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Effect of sodium tauroursodeoxycholate (UR-906) on liver dysfunction in bile duct-ligated rats

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Abstract

We investigated the effect of sodium tauroursodeoxycholate (UR-906) on cholestasis in common bile duct-ligated rats in comparison with the effect of dehydrocholic acid. UR-906 (30–180 μmol/kg) and dehydrocholic acid (180 μmol/kg) were intravenously given once daily for consecutive 20 days in rats and the common bile duct was ligated for the last 10 days. On the next day after the last test drug administration, serum biochemical and plasma hemostatic variables were determined. UR-906 significantly ameliorated the elevation of serum cholesterol, phospholipid, bilirubin and bile acid concentrations in bile duct-ligated rats. UR-906 significantly suppressed the prolongation of plasma prothrombin time and activated partial thromboplastin time. Furthermore, UR-906 significantly suppressed the decreases in plasma coagulation factor II and X activities. However, dehydrocholic acid did not cause significant changes in any of the variables examined in this model. These results suggest that UR-906 has a beneficial effect against cholestasis induced by bile duct ligation in rats and that this drug may be useful in the treatment of clinical cholestatic disorders. © 1997 Elsevier Science B.V.

Keywords: Bile duct ligation; Sodium tauroursodeoxycholate (UR-906); Dehydrocholic acid; Blood coagulation factor; Cholestasis; (Rat)

1. Introduction

It is well established that ursodeoxycholic acid can improve liver dysfunction in patients with cholestatic disorders including primary biliary cirrhosis (Leuschner et al., 1989; Poupon et al., 1991) and primary sclerosing cholangitis (Chazouillères et al., 1990). In experimental animals, ursodeoxycholic acid is also known to have an anticholestatic effect and a protective action against the hepatocyte toxicity induced by various agents such as estrogen (Bouchard et al., 1993) and taurochenodeoxycholic acid (Tsukahara et al., 1993). We have recently reported that ursodeoxycholic acid can exert a beneficial action against α-naphthylisothiocyanate-induced cholestasis in mice (Kinbara et al., 1993). Thus the beneficial effect of ursodeoxycholic acid against liver dysfunction in both humans and experimental animals has been confirmed by many investigators.

Sodium tauroursodeoxycholate (UR-906) is the taurine conjugate of ursodeoxycholic acid. UR-906 is known to prevent biliary protein excretion induced by taurochenodeoxycholic acid (Kitani et al., 1985), taurolithocholic acid-induced cholestasis (Schölmerich et al., 1990), α-naphthylisothiocyanate-induced cholestasis (Kinbara et al., 1993) and cyclosporin A-induced cholestasis (Queneau et al., 1993) in experimental animals and has a cytoprotective effect against taurochenodeoxycholic acid-induced toxicity in vitro studies (Heuman et al., 1991; Ohiwa et al., 1993). Furthermore, Krol et al. (1983) demonstrated that UR-906 significantly reduced ductular proliferation and portal inflammation in bile duct-ligated hamsters. These observations suggest that UR-906 as well as ursodeoxycholic acid can prevent cholestasis. However, the biochemical mechanisms of the protective effect of UR-906 against cholestasis and liver dysfunction are not fully understood. Dehydrocholic acid as well as UR-906 is known to have a potent choleretic effect (Utili et al., 1990; Yousef et al., 1990). Furthermore, dehydrocholic acid is clinically used as a choleretic (Mitchell and Torrance, 1966; Alkabes et

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al., 1980; Okolicsanyi et al., 1986) and is reported to be important in the postoperative care of patients with biliary atresia (Mochizuki et al., 1986). Therefore, the present study was carried out to further define the protective effect of intravenous treatment with UR-906 against cholestasis in comparison with the effect of dehydrocholic acid. For this purpose, we used common bile duct ligation to produce extrahepatic cholestasis in rats.

2. Materials and methods

2.1. Experimental animals and chemicals

Male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing 250–300 g (aged 12 weeks) were used in this study. The animals were allowed free access to standard laboratory chow (Japan CLEA CE-2, Tokyo, Japan) and tap water throughout the experiments. UR-906 and dehydrocholic acid were obtained from our laboratories (Tokyo Tanabe, Tokyo, Japan). These agents had a purity higher than 99%. UR-906 was dissolved in physiological saline. Dehydrocholic acid was dissolved in 0.5 M NaOH and adjusted to pH 9.0 with 0.5 M HCl.

2.2. Experimental procedures

2.2.1. Preliminary study

In order to determine the duration of common bile duct ligation, rats were anesthetized with sodium pentobarbital (Nembutal, 40 mg/kg i.p., Abbott, North Chicago, IL, USA). The abdomen was opened carefully and a 3 cm incision was made just below the xyphoid process. The common bile duct was exposed gently and a 1 cm section was excised after double ligature with two silk sutures. Pentcillin® (Toyama Chemical, Tokyo, Japan) at a dose of 0.8 g/kg was given intraperitoneally to protect against infection. Blood samples were obtained by cardiac puncture under light ether anesthesia on the 1st, 3rd, 5th and 10th day after bile duct ligation. Serum glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, leucine aminopeptidase and γ-glutamyl transpeptidase activities and cholesterol, phospholipid, bilirubin and bile acid concentrations were measured as described below. Each group consisted of three to six rats.

2.2.2. Drug therapy study

UR-906 (30, 90 and 180 μmol/kg), dehydrocholic acid (180 μmol/kg) and vehicle (saline) were administered intravenously (i.v.) into the tail vein of rats once daily for 10 consecutive days. On the next day, the common bile duct was ligated as described above. All groups then received the same dose of their respective test drug once daily for 10 consecutive days after ligation. Sham-operated animals were treated in the same manner except for the ligation of the bile duct and the treatment with drug. On the next day after the last treatment with test drugs, the rats were anesthetized with ether, and blood samples were collected by cardiac puncture directly into disposable syringes for determination of serum variables and into disposable syringes containing 3.8% sodium citrate solution to make 10% citrate blood for the determination of plasma hemostatic variables.

2.3. Analytical methods

Serum biochemical variables were measured with commercial kits (Daiichi Pure Chemicals, Tokyo, Japan). Serum glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, leucine aminopeptidase and γ-glutamyl transpeptidase activities and serum cholesterol, phospholipid, bile acid and bilirubin concentrations were all measured using a Hitachi automatic analyzer (Hitachi, Tokyo, Japan). Serum bile acid was measured by an enzymatic method (Osuga et al., 1977), using 3α-hydroxysteroid dehydrogenase. Plasma prothrombin time, activated partial thromboplastin time and coagulation factor X activities were measured using Neoplastin®, PTT reagent [BMY]® (Boeringer-Mannheim–Yamanouchi, Tokyo, Japan) and Testteam FX® (Daiichi Pure Chemicals), respectively. Factor II activity was determined by using a one-stage clotting assay with human plasma deficient in factor II (George King, USA). Factor II and factor X activities were expressed as percentages of those of sham-operated values (= 100%).

2.4. Statistical analysis

Data are expressed as means ± S.D. Statistical comparisons were made using Dunnett's multiple comparison test.

3. Results

3.1. Effect of bile duct ligation on serum biochemical variables

As shown in Table 1, serum glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase and leucine aminopeptidase activities and bile acid concentration showed a maximal elevation in rats on day 1 after bile duct ligation. Serum cholesterol and phospholipid concentrations showed a maximal elevation on day 2 after bile duct ligation. Thereafter, the values for these variables gradually decreased. However, a significant elevation in serum alkaline phosphatase activity and bile acid, cholesterol and phospholipid concentrations was still evident on day 10 after bile duct ligation. In contrast, serum bilirubin concentration and γ-glutamyl transpeptidase activity significantly increased throughout the experi-

Table 1
Effect of duration of bile duct ligation on serum variables in rats

	Intact (3)	1 day (6)	3 days (4)	5 days (5)	10 days (6)
Glutamic oxaloacetic transaminase (GOT) (U/L)	85 ± 15	1528 ± 428 [b]	714 ± 55	469 ± 83	859 ± 879
Glutamic pyruvic transaminase (GPT) (U/L)	31 ± 3	1020 ± 227 [b]	476 ± 56 [a]	193 ± 49	359 ± 372
Alkaline phosphatase (ALP) (U/L)	642 ± 55	2807 ± 368 [b]	1499 ± 186 [b]	1260 ± 124 [a]	1329 ± 290 [b]
Leucine aminopeptidase (LAP) (U/L)	62.8 ± 4.4	87.7 ± 7.5 [b]	83.9 ± 2.9 [b]	86.5 ± 11.1 [b]	61.7 ± 8.2
γ-Glutamyl transpeptidase (γ-GTP) (U/L)	0.13 ± 0.12	5.19 ± 1.74	6.52 ± 2.21 [b]	10.6 ± 3.5 [b]	16.0 ± 5.5 [b]
Cholesterol (mg/dl)	49 ± 2	99 ± 4 [b]	203 ± 17 [b]	169 ± 23 [b]	130 ± 19 [b]
Phospholipid (mg/dl)	140 ± 3	232 ± 17 [a]	374 ± 28 [b]	346 ± 61 [b]	311 ± 84 [b]
Bilirubin (mg/dl)	0.04 ± 0.01	4.71 ± 0.48 [b]	7.70 ± 0.70 [b]	8.46 ± 1.11 [b]	10.0 ± 2.5 [b]
Bile acid (μmol/l)	3.1 ± 1.2	240 ± 48 [b]	170 ± 23 [b]	166 ± 40 [b]	104 ± 28 [b]

Values are expressed as means ± S.D. The number of animals is in parentheses.
[a] $P < 0.05$; [b] $P < 0.01$ different from corresponding values in intact animals (Dunnett's multiple comparison test).

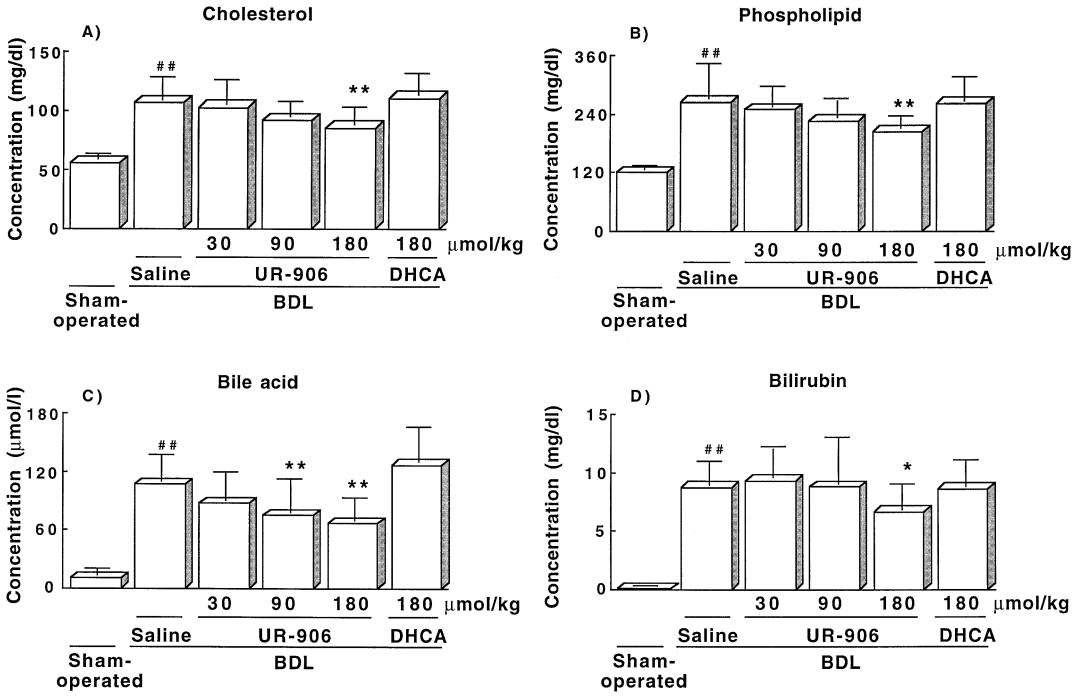

Fig. 1. Effect of UR-906 and dehydrocholic acid (DHCA) on serum cholesterol (A), phospholipid (B), bile acid (C) and bilirubin (D) concentrations in rats subjected to bile duct ligation (BDL). The rats were administered saline, UR-906 (30–180 μmol/kg, i.v.) and DHCA (180 μmol/kg, i.v.) daily for 10 days before and 10 days after BDL, respectively. Values are expressed as means ± S.D. $n = 10–14$. $^{*}P < 0.05$, $^{**}P < 0.01$ difference from corresponding values in saline + BDL animals; $^{\#\#}P < 0.01$ difference from corresponding values in sham-operated animals (Dunnett's multiple comparison test).

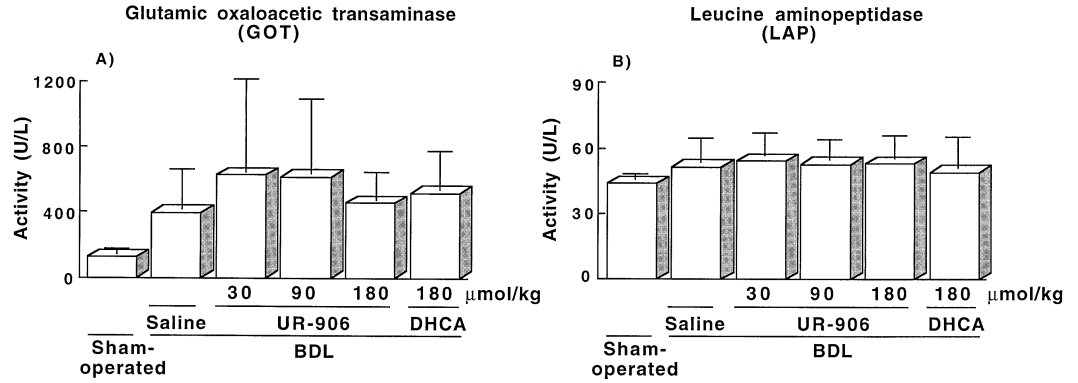

Fig. 2. Effect of UR-906 and dehydrocholic acid (DHCA) on serum glutamic oxaloacetic transaminase (GOT) (A) and leucine aminopeptidase (LAP) (B) activities in rats subjected to bile duct ligation (BDL). The rats were administered saline, UR-906 (30–180 μmol/kg, i.v.) and DHCA (180 μmol/kg, i.v.) daily for 10 days before and 10 days after BDL, respectively. Values are expressed as means ± S.D. $n = 10–14$.

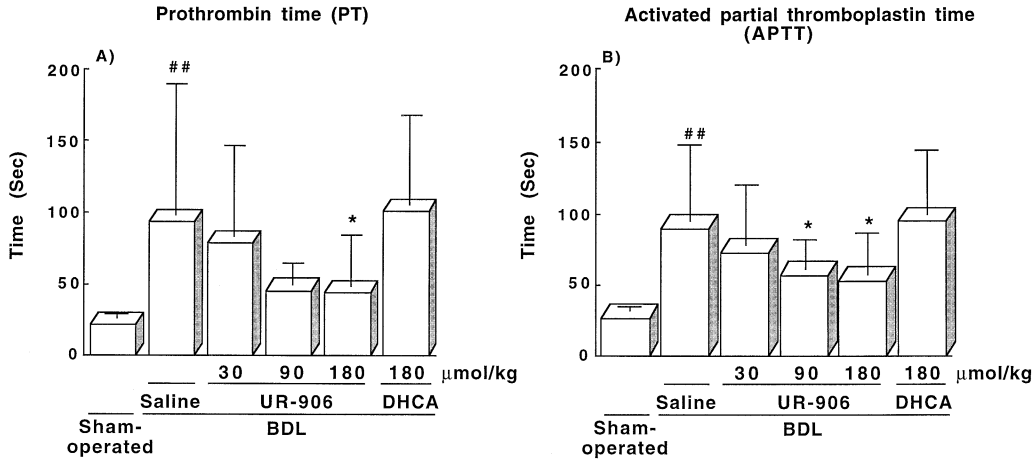

Fig. 3. Effect of UR-906 and dehydrocholic acid (DHCA) on plasma prothrombin time (PT) (A) and activated partial thromboplastin time (APTT) (B) in rats subjected to bile duct ligation (BDL). The rats were administered saline, UR-906 (30–180 μmol/kg, i.v.) and DHCA (180 μmol/kg, i.v.) daily for 10 days before and 10 days after BDL, respectively. Values are expressed as means ± S.D. $n = 10–14$. * $P < 0.05$ difference from corresponding values in saline + BDL animals; $^{\#\#}$ $P < 0.01$ difference from corresponding values in sham-operated animals (Dunnett's multiple comparison test).

ments, and the maximal elevation in serum bilirubin concentration and γ-glutamyl transpeptidase activity was observed in rats on day 10 after bile duct ligation.

3.2. Drug therapy study

3.2.1. Effect of UR-906 and dehydrocholic acid on serum biochemical variables in bile duct-ligated rats

As shown in Figs. 1 and 2, bile duct-ligated rats showed a significant increase in serum cholesterol, phospholipid, bile acid and bilirubin concentrations on day 10 after bile duct ligation as compared with sham-operated animals. However, no significant change in glutamic oxaloacetic transaminase and leucine aminopeptidase activities was seen in bile duct-ligated rats. In contrast, UR-906 prevented the significant elevation in cholesterol, phospholipid and bile acid concentrations in a dose-dependent manner. UR-906 at a dose of 180 μmol/kg also reduced the significant increase in bilirubin concentration caused by bile duct ligation. However, this drug produced no change in glutamic oxaloacetic transaminase and leucine aminopeptidase activities. Dehydrocholic acid did not cause significant changes in the values of any of the variables examined.

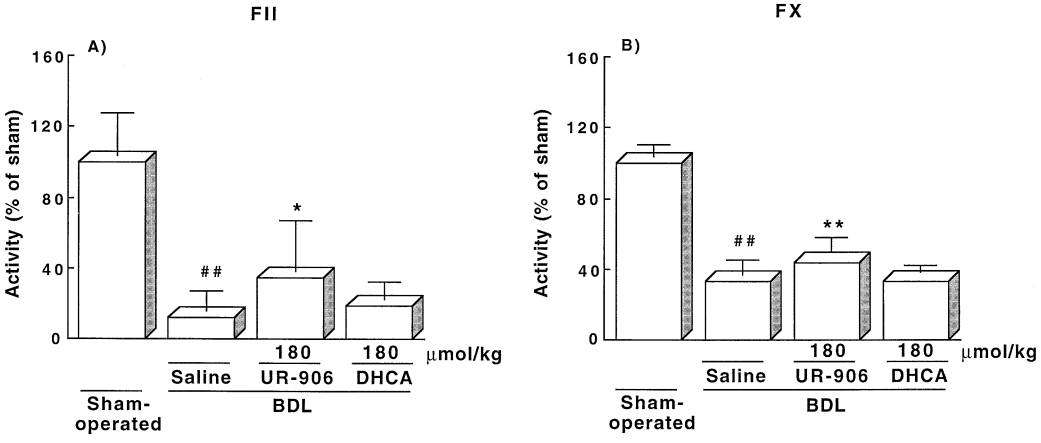

Fig. 4. Effect of UR-906 and dehydrocholic acid (DHCA) on plasma coagulation factor II (A) and factor X (B) activities in rats subjected to bile duct ligation (BDL). The rats were administered saline, UR-906 (180 μmol/kg, i.v.) and DHCA (180 μmol/kg, i.v.) daily for 10 days before and 10 days after BDL, respectively. Values are expressed as means ± S.D. $n = 9–10$. * $P < 0.05$, ** $P < 0.01$ difference from corresponding values in saline + BDL animals; $^{\#\#}$ $P < 0.01$ difference from corresponding values in sham-operated animals (Dunnett's multiple comparison test).

3.2.2. Effect of UR-906 and dehydrocholic acid on plasma hemostatic variables in bile duct-ligated rats

As shown in Fig. 3, bile duct-ligated rats showed a significant prolongation of plasma prothrombin time and activated partial thromboplastin time on day 10 after bile duct ligation as compared with sham-operated animals. In contrast, the administration of UR-906 dose-dependently reduced the significant prolongation of plasma prothrombin time and activated partial thromboplastin time. Individual plasma coagulation factor activity is depicted in Fig. 4. Bile duct-ligated rats showed a significant decrease in plasma factor II and X activities on day 10 after bile duct ligation as compared with sham-operated rats. UR-906 at a dose of 180 μmol/kg reversed the significant decrease in factor II and factor X activities in bile duct-ligated rats. Dehydrocholic acid did not significantly affect any of the plasma hemostatic variables in comparison with those of rats with bile duct ligation only.

4. Discussion

Many experimental studies have demonstrated that ligation of the common bile duct can easily cause severe cholestasis in rats. For example, marked changes in serum cholesterol, bile acids and bilirubin were seen in rats after ligation of the bile duct (Kountouras et al., 1984; Dueland et al., 1991). Therefore, bile duct-ligated animals are used as a good and reliable model of extrahepatic biliary obstruction in humans for assessing the therapeutic effect of pharmacological drugs (Krol et al., 1983; Frezza et al., 1993). Our preliminary study also indicated that bile duct-ligated rats showed a marked change in all serum variables (glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, leucine aminopeptidase, γ-glutamyl transpeptidase, cholesterol, phospholipid, bilirubin and bile acid) from 1 day after bile duct ligation. A significant change in most of these variables was still evident after 10 days. Furthermore, no animals died until 10 days after bile duct ligation. This finding was, at least in part, consistent with the report of Kinugasa et al. (1981), who demonstrated that the mean life span after bile duct ligation was 23.2 days in their rats. In order to evaluate the effect of UR-906 and dehydrocholic acid, therefore, we used rats subjected to bile duct ligation for 10 days as a model of acute cholestasis.

In the present study, UR-906 treatment caused a significant reduction in most of the biochemical variables as compared to those of bile duct-ligated rats. This effect of UR-906 was especially noted at high dose. These findings suggest that UR-906 can prevent the changes in biochemical variables induced by bile duct ligation. The present study also showed that serum bile acid concentration was low in UR-906-treated bile duct-ligated rats despite the administration of bile acid. In addition, we confirmed that the bile acid content of the liver significantly increased in rats after bile duct ligation, while UR-906 reduced the accumulation of bile acid. Furthermore, UR-906 significantly increased urinary bilirubin excretion in this model (data not shown). These findings suggest that UR-906 treatment may partially ameliorate acute cholestasis by enhancing an alternative pathway of bile salt excretion, for example, the renal route.

It is generally considered that there is clinically a significant correlation between the changes in the hemostatic variables and the degree of liver injury (Cornillon et al., 1985). The hemostatic variables such as prothrombin time and activated partial thromboplastin time are routinely used to screen patients with liver diseases including obstructive jaundice (Clark et al., 1973; Allison et al., 1979; Mba et al., 1990). For these hemostatic variables, Kambayashi et al. (1985) demonstrated that rats subjected to bile duct ligation for 7 days showed a marked reduction in the value of the hepaplastin test and prolongation of the activated partial thromboplastin time. A previous study (Cornillon et al., 1985) also reported that severe defects in coagulation factors, such as factor II and factor X, may be related to an impairment of protein synthesis in hepatocytes. The present study showed that bile duct ligation for 10 days caused marked changes in the hemostatic variables. In contrast, UR-906 treatment significantly suppressed these changes in bile duct-ligated rats. These findings seem to suggest that a significant prolongation of prothrombin time and activated partial thromboplastin time results from a decrease in coagulation factors produced by hepatocytes and that changes in these hemostatic variables may reflect impairment of liver function in our model.

Numerous biochemical studies have addressed whether ursodeoxycholic acid and its taurine conjugate have a beneficial effect on bile duct-ligated animals (Krol et al., 1983; Poo et al., 1992; Zimmermann and Reichen, 1992; Frezza et al., 1993). Recent studies reported that ursodeoxycholic acid has a beneficial effect on liver disease and cholestasis in bile duct-ligated rats (Poo et al., 1992; Frezza et al., 1993). In contrast, Zimmermann and Reichen (1992) reported that ursodeoxycholic acid had no beneficial effect on liver dysfunction and histological damage in bile duct-ligated rats. Thus, evidence for a protective effect against the liver dysfunction seen in bile duct-ligated rats is contradictory. In the present study, UR-906 treatment had a partially protective effect against liver dysfunction in bile duct-ligated rats. This finding is, at least in part, consistent with the findings of Poo et al. (1992) and Frezza et al. (1993). Therefore, we speculate that ursodeoxycholic acid and its taurine conjugate UR-906 may have a beneficial effect against the dysfunction and histological damage of the liver seen in bile duct-ligated rats.

Dehydrocholic acid had no effect on the changes in serum variables and on the alterations in hemostatic variables in bile duct-ligated rats. Dehydrocholic acid is thought to exert a choleretic effect associated with impairment of

the secretion of endogenous or exogenous bile acid, which results in impairment of biliary lipid secretion (Yousef et al., 1990; Utili et al., 1990). In contrast, UR-906 has a choleretic effect associated with enhancement of biliary bile acid secretion (Bouchard et al., 1993). Furthermore, Ohiwa et al. (1993) reported that UR-906, but not taurodehydrocholic acid, has a cytoprotective effect against taurochenodeoxycholic acid-induced toxicity in primary cultured rat hepatocytes. Our previous study also demonstrated that UR-906 but not dehydrocholic acid can exert a cytoprotective effect against taurochenodeoxycholic acid-induced toxicity (data not shown). In the present study, the beneficial effect of dehydrocholic acid was not observed in bile duct-ligated rats. Therefore, the physicochemical differences between UR-906 and dehydrocholic acid seem to suggest differences in potency between the two drugs in bile duct-ligated rats.

In conclusion, the present study demonstrates that bile duct ligation can cause marked increases in the levels of serum biochemical variables and changes in the hemostatic variables. UR-906 partially ameliorated the significant elevations in the values for the serum biochemical variables and partially reversed the changes in hemostatic variables. However, dehydrocholic acid did not cause significant changes in any of the variables examined in our model. These results indicate that UR-906 has a partial protective effect against cholestasis in bile duct-ligated rats. Furthermore, our results suggest that UR-906 may be useful in the treatment of clinical cholestatic disorders.

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