Biochemical Pharmacology, Vol. 39, No. 7, pp. 1175–1180, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

EFFECTS OF URSODEOXYCHOLATE ON MAXIMAL BILIARY SECRETION OF BILIRUBIN IN THE RAT

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(*Received* 11 *July* 1989; *accepted* 1 *October* 1989)

Abstract—The effect of sodium ursodeoxycholate (0.5 and 1.0 μmol/min/100 g) on the maximal biliary secretion (T_m) of bilirubin and on the concentration of bilirubin in liver and plasma at the end of a bilirubin load was studied in Wistar rats. Administration of ursodeoxycholate at 0.5 μmol/min/100 g caused a 0.8-fold increase in bile flow and a significant increase in the bilirubin T_m (+24%). This was associated with a significant reduction of liver and plasma bilirubin concentrations (−16% and −17%, respectively). Bilirubin UDP-glucuronosyltransferase activity was not significantly enhanced. There was a significant increase in the biliary excretion of bilirubin conjugates (+30%) and in the diconjugates/monoconjugates ratio in bile (+31%). When ursodeoxycholate was given at 1.0 μmol/min/100 g, it produced a 1.7-fold increase in bile flow, but the bilirubin T_m was significantly reduced (−21%). Liver bilirubin concentrations were decreased (−20%) and there was a significant enhancement in total pigment concentration in plasma (+19%). Both the excretion of unconjugated bilirubin and that of bilirubin conjugates were significantly reduced (−60% and −18%, respectively). There was a significant decrease in the bilirubin-UDP glucuronosyltransferase activity and the diconjugates/monoconjugates ratio in bile (−27% and −27%, respectively). These results indicate that ursodeoxycholate is able to increase maximal bilirubin secretion only when administered at low doses and that infusion at higher rates can significantly interfere with different steps in the hepatobiliary transport of the pigment.

Bilirubin is a potentially toxic compound that can accumulate in excess in plasma during cholestasis [1]. Metabolism and disposition of this molecule is a function of liver cells, that catalyse the formation of mono- and diglucuronide conjugates of bilirubin which are then excreted into bile [2]. Bile formation is largely dependent on the flux of bile acids through the liver and it is generally accepted that secretion of organic anions proceeds through two different pathways, one serving unsulfated bile acids and the other used by sulfated bile acids and most organic anions [2–4]. However, it is known that bile acids are able to influence the excretion of some organic anions, including bilirubin, although data reported are conflicting. Administration of taurocholate has been shown by some authors to increase endogenous bilirubin excretion or the T_m of bilirubin in some species [5–9], although others have described the absence of such an effect in the rat [10, 11]. Dehydrocholate appears to have no effect on bilirubin transport in this species [12], but it increases bilirubin T_m in the rabbit [13]. In man, even an inhibitory effect on bilirubin transport has been found after dehydrocholate administration [14]. For other organic anions, inhibitory effects of bile acids have sometimes been reported [15, 16], although the stimulatory effect is the best documented [17–21]. The exact nature of the relationship between transport mechanisms and interactions of bile acids and organic anions in the course of their hepatobiliary transport still remains to be fully elucidated.

The purpose of this study was to investigate the influence of ursodeoxycholate, a bile acid used in the treatment of different hepatic diseases [22–24], on the hepatobiliary transport of bilirubin in the rat. The T_m of bilirubin and the concentration and composition of bilirubin in bile, plasma and liver were studied under the effect of two different i.v. doses of the bile acid.

MATERIALS AND METHODS

Chemicals. Bilirubin, bovine albumin, digitonin, UDP-glucuronic acid and sodium ursodeoxycholate were obtained from the Sigma Chemical Co. (St Louis, MO). Ethylanthranilate and *p*-iodoaniline were supplied by Fluka A.G. (Buchs, Switzerland). All other reagents were of analytical reagent grade.

Experimental design. Male Wistar rats weighing 200–250 g were used. The animals were kept on a standard rat chow and tap water *ad lib.* on a 12 hr dark/light cycle.

Non-fasted rats were anaesthetized by intraperitoneal injection of pentobarbital (50 mg/kg body wt). After laparotomy, a PE-50 catheter was inserted into the common bile duct. Catheters of equal size were inserted into the left jugular vein and left carotid artery. Body temperature was monitored continuously and kept at 37° by a thermostatically controlled heating table.

After two 10-min collections of bile, sodium ursodeoxycholate was infused at two different doses of 0.5 and 1.0 μmol/min/100 g and bile was collected for 10 additional 10-min periods. Ursodeoxycholate was dissolved in a previously prepared solution of 0.154 M NaCl, 0.1 M Na_2CO_3 (1:1, v/v), containing

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3% bovine albumin. The solution was filtered (pore diameter 0.45 μm) and its pH adjusted to 8.3 before dissolving the bile acid. Control animals received the solution without bile acid. Forty minutes after the beginning of ursodeoxycholate infusion, unconjugated bilirubin, 3.4 μmol/100 g, dissolved in 0.1 M NaOH and brought to pH 8.5 with 0.05 M HCl was injected, followed by an infusion of 256 nmol/min/100 g administered over a period of 1 hr. The bilirubin output during the period 30–60 min was taken as representative of the maximal bilirubin secretion (T_m). At the end of the experiments the rats were killed by exsanguination via the carotid artery. The livers were flushed with ice-cold 0.154 M NaCl injected via the hepatic veins and stored in the dark at −20° for further analysis.

Analytical methods. Bile volume was measured gravimetrically without correction for density. Total bilirubin in liver, serum and bile was determined after diazo cleavage with *p*-iodoaniline [25, 26]. The composition of bile pigments in plasma and bile was analysed by thin layer chromatography of methyl ester derivatives after alkaline methanolysis [27]. UDP-glucuronosyltransferase activity was assayed with digitonin-activated liver homogenates using bilirubin as acceptor substrate [28]. Protein concentration in liver homogenates was measured by the method of Lowry *et al.* [29]. Bile acid concentration in bile was determined with a 3α-hydroxysteroid dehydrogenase [30].

Results were expressed as means ± SE. Statistical significance of the differences was calculated by the non-parametric Mann-Whitney U test. P values of less than 0.05 were considered significant.

RESULTS

Effect of ursodeoxycholate on bile flow and bile acid secretion

Sequential changes in bile flow and bile acid secretion in control rats and rats with ursodeoxycholate infusion are shown in Fig. 1. Administration of the bile acid caused a choleretic effect with a maximum at 30–40 min following the beginning of the infusion, that represented a 0.8-fold or 1.7-fold increase for the doses of 0.5 μmol/min/100 g or 1.0 μmol/min/100 g respectively. Bile acid secretion was also significantly increased. At a bile acid infusion of 0.5 μmol/min/100 g, bile acid output was equal to the sum of baseline excretion plus ursodeoxycholate infusion. When ursodeoxycholate was increased to 1.0 μmol/100 g/min, bile acid output fell to approximately 80% of this amount.

Effect of ursodeoxycholate on the secretion of bilirubin

Basal bilirubin concentration was significantly reduced following both doses of the bile acid, with no significant modification in bilirubin output with respect to the control animals (Table 1). During the saturating load of bilirubin, the maximal bilirubin concentration was reduced in animals receiving ursodeoxycholate at 0.5 μmol/min/100 g (−39%), with a 24% increase in the T_m of bilirubin. When ursodeoxycholate was given at 1.0 μmol/min/100 g, both the maximal bilirubin concentration and T_m were significantly reduced (−73% and −21%, respectively) (Table 1, Fig. 2).

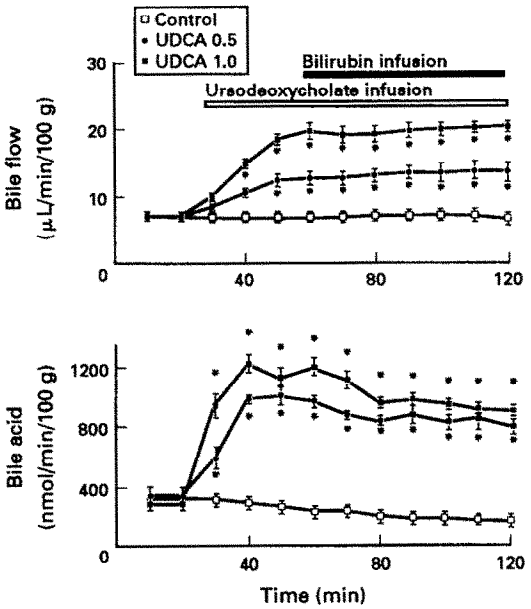

Fig. 1. Bile flow and bile acid secretion in control and ursodeoxycholate-infused rats (UDCA 0.5 μmol/min/100 g and UDCA 1.0 μmol/min/100 g). Values are means ± SE from five to eight rats. *P < 0.05 significantly different from control value.

During the 60 min period of bilirubin infusion, 18.8 μmol of unconjugated bilirubin were given per 100 g body wt. The cumulative excretion of bilirubin in bile over the same period is represented in Table 2. Infusion of ursodeoxycholate at 0.5 μmol/min/100 g caused a 25% increase in the cumulative excretion of the pigment. However, when the bile acid was increased to 1.0 μmol/min/100 g, excretion fell by 18% with respect to the controls. The recovery of infused bilirubin was a 50.9% in the controls vs 63.8% and 41.9% for the low and high doses of ursodeoxycholate, respectively. Figure 3 shows the maximal biliary secretion rate of the various bile pigments in control and bile acid-infused rats. In animals with a bile acid infusion of 0.5 μmol/min/100 g the output of unconjugated bilirubin fell by 56% with respect to the controls, while there was a significant increase in the output of conjugates (+30%) and in the diconjugates/monoconjugates ratio (+31%). Following administration of ursodeoxycholate at 1.0 μmol/min/100 g both the excretion of unconjugated bilirubin and that of bilirubin conjugates were significantly reduced (−60% and −18%, respectively). The diconjugates/monoconjugates ratio fell by 27%.

Effects of ursodeoxycholate on bilirubin UDP-glucuronosyltransferase activity and on bile pigments in serum and liver

Bilirubin UDP-glucuronosyltransferase activity was slightly and non-significantly reduced at the ursodeoxycholate dose of 0.5 μmol/min/100 g (Table

Table 1. Bilirubin conjugation and biliary secretion in control and ursodeoxycholate-infused rats

	Control	Ursodeoxycholate	
		0.5 μmol/min/100 g	1.0 μmol/min/100 g
UDP-glucuronosyltransferase activity			
(nmol/min/g liver)	59 ± 4	54 ± 4	45 ± 3*
(nmol/min/mg protein)	0.40 ± 0.02	0.36 ± 0.02	0.29 ± 0.03*
Biliary bilirubin concentration			
Basal (μmol/L)	125 ± 12	68 ± 4*	46 ± 7*
At T_m (mmol/L)	26.3 ± 1.6	15.9 ± 1.4*	7.0 ± 0.4*
Biliary bilirubin excretion			
Basal (nmol/min/100 g)	0.80 ± 0.06	0.84 ± 0.07	0.85 ± 0.10
At T_m (nmol/min/100 g)	176 ± 8	218 ± 11*	139 ± 8*

Values are means ± SE from five to eight rats. Basal values correspond to the last 20 min of ursodeoxycholate infusion alone and T_m values to the 30–60 min of bilirubin plus ursodeoxycholate infusion.

* $P < 0.05$ significantly different from control value.

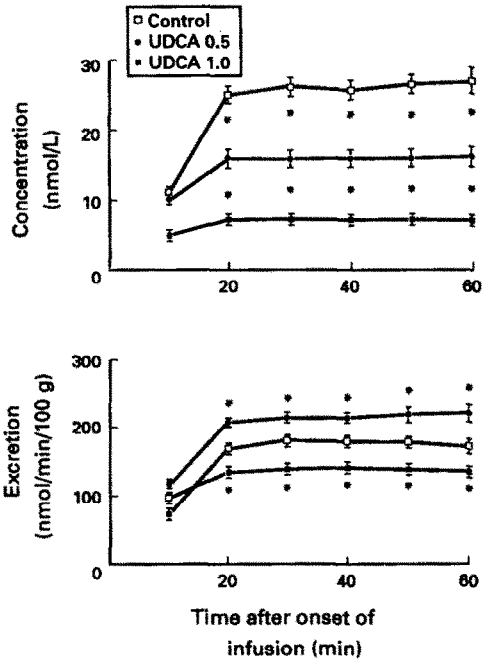

Fig. 2. Biliary bilirubin concentration and secretion in control and ursodeoxycholate-infused rats (UDCA 0.5 μmol/min/100 g and UDCA 1.0 μmol/min/100 g) during an already established condition of maximal bilirubin secretion. Values are means ± SE from five to eight rats.
*$P < 0.05$ significantly different from control value.

1). The higher dose of the bile acid caused a significant reduction of the enzyme activity (−24%) (Table 1). At the end of the experiments total bilirubin concentration in serum fell by 17% in animals receiving ursodeoxycholate at 0.5 μmol/min/100 g (Table 2). This decrease corresponded to a pronounced reduction of the concentration of unconjugated bilirubin, whose proportion amounted to 80% of total pigment in serum in contrast to the 86% found in the controls. At the higher infusion of bile acid, total serum bilirubin was increased with respect to the controls by 19%. Both unconjugated and conjugated bilirubin concentration were significantly increased (Table 2).

Table 2 also summarizes the bilirubin concentrations in liver at the end of the experiments. Control rats had the highest concentration of bilirubin in the liver. Rats infused with both doses of ursodeoxycholate showed a significant reduction of bilirubin concentration (−16% and −20%, respectively, for the low and high dose).

DISCUSSION

The interaction between bile acids and other organic anions in the course of their hepatobiliary transport is not clear. Conflicting results have been obtained that could partially be accounted for in terms of differences in species, substrate or the experimental conditions employed. Among the bile acids, ursodeoxycholate is one that is used in clinical practice for the treatment of different hepatic pathologies such as hepatolithiasis, primary biliary cirrhosis or primary sclerosing cholangitis [22–24]. However, the information regarding its physiological properties is scarce compared with that dealing with other bile acids.

In this study, it was seen that following administration of a dose of 0.5 μmol/min/100 g a stimulation occurred in the T_m of bilirubin, accompanied by reductions in the plasma and hepatic concentrations of the compound. Previous studies have shown that tauroursodeoxycholate is able to facilitate the excretion of taurocholate when both bile acids are perfused simultaneously [31]. The administration of tauroursodeoxycholate also stimulates the T_m of sulfobromophthalein in the rat [19]. Although in the hamster both ursodeoxycholate and tauroursodeoxycholate have been shown to be lacking in a stimulatory effect on the biliary excretion of sulfobromophthalein [17], this is probably related to

Table 2. Parameters of bilirubin metabolism during maximal biliary secretion (T_m) in control and ursodeoxycholate-infused rats

	Control	Ursodeoxycholate	
		0.5 μmol/min/100 g	1.0 μmol/min/100 g
Cumulative biliary excretion of infused bilirubin (μmol/100 g)	9.6 ± 0.9	12.0 ± 0.8*	7.9 ± 0.6*
Hepatic bilirubin concentration (μmol/g liver)	0.55 ± 0.03	0.46 ± 0.03*	0.44 ± 0.02*
Plasma bilirubin concentration (nmol/mL)			
Unconjugated	686 ± 24	543 ± 19	791 ± 38*
Conjugated	100 ± 16	111 ± 18	145 ± 12*

Values are means ± SE from four to six rats. Plasma and liver values were obtained after the 1-hr infusion of bilirubin.
* $P < 0.05$ significantly different from control value.

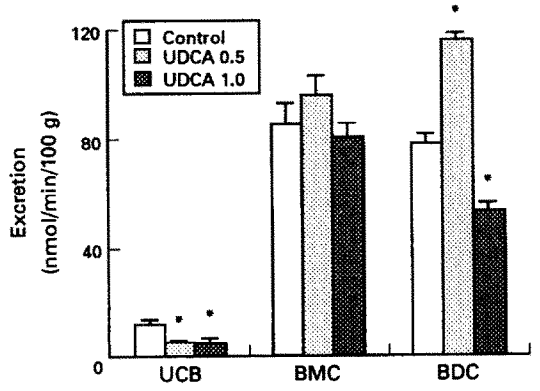

Fig. 3. Biliary excretion of unconjugated bilirubin (UCB) and bilirubin mono- (BMC) and diconjugates (BDC) during bilirubin T_m in control and ursodeoxycholate-infused rats (UDCA 0.5 μmol/min/100 g and UDCA 1.0 μmol/min/100 g). Values are means ± SE from five to eight rats and correspond to the 30–60 min of ursodeoxycholate plus bilirubin infusion. *$P < 0.05$ significantly different from control value.

the low capacity of this animal species to excrete these bile acids [19].

The exact mechanism by which ursodeoxycholate stimulates the T_m of bilirubin is difficult to elucidate. Several hypotheses have been put forward to explain the increase detected in the biliary excretion of the pigment following the administration of other bile acids: uptake into mixed micelles [32], recruitment of centrilobular hepatocytes [8] or intracellular interaction of bile acids and bile pigments [9]. It has been proposed that the maximal rate at which bilirubin and other organic anions can be excreted depends on the balance between the intrinsic secretory capacity of the liver and the potential toxicity of the organic anion [33] and that near physiological doses of bile acid could counteract the possible toxic effects exerted by the organic anions in the excretory mechanisms [21]. Recently, Crawford and Gollan [5] have suggested that following bile acid infusion, a microtubule-dependent vesicular system able to increase the transhepatic transport and biliary excretion of pigments would be recruited. This system would only play a minor role in the transport and metabolism of bile pigments in the basal state. This hypothesis is based on the fact that colchicine administration to bile acid-depleted/infused rats significantly delays the excretion of bilirubin conjugates, an effect that is not observed in control animals [5]. In this sense it has been recently demonstrated by gel permeation chromatography that conjugated bilirubin presents a high degree of vesicular association in bile [34]. If confirmed, the mechanism could be applied to our results, assuming that part of the conjugates generated inside the hepatocytes could be cotransported together with ursodeoxycholate to the canalicular membrane via a microtubule-dependent system.

In our experiments not only did an increase in the bilirubin occur after infusion of the bile acid at a dose of 0.5 μmol/min/100 g but also there was a significant increase in the bilirubin diconjugates/monoconjugates ratio. Different studies have described a relationship between this ratio and UDP-glucuronosyltransferase activity, which confirms the important role of the enzyme in the regulation of the synthesis of bilirubin glucuronides and in their pattern of biliary excretion [35]. However, certain other factors, such as intracellular binding and biliary secretion may also affect this pattern [36]. In fact, in many species, the diconjugates/monoconjugates ratio is greater in bile than in plasma owing to the preferential excretion of diconjugates; this has been attributed to an interaction of the latter with the secretory apparatus or a lower degree of binding to ligandin [36]. Both bilirubin and bile acids are able to interact with glutathione *S*-transferases [37] and may even compete for common binding sites [38]. Following ursodeoxycholate infusion, therefore, one could think in terms of a reduction of bilirubin binding, which together with the above-mentioned recruitment of secretory mechanisms would stimulate the secretion of diconjugates to a greater extent that that of monoconjugates.

When ursodeoxycholate is administered at a dose

of 1.0 μmol/min/100 g a reduction occurs in the T_m of bilirubin accompanied by an increase in the plasma levels and a decrease in the hepatic concentration of the pigment. The fact that both biliary excretion and hepatic levels are reduced and that the levels of unconjugated bilirubin in plasma rise suggest that the hepatic removal rate of the pigment is reduced. A similar effect has been reported with respect to the hepatic transport of sulfobromophthalein after infusion of high doses of tauroursodeoxycholate [19]. Along the same lines, the studies of different authors suggest that high loads of taurocholate are able to inhibit the process of hepatic uptake of different organic anions such as ioglycamide [21], sulfobromophthalein or indocyanine green [18].

In any case, this is not the only possible explanation of the inhibitory effect of ursodeoxycholate on the hepatic transport of bilirubin and conjectures could also be made about effects on conjugation with the glycosyl moieties and/or of transport to the canaliculi and biliary excretion. In our experiments, a significant reduction was observed in glucuronosyl transferase activity and hence in the conjugation process of the pigment. Conjugation is the limiting factor in hepatobiliary transport, as shown in the relationship between T_m and enzyme activity under different experimental conditions [35, 39]. The inhibition of conjugation would furthermore help to explain the lower diconjugates/monoconjugates ratio observed after a high load of ursodeoxycholate. This ratio has been shown to increase in bile and plasma following administration of glucuronosyltransferase inducers such as phenobarbital or glutethimide [35] and a linear correlation between enzyme activity and the diconjugates/monoconjugates ratio in liver homogenates of hypo- or hyperthyroid rats has been reported [36]. It is known that glucuronidation of bilirubin in rat liver microsomes is modulated by physicochemical properties of the microsomal membrane [40]. Alterations in the lipid composition/fluidity of these membranes induced by ursodeoxycholate could help to explain the reduced activity of the enzyme found in our experiments.

Finally, a reduction in biliary excretion by the high dose of the bile acid could be proposed. This would contribute to the decrease in the T_m and hepatic removal rate of the pigment. Such a mechanism could account for the higher concentration of conjugated bilirubin detected in plasma as a consequence of its reflux from the liver compartment. However, the direct inhibitory effect on biliary excretion would not be unique, since if this were the case one would expect an increase and not a decrease in the hepatic concentrations of the pigment.

The cause of the inhibitory effect of ursodeoxycholate on the different phases of the hepatobiliary transport of bilirubin remains unresolved. In our experiments the dose of 1.0 μmol/min/100 g of the bile acid was close to amounts that lead to saturation of its biliary excretory capacity. Nevertheless, it would not appear to lead to a non-specific toxicity at membrane level since the choleretic effect induced by the bile acid persisted. It has been recently reported that under certain conditions increased amounts of glucuronide conjugates of ursodeoxycholate, whose biliary secretory processes may differ from those of the amino acid conjugates, can be formed in rat liver [41, 42]. Although the synthesis and secretion of these metabolites could be responsible for the impairment detected in bilirubin conjugation or excretion, this hypothesis needs further experimental support.

In summary, our results corroborate the complexity of the interactions between bile acids and the hepatic transport of organic anions. Ursodeoxycholate administration leads to dose-dependent alterations in the biliary excretion of bilirubin, with both stimulatory and inhibitory effects on the different steps of the hepatobiliary transfer of the pigment.

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