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Effect of sodium tauroursodeoxycholate on phalloidin-induced cholestasis in rats

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Abstract

We investigated the therapeutic effect of tauroursodeoxycholate on phalloidin-induced cholestasis in rats. Intrahepatic cholestasis was induced by administration of phalloidin (500 μg/kg, i.p.) for 7 days. From the day of the last phalloidin injection, tauroursodeoxycholate (60–360 μmol/kg) was given intravenously twice a day for 4 days. On the next day after the last tauroursodeoxycholate administration, bile flow, serum biochemical parameters and biliary lipid excretion rates were determined. Tauroursodeoxycholate significantly suppressed the decrease in bile flow and increases in serum alkaline phosphatase, leucine aminopeptidase and glutamic pyruvic transaminase activities, cholesterol, phospholipid and bile acid concentrations observed in phalloidin-induced cholestasis in rats. Furthermore, tauroursodeoxycholate significantly improved the biliary cholesterol and phospholipid excretion rates in phalloidin-induced cholestasis in rats. These results demonstrate the usefulness of tauroursodeoxycholate as a therapeutic agent in intrahepatic cholestasis.
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Keywords: Bile flow; Tauroursodeoxycholate; Phalloidin; Cholestasis; (Rat)

1. Introduction

Ursodeoxycholic acid is widely used for the treatment of liver dysfunction in patients with acute and chronic intrahepatic cholestatic disorders (Fabris et al., 1999; Friman and Svanvik, 1994), primary biliary cirrhosis (Combes et al., 1999; Pares et al., 2000; Verma et al., 1999; Poupon et al., 1997) and primary sclerosing cholangitis (Van de Meeberg et al., 1996).

Tauroursodeoxycholate, which is a taurine conjugate of ursodeoxycholic acid, is also known to have a powerful choleretic action. Several studies have shown that pretreatment with tauroursodeoxycholate as well as ursodeoxycholic acid has beneficial effects against cholestasis induced by various agents (Kinbara et al., 1993, 1997; Queneau et al., 1994; Tsukahara et al., 1993; Jacquemin et al., 1993; Bouchard et al., 1993; Azer et al., 1995) and bile duct ligation-induced liver dysfunction (Ishizaki et al., 1997a) in experimental animals. However, little is known about the effects of post-treatment with tauroursodeoxycholate against intrahepatic cholestasis in animals.

There are many investigations (Elias et al., 1980; Vonk et al., 1982; Watanabe et al., 1983; Dubin et al., 1980; Dancker et al., 1975) showing that phalloidin, a cyclic peptide isolated from the mushroom *Amanita phalloides*, accelerates the polymerization of actin into microfilaments and produces physiological and morphological cholestasis in various experimental animals. Furthermore, a previous report suggested that widening of the actin microfilamentous zone in phalloidin-induced cholestasis in rats is consistent with the observations in the cholestatic liver of patients suffering from a familial form of the disease (Weber et al., 1981). In addition, we have recently observed (Ishizaki et al., 1997b) that elevated serum markers of cholestasis and other alterations in biliary secretion (serum alkaline phosphatase, cholesterol, bile flow, etc.), persisted even at 4 days after the last administration of phalloidin in rats. Thus, this rodent model is suitable to test therapeutic effects of tauroursodeoxycholate on intrahepatic cholestasis. In the present study, we examined the

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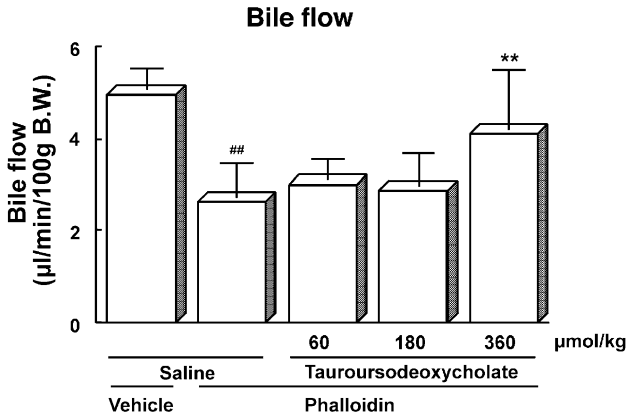

Fig. 1. Effect of tauroursodeoxycholate on bile flow in phalloidin-induced cholestasis rats. The rats were given phalloidin (500 μg/kg B.W. i.p.) for 7 days daily. Tauroursodeoxycholate (60–360 μmol/kg, i.p.) and saline were administered twice a day for 4 days from the day the last phalloidin administration, respectively. Values are expressed as means ± S.D. $n = 9–10$. $^{**}P < 0.01$ difference from value in saline + phalloidin animals; $\#\#P < 0.01$ difference from value in saline + vehicle (2% dimethylformamide-saline solution) animals (Dunnett's multiple comparison test).

therapeutic effects of intravenous administration of tauroursodeoxycholate on bile flow, serum biochemical parameters and biliary lipid excretion rates in phalloidin-treated 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 to free access to a standard laboratory chow (Japan CLEA CE-2, Tokyo, Japan) and tap water throughout the experiments. Phalloidin (Sigma, St. Louis, MO) was dissolved in 2% dimethylformamide-saline solution. Sodium tauroursodeoxycholate was obtained from our laboratories (Mitsubishi-Tokyo Pharmaceuticals, Tokyo, Japan), and its purity was higher than 99%. Tauroursodeoxycholate was dissolved in physiological saline.

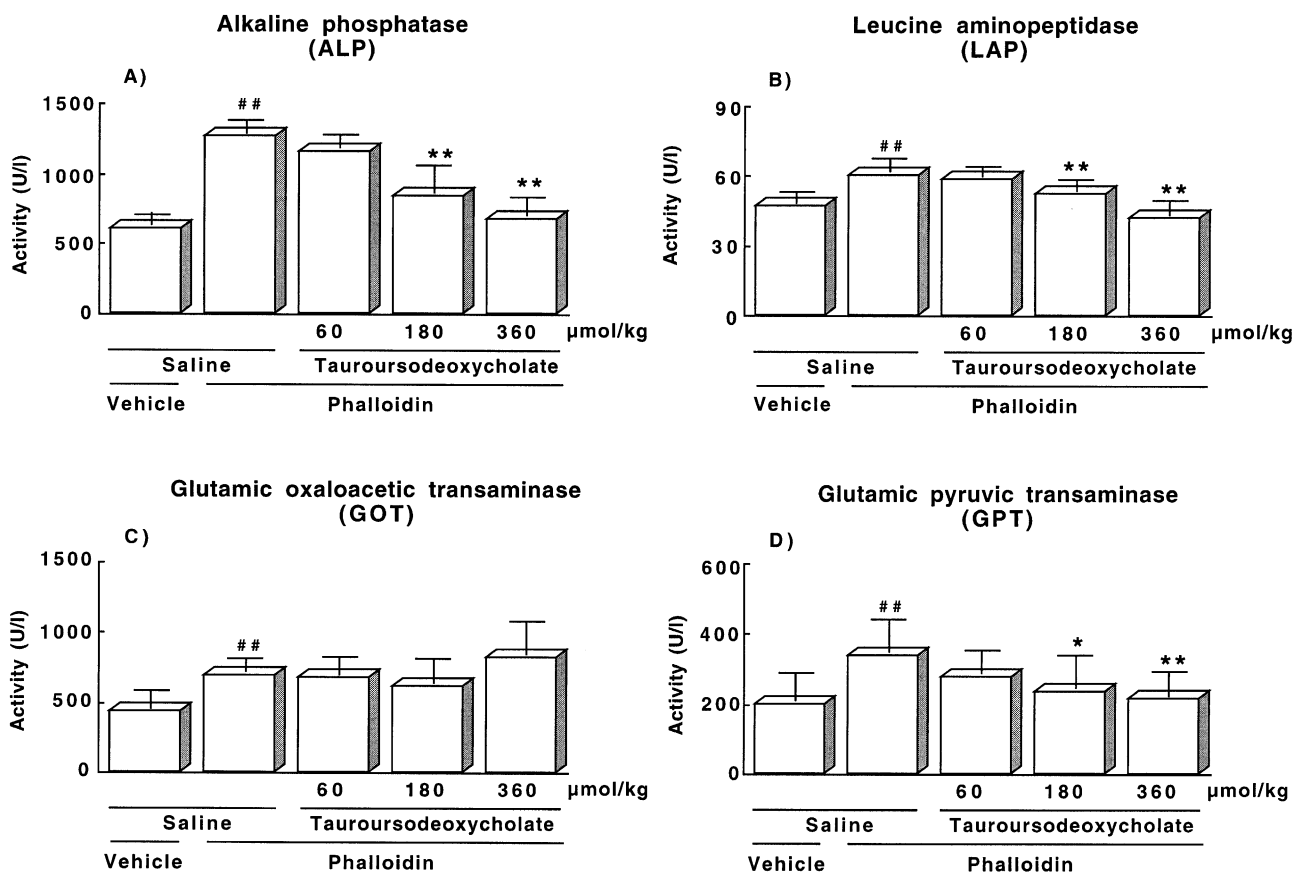

Fig. 2. Effect of tauroursodeoxycholate on serum alkaline phosphatase (ALP) (A), leucine aminopeptidase (LAP) (B), glutamic oxaloacetic transaminase (GOT) (C) and glutamic pyruvic transaminase (GPT) (D) activities in phalloidin-induced cholestasis rats. The rats were given phalloidin (500 μg/kg B.W. i.p.) for 7 days daily. Tauroursodeoxycholate (60–360 μmol/kg, i.p.) and saline were administered twice a day for 4 days from the day the last phalloidin administration, respectively. Values are expressed as means ± S.D. $n = 9–10$. $^{*}P < 0.05$, $^{**}P < 0.01$ difference from corresponding values in saline + phalloidin animals; $\#\#P < 0.01$ difference from corresponding values in saline + vehicle (2% dimethylformamide-saline solution) animals (Dunnett's multiple comparison test).

2.2. Experimental procedures

Phalloidin at a dose of 500 μg/kg was given intraperitoneally (i.p.) for 7 days in rats. Control animals received the vehicle (2% dimethylformamide-saline solution) for 7 days instead of phalloidin. Tauroursodeoxycholate (60, 180 and 360 μmol/kg) or vehicle (saline) were administered intravenously (i.v.) from the tail vein twice a day for 4 days from the day of the last phalloidin administration. On the next day after the last tauroursodeoxycholate administration, the rats were anesthetized with urethane (0.8 g/kg, i.p.). The common bile duct was then cannulated with a polyethylene SP 28 catheter (Natume, Japan) and 30 min later, bile was collected for 30 min to measure the bile flow. No correction was made for specific gravity. Rectal temperature was maintained at 37–38°C on a heating mat throughout the experiment. After the end of bile collection, the rats were anesthetized with light ether, and blood samples were obtained by cardiac puncture.

2.3. Analytical methods

For determination of biochemical parameters, commercial kits (Daiichi Chemicals, Tokyo, Japan) were used. Glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase and leucine aminopeptidase activities, cholesterol, phospholipid, bile acid and bilirubin concentrations were all measured using the Hitachi automatic analyzer (Hitachi, Japan). In order to obtain the excretion rate for biliary parameters, cholesterol, phospholipid, bile acid and bilirubin concentrations were multiplied by bile flow.

2.4. Statistical analysis

Results are expressed as means ± S.D. Statistical significances were assessed using analysis of variance (ANOVA) followed by the Dunnett's multiple comparison test. Each group contained 9–10 animals.

3. Results

3.1. Changes in bile flow

As shown in Fig. 1, phalloidin-treated rats showed a significant decrease in bile flow (52.8%) on day 4 after the last phalloidin administration as compared with control animals. In contrast, tauroursodeoxycholate at a dose of 360 μmol/kg prevented the significant decrease in bile flow (85.4%).

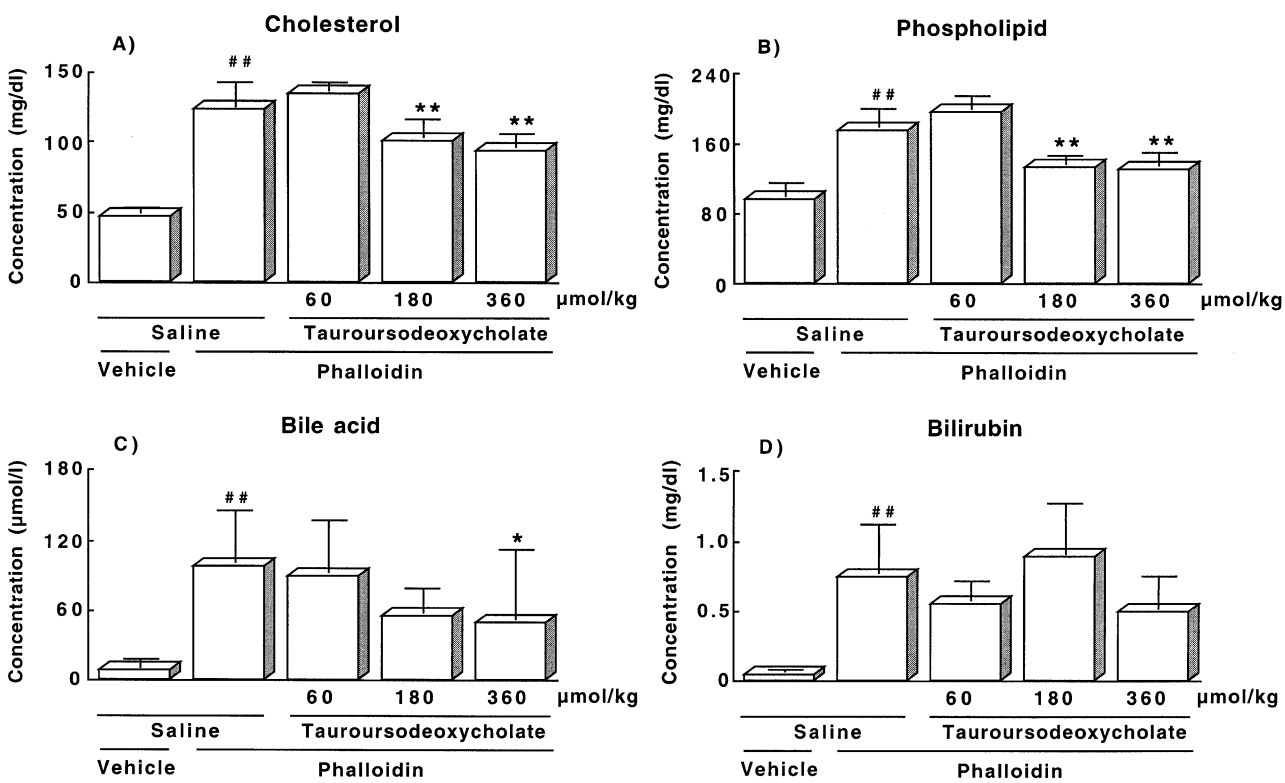

Fig. 3. Effect of tauroursodeoxycholate on serum cholesterol (A), phospholipid (B), bile acid (C) and bilirubin (D) concentrations in phalloidin-induced cholestasis rats. The rats were given phalloidin (500 μg/kg B.W. i.p.) for 7 days daily. Tauroursodeoxycholate (60–360 μmol/kg, i.p.) and saline were administered twice a day for 4 days from the day the last phalloidin administration, respectively. Values are expressed as means ± S.D. $n = 9–10$. $^{*}P < 0.05$, $^{**}P < 0.01$ difference from corresponding values in saline + phalloidin animals; $\#\#P < 0.01$ difference from corresponding values in saline + vehicle (2% dimethylformamide-saline solution) animals (Dunnett's multiple comparison test).

3.2. Changes in serum biochemical parameters

As shown in Fig. 2, phalloidin-treated rats showed significant increases in serum alkaline phosphatase, leucine aminopeptidase, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase activities on day 4 after the last phalloidin administration as compared with control animals. In contrast, tauroursodeoxycholate ameliorated the significant elevations in alkaline phosphatase, leucine aminopeptidase and glutamic pyruvic transaminase activities in a dose-dependent manner, except for glutamic oxaloacetic transaminase activity.

As shown in Fig. 3, phalloidin-treated rats showed significant increases in serum cholesterol, phospholipid, bile acid and bilirubin concentrations on day 4 after the last phalloidin administration as compared with control animals. Treatment with tauroursodeoxycholate led to significant reductions in cholesterol, phospholipid and bile acid concentrations, and the effects of tauroursodeoxycholate were observed when given at doses of 180 and 360 μmol/kg. However, this drug produced no significant change in bilirubin concentration.

3.3. Changes in biliary parameters excretion rates

As shown in Fig. 4, phalloidin-treated rats showed significant decreases in biliary cholesterol and phospholipid excretion rates on day 4 after the last phalloidin administration as compared with control animals. In contrast, tauroursodeoxycholate significantly facilitated the biliary cholesterol and phospholipid excretion rates at a dose of 360 μmol/kg. Phalloidin-treated rats showed a significant increase in biliary bilirubin excretion rate as compared with control animals. Tauroursodeoxycholate significantly increased biliary bilirubin excretion rate at doses of 180 and 360 μmol/kg. However, this drug produced no significant change in biliary bile acid excretion rate.

4. Discussion

There are some reports on the beneficial effects of various drugs such as diltiazem and indomethacin against phalloidin-induced hepatotoxicity (Vogel et al., 1984; Kobusch and Du Souich, 1990; Barriault et al., 1994; Iwu et al., 1987). However, they only evaluated lethality and/or increases in serum biochemical parameters induced by phalloidin administration. In the present study, we evaluated the therapeutic effect of tauroursodeoxycholate using the phalloidin-induced cholestasis model. Results showed that a significant decrease in bile flow was observed at 4 days after last phalloidin administration as compared with

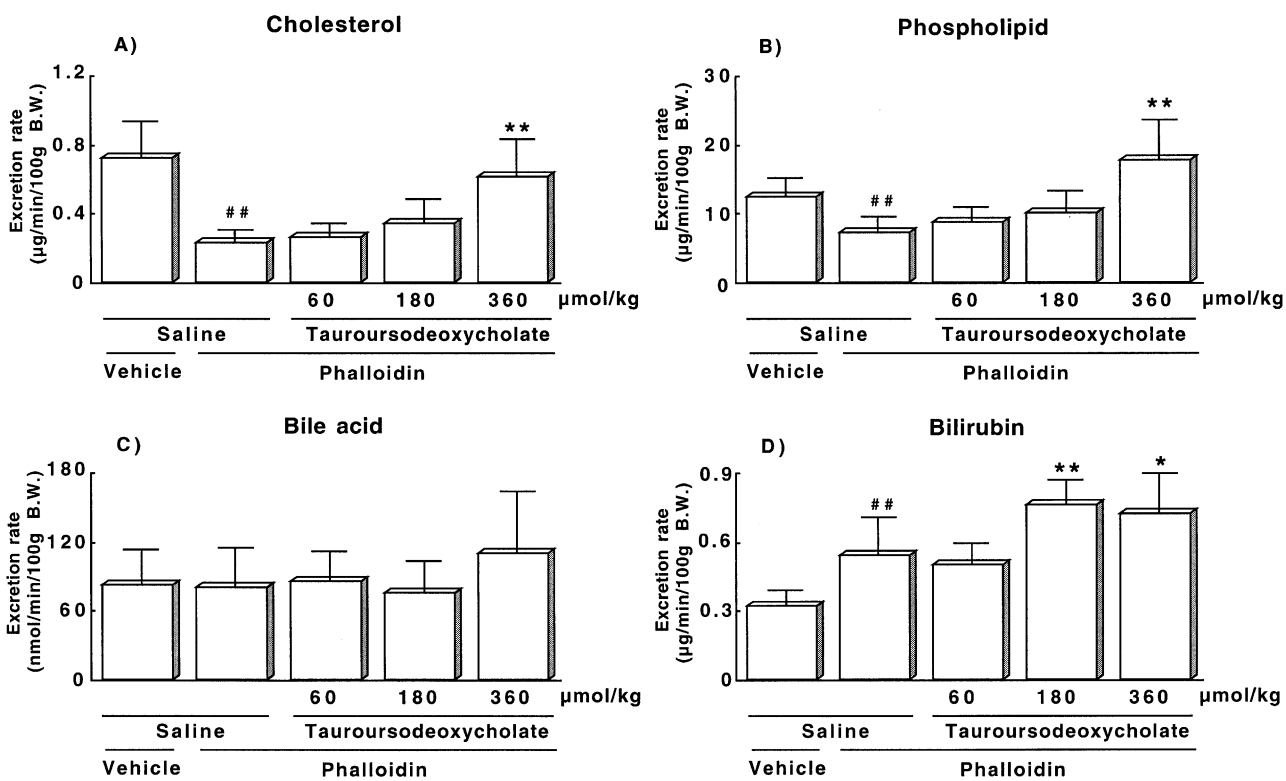

Fig. 4. Effect of tauroursodeoxycholate on biliary cholesterol (A), phospholipid (B), bile acid (C) and bilirubin (D) excretion rates in phalloidin-induced cholestasis rats. The rats were given phalloidin (500 μg/kg B.W. i.p.) for 7 days daily. Tauroursodeoxycholate (60–360 μmol/kg, i.p.) and saline were administered twice a day for 4 days from the day the last phalloidin administration, respectively. Values are expressed as means ± S.D. $n = 9–10$. $^{*}P < 0.05$, $^{**}P < 0.01$ difference from corresponding values in saline + phalloidin animals; $\#\#P < 0.01$ difference from corresponding values in saline + vehicle (2% dimethylformamide-saline solution) animals (Dunnett's multiple comparison test).

control rats. In addition, animals exhibited significant increases in serum alkaline phosphatase, leucine aminopeptidase, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase activities, cholesterol, phospholipid, bile acids and bilirubin concentrations and a decrease in biliary cholesterol, phospholipid excretion rates. These observations are almost consistent with our previous study (Ishizaki et al., 1997b).

Tauroursodeoxycholate is known to have a powerful choleretic action (Tsukahara et al., 1993; Kinbara et al., 1997; Queneau et al., 1994) in various experimental cholestatic conditions. We have previously reported that tauroursodeoxycholate infusion at a rate of 0.3 μmol/min/100 g for 2 h (= 360 μmol/kg/2 h) induced a remarkable increase in bile flow, biliary cholesterol and phospholipid excretion rates in normal rats (Iwaki et al., 1998). Thus, there is the possibility that tauroursodeoxycholate at a dose of 360 μmol/kg, as given here, results in similar effects. In the present study, tauroursodeoxycholate treatment also ameliorated the decrease in bile flow induced by phalloidin, which was accompanied with enhanced biliary cholesterol and phospholipid excretion.

Our previous study (Kinbara et al., 1997) reported that intravenous administration of tauroursodeoxycholate caused a significant increase in tauroursodeoxycholate concentration in bile. Furthermore, Ohiwa et al. (1993) reported that tauroursodeoxycholate exerted a cytoprotective effect against taurochenodeoxycholic acid-induced toxicity in primary cultured rat hepatocytes. In the present study, tauroursodeoxycholate treatment caused a significant reduction in serum alkaline phosphatase, leucine aminopeptidase and glutamic pyruvic transaminase activities. This finding may be attributed to a cytoprotective properties of tauroursodeoxycholate and would occur as a consequence of stimulation of the efflux of cytotoxic bile acids from hepatocytes rather than by replacement of cytotoxic bile acids (Ohiwa et al., 1993).

Tauroursodeoxycholate treatment caused a significant reduction in serum cholesterol, phospholipid and bile acid concentrations (Fig. 3) and significantly increased the biliary cholesterol and phospholipid excretion rates (Fig. 4). These phenomena suggest that tauroursodeoxycholate can improve serum parameters by facilitating biliary lipid excretion rates based on its choleretic action.

In conclusion, the present study shows that tauroursodeoxycholate exerts a beneficial therapeutic effect against phalloidin-induced cholestasis in rats. It is also suggested that tauroursodeoxycholate may be useful as a therapeutic agent against intrahepatic cholestasis in humans.

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