic acid were dissolved in saline, mixed with their respective ^{3}H- and/or ^{14}C-labeled bile acid and administered at 9.3 μmoles/kg, which corresponds to the dose of 5 mg/kg of taurocholic acid. This dose was selected because in preliminary experiments a linear relationship was demonstrated between dose (either femoral or portal vein administration) and AUC in the dose range of 2–20 mg/kg (unpublished data).

The concentration of ^{3}H- and/or ^{14}C-labeled bile acids was determined in a Packard Tri-Carb counter (Packard Instruments Corp., Downers Grove, IL)

after an aliquot (50–100 μl) was placed in a scintillation vial containing 10 ml of 3a70B scintillation fluid (Research Products International Corp., Elk Grove Village, IL).

Bile volume was measured gravimetrically, assuming a density of 1.0 for bile. The concentration of each bile acid in bile was quantitated as described above using 25 μl of bile. Quenching was determined by automatic external standardization.

$AUC_{0\to\infty}$ (area under the plasma concentration time curve) was calculated by mathematical integration after curve fitting using the "CSTRIP" program developed by Sedman and Wagner [15] as previously described [16]. In the present study, the best fitting exponential number was judged by both the regression coefficient ($r > 0.995$) and by the percent deviation at each time point.

The mean plasma clearance (Cl_p) of each bile acid was calculated using the following equation:

$$Cl_p = \text{Dose}_{\text{femoral}}/AUC_{0\to\infty} \quad (1)$$

Under linear and dose-independent conditions, systemic availability (f) and hepatic extraction ratio (mean or steady-state) (E_h) are given by equations 2 and 3.

$$\text{Systemic availability } (f) = \frac{AUC_{\text{portal}}}{AUC_{\text{femoral}}} \quad (2)$$

$$E_h = 1 - f = 1 - \frac{AUC_{\text{portal}}}{AUC_{\text{femoral}}} \quad (3)$$

In this study E_h was calculated by equation 3 using the mean values of both AUC_{portal} and AUC_{femoral} in the unsaturable dose range of the portal vein administration (unpublished data).

Statistical analysis was performed with Student's t-test with $P = 0.05$ as the minimal level of significance.

RESULTS

The plasma disappearance of five bile acids (taurocholic, glycocholic, cholic, deoxycholic and

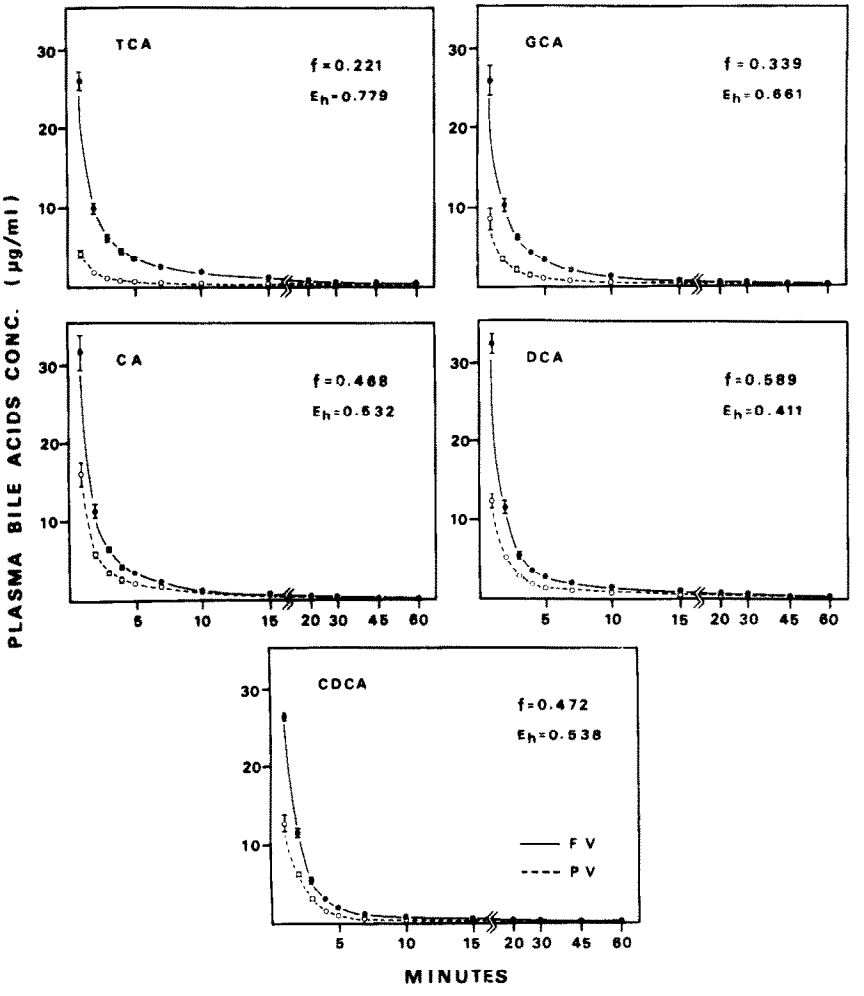

Fig. 1. Plasma disappearance curves of (a) taurocholic acid (TCA), (b) glycocholic acid (GCA), (c) cholic acid (CA), (d) deoxycholic acid (DCA), and (e) chenodeoxycholic acid (CDCA). Closed circles represent femoral vein (FV) injection while open circles represent portal vein (PV) injection. Each point and vertical bar represent the mean and S. E. of five rats. Curves were calculated by the "CSTRIP" method, using a digital computer.

Table 1. Comparison of pharmacokinetic parameters of a series of bile acids*

Bile acids†	AUC ($\mu g \cdot ml^{-1} \cdot min^{-1}$) Femoral vein	Portal vein	Systemic availability (f)	Hepatic extraction ratio (E_h)	Mean plasma clearance (Cl_p) ($ml \cdot min^{-1} \cdot kg^{-1}$)
TCA	136.3 ± 11.4	30.2 ± 3.7‡	0.221	0.779	36.9 ± 3.2
GCA	132.4 ± 14.4	44.8 ± 5.1‡	0.339	0.661	35.4 ± 4.4
CA	156.6 ± 12.5	73.3 ± 5.1‡	0.468	0.532	26.3 ± 2.4
DCA	170.8 ± 5.1	100.7 ± 4.9‡	0.589	0.411	22.9 ± 0.7
CDCA	197.7 ± 6.4	93.3 ± 6.7‡	0.472	0.538	19.8 ± 0.7

* Each value is the mean ± S.E. of five rats.

† Dose: 9.3 μmoles/kg (corresponds to 5 mg/kg of taurocholic acid). Abbreviations: TCA, taurocholic acid; GCA, glycocholic acid; CA, cholic acid; DCA, deoxycholic acid; and CDCA, chenodeoxycholic acid.

‡ Significantly different from femoral vein administration ($P < 0.05$).

chenodeoxycholic acid) after intravenous administration of 9.3 μmoles/kg into the femoral and portal veins was very rapid and is shown in Fig. 1. In the early sampling periods, significant differences were evident between the plasma concentration of bile acids when administered portally and systemically.

The calculated pharmacokinetic parameters of these bile acids are summarized in Table 1. For all the bile acids, the AUC after portal vein injection was significantly less than those after femoral vein injection. Of the five bile acids examined in this study, the conjugated bile acids had the highest hepatic extraction. TCA had an hepatic extraction ratio of about 80 per cent, and for GCA it was more than 65 per cent.

The mean plasma clearance values of TCA and GCA were very high (Table 1), being about 35 $ml \cdot min^{-1} \cdot kg^{-1}$ while those of CA, DCA and CDCA were about 20–25 $ml \cdot min^{-1} \cdot kg^{-1}$. Thus, the plasma clearance values for the unconjugated bile acids were about two-thirds of those of the conjugated bile acids.

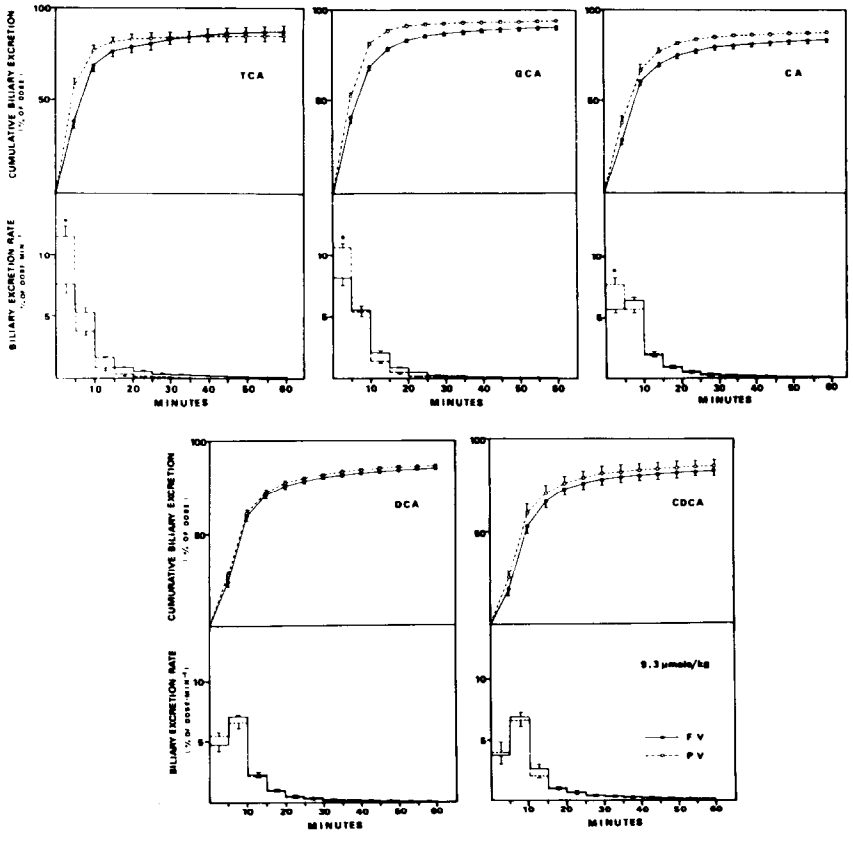

Fig. 2. Biliary excretion profile of (a) taurocholic acid (TCA), (b) glycocholic acid (GCA), (c) cholic acid (CA), (d) deoxycholic acid (DCA), and (e) chenodeoxycholic acid (CDCA) after 9.3 μmoles/kg. Upper panel represents cumulative biliary excretion curves. Lower panel represents biliary excretion rate. Values were statistically significant at $P < 0.05$ (*) when compared with the biliary excretion rate after femoral injection. Each point and bar represent the mean and S.E. of five rats.

The biliary excretion profiles of the bile acids excreted over 60 min after injection of TCA, GCA, CA, DCA and CDCA into the femoral and portal veins are shown in Fig. 2. The biliary excretion rate of TCA, GCA and CA after portal vein injection was significantly higher than when injected via the femoral vein for the first 5 min after injection. However, the two dihydroxy bile acids, DCA and CDCA, showed no significant difference in the biliary excretory rate between the two routes of administration. Also, the maximal biliary excretion rate for the two dihydroxy bile acids was observed during the second collection period while the maximal biliary excretion rate for both the unconjugated and the conjugated trihydroxy bile acids was observed during the first collection period. For all bile acids, more than 80 per cent of the dose was excreted into the bile within 1 hr after administration, and no significant difference was observed in the cumulative biliary excretion between the two routes of administration. Also no significant differences were shown in the average bile flow rate after administration of the five bile acids or between the two routes of administration. Bile flow rates after the administration of these bile acids through the femoral and portal veins were 67.0 ± 1.8 (mean $\pm$ S.E., $N = 25$) and 66.6 ± 2.4 ($N = 27$) $\mu l \cdot min^{-1} \cdot kg^{-1}$ respectively.

DISCUSSION

In this study, we determined the mean hepatic extraction ratio (E_h) of five common bile acids, which are slightly different in chemical structure and physical properties, by use of the AUC after portal and femoral vein administration. Three trihydroxy bile acids (TCA, GCA and CA) and two dihydroxy bile acids (DCA and CDCA) were studied.

We compared the plasma disappearance, hepatic extraction, and biliary excretion of five common bile acids using an equimolar dose of 9.3 μmoles/kg (which corresponds to 5 mg/kg of TCA which is an unsaturable dose after both routes of administration of TCA) in an attempt to determine the effect of conjugation and position of hydroxyl groups on the hepatic clearance of bile acids. Recently, Ng and Hofmann [17] reported that more than 70 per cent of intravenously administered GCA was distributed in bile and liver within 10 min. These findings suggest a rapid and efficient hepatic uptake and biliary excretion for this bile acid. In the present study the plasma disappearance of all the bile acids examined was found to be very rapid after both systemic and intraportal administration but their concentration in the plasma was significantly lower in the early time periods after intraportal than systemic administration (Fig. 1).

TCA, the taurine conjugate and predominant bile acid in rat bile, had the highest extraction ratio (about 80 per cent) and GCA, the glycine conjugate but a minor constituent of rat bile, also had a high extraction ratio (about 65 per cent) (Table 1). However, the non-conjugated bile acids had a lower hepatic extraction: the free trihydroxy bile acid (CA) and 3α,7α-dihydroxy cholic acid (CDCA) had a lower extraction ratio (about 55 per cent), and 3α,12α-dihydroxy cholic acid (DCA) had the lowest ratio (only 40 per cent). From these findings, it is clear that the conjugated form of bile acid is more efficiently extracted by liver than free cholic acid.

A remarkable difference in the biliary excretion profile was exhibited between the trihydroxy and the dihydroxy bile acids. A delay in the maximal biliary excretion rate was observed in that cholic acid and its conjugates reached their maximal rate of excretion in the first collection period (0–5 min) while for the dihydroxy bile acids (DCA and CDCA) it was not achieved until the second collection period (5–10 min). A possible explanation for this effect is that the transport processes (including conjugation) have a higher capacity for the trihydroxy bile acids than for the dihydroxy bile acids. It is also possible that the dihydroxy bile acids produce a structural change in the sinusoidal and/or bile canalicular membranes as has been reported for the liver plasma membrane [18].

The number of hydroxyl groups appeared to be less important than conjugation for hepatic extraction since the dihydroxy CDCA and trihydroxy CA were cleared at the same rate. However, DCA (3α,12α-dihydroxy cholic acid) was extracted less efficiently than CDCA (3α,7α-dihydroxy cholic acid), suggesting that the 12 OH might be an important determinant for the rate of hepatic extraction of bile acids. The percent of the bile acids excreted into the bile during the first 5 min after administration was also highest for the conjugated bile acids, TCA (12 per cent) and GCA (11 per cent), and lower for the unconjugated bile acids, CA (8 per cent), DCA (5 per cent) and CDCA (4 per cent). However, for the dihydroxy bile acids there appears to be a lag in their transport across the liver in that both the time for appearance in the bile is longer for the dihydroxy than the trihydroxy and the peak excretory rate and hepatic extraction do not correlate well for the dihydroxy bile acids.

In a previous paper [16] we determined the E_h of several non-metabolizable organic anions which are often used to measure the hepatic excretory function in experimental laboratory animals. Phenol 3,6-dibromphthalein disulfonate (DBSP) and amaranth (AM) had E_h values of 40–45 per cent, while indocyanine green (ICG) and rose bengal (RB) showed extremely low E_h of 5 per cent in the dose range of 1–2 mg/kg. Thus, in comparison, the bile acids are much more efficiently extracted by the liver than most of the compounds used to evaluate hepatic excretion function in rats and, thus, difference in blood flow [19, 20] would be expected to have a greater effect on the clearance of bile acids than on the other organic anions.

In conclusion, our results demonstrate that both taurine and glycine conjugation increase the hepatic extraction of bile acids, but taurine appeared to be more effective than glycine.

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