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Effects of high taurocholate load on activities of hepatic alcohol metabolizing enzymes

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Abstract

Membrane-associated cytotoxicity induced by hydrophobic bile salts is a major contributing factor leading to liver diseases. Administration of ursodeoxycholate reduces serum liver enzymes in chronic liver diseases but the nature of this effect is still unclear. Using alcohol metabolising enzymes as cellular markers, the hepatotoxic properties of hydrophobic bile salts and the putative hepatoprotective effect of ursodeoxycholate was examined. Two animal models of biliary retention, bile duct obstruction and choledochocaval fistula was used to investigate the effect of taurocholate on the hepatic alcohol metabolizing enzymes: cytosolic alcohol dehydrogenase, microsomal ethanol oxidizing system, catalase and aldehyde dehydrogenase before and after the infusion of taurocholic acid or tauroursodeoxycholic acid for two days period. Bile duct obstruction was found to be similar to or slightly exceeds choledochocaval fistula in the degree of retention. Following the taurocholic acid infusion, the serum alcohol dehydrogenase activity as well as microsomal ethanol oxidizing system and aldehyde dehydrogenase were greatly increased but the level of cytosolic alcohol dehydrogenase and catalase activities was found to be lower in either or both models in comparison with the control animals. However, the tauroursodeoxycholic acid infusion did not induce any significant changes in the levels of all the alcohol metabolizing enzyme activities in either or both models. These findings suggest that hydrophobic taurocholic acid (7α) affects the plasmalemma to allow leakage of cytosolic alcohol dehydrogenase into the blood circulation, stimulates the biosynthesis of microsomal ethanol oxidizing system and aldehyde dehydrogenase, and suppresses the biosynthesis of alcohol dehydrogenase and catalase. But in contrast, the hydrophilic tauroursodeoxycholic acid (7β) provided hepatoprotective effect.

Keywords: Alcohol metabolizing enzymes, Bile acid cytotoxicity, Choledochocaval fistula rat

Introduction

Some 80% to 90% of ingested alcohol is oxidized in the liver (Vidal *et al*., 1990) where ethanol is metabolized in a two stage oxidative process first to acetaldehyde and to acetate (Horton and Mills, 1979). The enzymes that catalyze these two oxidations are alcohol dehydrogenase (ADH), located in the cytoplasm, catalase in the peroxisomes, the microsomal ethanol oxidizing system (MEOS) in the endoplasmic reticulum (Lieber, 1985), and aldehyde dehydrogenase (ALDH) which is located in the soluble and insoluble fractions of hepatocytes (Crow *et al*., 1974; Harada, 2001). Although ADH catalyzes the rate-limiting step in the ethanol metabolism (Plapp *et al*., 1984; Vidal *et al*., 1990), its physiological role is uncertain (Pietruszko, 1975). However, the serum activity of this enzyme is a specific reflexion of hepatocellular necrosis (Mezey *et al*., 1968; Skursk *et al*., 1979) because the highest ADH activities have been found in the liver (Skursk *et al*., 1979), and the reduction of ADH activity is proportional to the severity of liver disease (Pan *et al*., 1989; Vidal *et al*., 1989); that being so, the appearance of this enzyme in serum can indicate liver disease with comparatively good selectivity (Skursk *et al*., 1979).

Bile acids are cytotoxic when present in abnormally high concentrations (Hofmann, 1999). Bile acids cytotoxicity is thought to be dependent on their detergent property (Hardison *et al*., 1981; Kitani *et al*., 1986; Morgan *et al*., 1998) or lipid-solubilizing properties (Ogawa *et al*., 1990). At concentration near the critical micellar concentration, or at high perfusion rate, bile salts are capable of solubilizing cell membrane components (Morgan *et al*., 1998) leading to cell lysis (Drew and Priestly, 1963). On the other hand, bile acids stimulate biliary membrane-bound enzymes, alkaline phosphatase (Ogawa *et al*., 1990) and γ-glutamyl transpeptidase (Kim and Kim, 1997), and competitively inhibit the cytochrome P 450-dependent microsomal biotransforming enzymes (Greim *et al*., 1972), arylesterase (Han and Kim, 1997) and carboxylesterase (Han and Kim, 1998). The most common model used to study abnormalities associated with cholestasis is the animal with complete biliary obstruction, most often the rat. Using this model of cholestasis, the increase in the activities of MEOS and aldehyde dehydrogenase, and the decrease in the

activities of alcohol dehydrogenase and catalase were observed (Kwak *et al*., 1988). However, due to the impairment of transhepatocellular transport and increased biliary pressure (Hardison *et al*., 1983), this model may not be appropriate to clarify whether the activity change of the enzymes is caused by retention of biliary constituents. Creation of a shunt between the common bile duct and the superior vena cava, a choledochocaval fistula (CCF), causes biliary retention without obstruction (Toyota *et al*., 1984). Many laboratories have studied the bile acid cytotoxicity by determining the cytotoxic parameters such as alkaline phosphatase, lactate dehydrogenase, 5'-nucleotidase and aminotransferases (Hardison *et al*., 1983; Kitani *et al*., 1986; Ogawa *et al*., 1990); however, the possible changes of alcohol dehydrogenase, as the cytotoxic parameter, induced by different bile constituents load have not been investigated. In the present study, we have examined the leakage of cytosolic ADH into the blood stream using two animal models of retention of biliary constituents to investigate *in vivo* cytotoxicity of bile acids. Activities of MEOS, catalase and ALDH during 2 days experiment were measured in rat livers after BDO and in the liver from a CCF rat. Activities of these alcohol metabolzing enzymes were determined following the infusion of either taurine amidate of cholic acid (3, 7, 12-trihydroxy-5-cholanoic acid) (TCA) or ursodeoxycholic acid (3, 7-dihydroxy-5-cholanoic acid) (TUDC) at high perfusion rate (450 μmol/kg) for 15 min (Ogawa *et al*., 1990).

Materials and Methods

Chemicals

β-nicotinamide adenine dinucleotide (β-NAD^+), β-nicotinamide adenine dinucleotide reduced form disodium salt (β-NADH, from yeast), N, N', dimethyl-4-nitrosoanilline, β-nicotinamide adenine dinucleotide phosphate sodium salt ($NADP^+$, from yeast), glycine, pyrazole, propionaldehyde, deoxycholic acid sodium salt, nicotinamide, glucose 6-phosphate, semicarbazide hydrochloride, 2-butanol, taurocholic acid sodium salt (from ox bile), tauroursodeoxycholic acid sodium salt, alcohol dehydrogenase from horse liver, aldehyde dehydrogenase from baker's yeast, catalase from bovine liver and reference protein (10 g/100 ml bovine serum albumin) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Absolute ethanol was purchased from Merck (Damstadt, Germany). Unless specified otherwise, additional reagents used were of analytical grade.

Animals

Normal male rats of Sprague-Dawley strain, weighing 280-320 g, were used for the experiments. All animals were maintained on a pellet diet obtained commercially (Sam Yang Foods Co., Wonju, Korea) and tap water. All the experimental groups, with five rats in each group, were divided into 15 groups as follows: One normal group (group 1); Sham-operated control groups (group 2A and 2B): the rats were sacrificed on the 1st and 2nd day after sham operation, midline laparatomy; BDO groups (group 3A and 3B): the rats were sacrificed on the 1st and 2nd day after common bile duct ligation; BDO groups received TCA infusion (group 4A and 4B): the rats were infused with TCA (450 μmol//kg) intravenously through the external jugular vein shortly after BDO, and were sacrificed on the 1st and 2nd day post-ligation; the BDO group received TUDCA infusion (group 5A and 5B): the rats were infused with TUDCA (450 μmol//kg) intravenously through the external jugular vein shortly after BDO, and were sacrificed on the 1st and 2nd day post-ligation; the CCF group (group 6A and 6B): the rats were sacrificed on the 1st and 2nd day after CCF implantation according to the method of Hardison *et al*. (1983); the CCF group received TCA infusion (group 7A and 7B): immediately after the implantation of fistula, the rats were infused with TCA (450 μmol//kg) into cannula, and were sacrificed on the 1st and 2nd day post-implantation; the CCF group received TUDCA infusion (group 8A and 8B): shortly after CCF implantation, the rats were infused with TUDCA (450 μmol/kg) into cannula, and were sacrificed on the 1st and 2nd day post-implantation. All animals had fasted 12 h prior to sacrifice or surgery in which rats were anesthesized lightly with ether. Bile duct obstruction was performed as previously described (Kim and Joo, 2001). A CCF was implanted according to the method of Hardison *et al*. (1983). Briefly, after a midline incision, the common bile duct was cannulated close to the liver hilus with a PE-50 tube. The cannula was looped outside the abdominal cavity by crossing the incision and tunneled subcutaneously to the neck of the rat. A second catheter consisting of a silastic tube connected to a PE-90 tube was inserted 2.7 cm into the interior jugular vein, and both cannulas were connected behind the animal's neck. Bile salts were infused by using the syringe pump (Sage Instruments, model 341A) according the methods of Ogawa *et al*. (1990) for 15 min. The livers were excised following perfusion (see below), and blood was collected from the abdominal aorta. The serum was separated by centrifugation and stored at -20°C until use.

Preparation of subcellular fractions

Subcellular fractionation was performed as described previously (Ihm and Kim, 1997). Briefly, the livers were perfused *via* portal vein with cold 0.25 M sucrose, and then excised, blotted, weighed, minced and homogenized in 9 volume of 0.25 M sucrose. Each homogenate was

subjected to cell fractionation. Cytosol, mitochondria and microsomes were isolated by the sucrose linear density gradient centrifugation method (Kwak and Kwak, 1986), and stored at -80°C. All the isolation procedures were performed at 2 to 4°C. The hepatic cytosolic preparations were used for ADH and ALDH assays without any treatment. For the studies on the subcellular distribution of ALDH activity, the microsomal and mitochondrial pellet were suspended in 0.25 M sucrose to yield a final protein concentration of 5 mg/ml. The suspensions was diluted with one half of 1% sodium bicarbonate (w/v) solubilizing buffer containing 1% sodium deoxycholate (w/v). The suspension was sonicated at 2 min-intervals for a total 10 min using a Fischer model 300 ultrasonic dismembrator at 20 ± 0.4 kHz. This microsomal preparation was also used for determination of MEOS activity. Another portion of the liver was homogenized in 9 volume of 5 mM potassium phosphate buffer, pH 6.8 and sonicated to determine catalase activity.

Enzyme assays

Alcohol dehydrogenase

Cytosolic ADH activities were determined with the hepatic cytosolic preparation according to the method of Koivula *et al*. (1975). The assay mixture contained 196 μM NaOH-glycine buffer, pH 9.6, 40 μM ethanol, 2.68 μM NAD^+ and 0.1 ml of sample, in a final volume of 4.0 ml. The mixture was incubated for 3 min at 37°C. ADH oxidizes ethanol to acetaldehyde and NADH at the expense of NAD^+. The absorbance of NADH was measured at 340 nm using a Varian Cary 210 spectrophotometer. The enzyme activities were expressed as nM of NADH formed per min per mg protein of hepatic cytosolic preparations. Alcohol dehydrogenase activties in sera were measured using *n*-butanol and *N*, *N'*-dimethyl-4-nitrosoaniline as the substrates according to the method of Skurk (1979). 0.25 ml of serum was incubated with the reaction mixture containing 0.25 μM NAD^+, 2.5 μM *N*, *N*-dimethyl-4-nitrosoaniline, 10 μM NADH, 50 mM *n*-butanol in 0.1 M sodium phosphate buffer, pH 8.5, in a final volume of 1.0 ml for 20 min at 25°C. The incubations were terminated by the addition of 0.25 ml of 50 mM pyrazole. The absorbance of butylaldehyde was measured at 440 nm using a Varian Cary 210 spectrophotometer. The enzyme activities were expressed as μM of butylaldehyde formed per min per ml of sera.

Microsomal ethanol oxidizing system

The activities were determined according to the modified method of Lieber and Decarli (1968). Each 0.5 ml of sonicated microsomal suspensions (see above) containing a final protein concentration of 2.5 mg/ml and 0.5 ml of the cytosolic preparations was incubated in a medium containing 0.3 μM $NADP^+$, 5 μM $MgCl_2$, 20 μM nicotinamide, 8 μM glucose 6-phosphate, 50 μM ethanol and 80 μM of phosphate buffer, pH 7.4. The incubations were carried out in main chambers of stoppered 50 Erlenmeyer flasks, with a center wall containing 0.6 ml of 15 mM semicarbazide-HCl in 0.16 M potassium phosphate buffer, pH 7.0 for 30 min at 37°C. The reaction was stopped with 0.5 ml of 70% trichloroacetic acid. After an overnight diffusion period at room temperature, the concentration of acetaldehyde semicarbazide was determined spectrophotometrically at 224 nm. The enzyme activities were expressed as nM of acetaldehyde formed per min per mg protein of hepatic microsomal preparations.

Catalase

Catalase activity was determined using hydrogen peroxide as the substrate according to the method of Nelson and Kiesow (1972). The reaction mixture consisted of 31.5 μM hydrogen peroxide in 150 μM KH_2PO_4 buffer, pH 6.8 and 10% (W/V) homogenate (see above) 20 μl, in a final volume of 3.2 ml. Extinction of hydrogen peroxide was scanned for 1 min at 25°C. The decomposition of hydrogen peroxide was measured at 240 nm. The enzyme activity was expressed as μM decomposed hydrogen peroxide (ΔH) per min per mg protein of liver homogenate.

Aldehyde dehydrogenase

Aldehyde dehydrogenase activity was measured spectrophotometrically using propionaldehyde and NAD^+ as the substrates by following the NADH production at 340 nm according to the method of Koivula and Koivulsalo (1975). The reaction mixture contained 168 μM tetrasodium pyrophosphate, 6.68 μM pyrazol, 5.32 μM NAD^+, 24 μM propionaldehyde with 0.1 ml of hepatic subcellular preparations in final volume of 4.0 ml was incubated for 3 min at 37°C. The enzyme activities were expressed as in the ADH assay.

Determination of protein

The protein concentration was determined by the Biuret reaction (Gornal *at al*., 1949), using bovine albumin as the standard.

Statistical analysis

Values are expressed as mean ±SD. Statistical significance was calculated using Student's *t*-test at $P \leq 0.05$.

Results

Alcohol dehydrogenase

The level of cytosolic ADH activities did not change significantly in either or both models in comparison with those levels of activities from normal or sham-operated control (Table 1). However, the level of the serum ADH activity was increased markedly soon after the BDO or CCF construction in comparison with those levels of

Table 1. Effects of time and model of biliary retention on liver cytosolic alcohol dehydrogenase (ADH) activities in rats

Experimental groups	ADH activites (nM NADH min^{-1} mg $protein^{-1}$)
Normal	77.6 ± 10.8
Sham 1 day	76.7 ± 11.4
Sham 2 days	78.3 ± 12.1
CCF 1 day	75.2 ± 10.3
CCF 2 days	73.2 ± 11.6
BDO 1 day	72.7 ± 10.9
BDO 2 days	68.3 ± 11.3

The data are expressed as mean ±SD with 5 rats in each group; Sham 1 day or Sham 2 days: Sacrificed on the 1st day or 2nd day after sham operation, CCF 1 day or CCF 2 days: Sacrificed on the 1st day or 2nd day after choledochocaval fistula construction, BDO 1 day or BDO 2 days: Sacrificed on the 1st day or 2nd day after common bile duct ligation.

Table 2. Effects of taurocholic acid (TCA), and tauroursodeoxycholic acid (TUDCA) infusions after bile duct obstruction (BDO) on liver cytosolic alcohol dehydrogenase (ADH) activities in rats

Experimental groups	ADH activites (nM NADH min^{-1} mg $protein^{-1}$)
BDO 1 day	72.7 ± 10.9
BDO 1 day + TCA	35.4 ± 6.8^{r}
BDO 1 day + TUDCA	74.2 ± 11.4
BDO 2 days	68.3 ± 11.3
BDO 2 days + TCA	27.5 ± 5.3^{u}
BDO 2 days + TUDCA	70.6 ± 10.5

The data are expressed as mean ±SD with 5 rats in each group; BDO 1 day + TCA or BDO 1 day + TUDCA, and BDO 2 days + TCA or BDO 2 days + TUDCA: One of the following bile acids were administered intravenously through the superior vena cava: TCA or TUDCA (45 moles/100 *g* body weight) at the time of common bile duct ligation in rats. Then the rats were sacrificed 1 or 2 days after the ligation.
r; P < 0.001 vs. BDO 1 day, u; P < 0.001 vs. BDO 2 days

Table 3. Effects of taurocholic acid (TCA), and tauroursodeoxycholic acid (TUDCA) infusions after choledochocaval fistula (CCF) on liver cytosolic alcohol dehydrogenase (ADH) activities in rats

Experimental groups	ADH activites (nM NADH min^{-1} mg $protein^{-1}$)
CCF 1 day	75.2 ± 10.3
CCF 1 day + TCA	46.2 ± 6.9^{l}
CCF 1 day + TUDCA	76.4 ± 11.2
CCF 2 days	73.2 ± 11.6
CCF 2 days + TCA	36.6 ± 7.4^{o}
CCF 2 days + TUDCA	77.3 ± 12.3

The data are expressed as mean ±SD with 5 rats in each group; CCF 1 day + TCA or CCF 1 day + TUDCA, and CCF 2 days + TCA or CCF 2 days + TUDCA: One of the following bile acids were administered intravenously through the superior vena cava: TCA or TUDCA (45 moles/100 *g* body weight) at the time of CCF construction in rats. Then the rats were sacrificed 1 or 2 days after CCF construction.
l; P < 0.001 vs. CCS 1 day, o; P < 0.001 vs. CCS 2 days

activities from either normal or sham-operated control ($P \leq 0.001$) as shown in Table 4. Subsequent to the

Table 4. Effects of time and model of biliary retention on serum alcohol dehydrogenase (ADH) activities in rats

Experimental groups	ADH activites (μM butylaldehyde min^{-1} ml^{-1})
Normal	4.9 ± 1.3
Sham 1 day	5.3 ± 1.4
Sham 2 days	5.1 ± 1.1
CCF 1 day	32.6 ± 10.7^{c,f}
CCF 2 days	37.2 ± 12.2^{c,i}
BDO 1 day	50.4 ± 15.8^{c,f}
BDO 2 days	52.6 ± 17.2^{c,i}

The data are expressed as mean ±SD with 5 rats in each group; Experimental groups are descibed in Table 1 and text.
c; P < 0.001 vs. Nomal, f; P < 0.001 vs. Sham 1 day, i; P < 0.001 vs. Sham 2 days

Table 5. Effects of taurocholic acid (TCA), and tauroursodeoxycholic acid (TUDCA) infusions after bile duct obstruction (BDO) on serum alcohol dehydrogenase (ADH) activities in rats

Experimental groups	ADH activites (μM butylaldehyde min^{-1} ml^{-1})
BDO 1 day	50.4 ± 15.8
BDO 1 day+TCA	80.7 ± 23.6^{p}
BDO 1 day+TUDCA	51.2 ± 16.5
BDO 2 days	52.6 ± 17.2
BDO 2 days+TCA	91.6 ± 28.4^{s}
BDO 2 days+TUDCA	51.8 ± 15.1

The data are expressed as mean ±SD with 5 rats in each group; Experimental groups are descibed in Table 3 and text.
p; P < 0.05 vs. BDO 1 day, s; P < 0.05 vs. BDO 2 days

Table 6. Effects of taurocholic acid (TCA), and tauroursodeoxycholic acid (TUDCA) infusions after choledochocaval fistula (CCF) on serum alcohol dehydrogenase (ADH) activities in rats

Experimental groups	ADH activites (μM butylaldehyde min^{-1} ml^{-1})
CCF 1 day	32.6 ± 10.7
CCF 1 day+TCA	50.3 ± 16.2
CCF 1 day+TUDCA	30.8 ± 11.6
CCF 2 days	37.2 ± 12.2
CCF 2 days+TCA	61.4 ± 18.9^{m}
CCF 2 days+TUDCA	35.7 ± 11.7

The data are expressed as mean ±SD with 5 rats in each group; Experimental groups are descibed in Table 2 and text.
m; P < 0.05 vs. CCF 2 days

TCA infusion, the activities of cytosolic ADH in the rat liver decreased significantly on the 2nd day after the bile duct ligation or CCF construction ($P \leq 0.001$) (Table 2 and 3). The level of ADH activity in the serum was significantly increased from the 1st to the 2nd day post-ligation or post-implantation following the TCA infusion ($P \leq 0.001$) as shown in Tables 5 and 6. After TUDC infusion, the levels of the cytosol ADH activities in the rat liver as well as in serum did not show any significant change by either or both models in comparison with

those activities from the BDO or CCF control group (Tables 2, 3, 5 and 6).

MEOS

The levels of MEOS activities did not increase significantly in the rat liver in either or both models in comparison with those levels of activities from the sham-operated control or normal control group except on the 2nd day after BDO, where the enzyme activity level was greatly elevated in the cholestatic rat liver on the 2nd day post-ligation in comparison with those level of activities from the sham-operated or normal control ($P \leq 0.05$) (Table 7). Subsequent to the TCA injection, the activities of MEOS in the rat liver increased from the 1st to the 2nd day post-ligation or on the 2nd day after CCF construction in comparison with those activities from each BDO or CCF control group ($P \leq 0.05$) (Tables 8 and 9). Subsequent to the TUDC injection, activities of MEOS in the microsomal preparation of rat liver did not show any significant increase in either or both models in comparison with those activities from the BDO or CCF control group (Tables 8 and 9).

Table 7. Effects of time and model of biliary retention on hepatic microsomal ethanol oxidizing system (MEOS) activities in rats

Experimental groups	MEOS activites (nM acetaldehyde min^{-1} mg $protein^{-1}$)
Normal	5.38 ± 0.76
Sham 1 day	5.41 ± 0.80
Sham 2 days	5.45 ± 0.78
CCF 1 day	5.62 ± 0.87
CCF 2 days	5.84 ± 0.82
BDO 1 day	5.88 ± 0.85
BDO 2 days	$6.73 \pm 0.96^{a,g}$

The data are expressed as mean ±SD with 5 rats in each group; Experimental groups are descibed in Table 1 and text.
a; $P < 0.05$ vs. Nomal, g; $P < 0.05$ vs. Sham 2 days

Table 8. Effects of taurocholic acid (TCA), and tauroursodeoxycholic acid (TUDCA) infusions after bile duct obstruction (BDO) on hepatic microsomal ethanol oxidizing system (MEOS) activities in rats

Experimental groups	MEOS activites (nM acetaldehyde min^{-1} mg $protein^{-1}$)
BDO 1 day	5.88 ± 0.85
BDO 1 day+TCA	7.53 ± 1.32^{p}
BDO 1 day+TUDCA	5.96 ± 0.83
BDO 2 days	6.73 ± 0.96
BDO 2 days+TCA	8.62 ± 1.41^{s}
BDO 2 days+TUDCA	6.87 ± 1.07

The data are expressed as mean ±SD with 5 rats in each group; Experimental groups are descibed in Table 3 and text.
p; $P < 0.05$ vs. BDO 1 day, s; $P < 0.05$ vs. BDO 2 days

Table 9. Effects of taurocholic acid (TCA), and tauroursodeoxycholic acid (TUDCA) infusions after choledochocaval fistula (CCF) on hepatic microsomal ethanol oxidizing system (MEOS) activities in rats

Experimental groups	MEOS activites (nM acetaldehyde min^{-1} mg $protein^{-1}$)
CCF 1 day	5.62 ± 0.87
CCF 1 day+TCA	6.70 ± 0.92
CCF 1 day+TUDCA	5.73 ± 0.84
CCF 2 days	5.84 ± 0.82
CCF 2 days+TCA	7.71 ± 1.14^{m}
CCF 2 days+TUDCA	6.04 ± 0.93

The data are expressed as mean ±SD with 5 rats in each group; Experimental groups are descibed in Table 2 and text.
m; $P < 0.05$ vs. CCF 2 days

Table 10. Effects of time and model of biliary retention on hepatic catalase activities in rats

Experimental groups	Catalase activites (μM H_2O_2 reduced min^{-1} mg $protein^{-1}$)
Normal	32.7 ± 3.6
Sham 1 day	33.5 ± 3.8
Sham 2 days	33.8 ± 4.1
CCF 1 day	31.4 ± 3.9
CCF 2 days	29.7 ± 3.5
BDO 1 day	28.2 ± 4.3
BDO 2 days	$26.3 \pm 3.7^{a,g}$

The data are expressed as mean ±SD with 5 rats in each group; Experimental groups are descibed in Table 1 and text.
a; $P<0.05$ vs. Nomal, g; $P<0.05$ vs. Sham 2 days

Table 11. Effects of taurocholic acid (TCA), and tauroursodeoxycholic acid (TUDCA) infusions after bile duct obstruction (BDO) on hepatic catalase activities in rats

Experimental groups	Catalase activites (μM H_2O_2 reduced min^{-1} mg $protein^{-1}$)
BDO 1 day	28.2 ± 4.3
BDO 1 day+TCA	21.8 ± 3.1^{p}
BDO 1 day+TUDCA	30.8 ± 3.9
BDO 2 days	26.3 ± 3.7
BDO 2 days+TCA	17.0 ± 2.3^{t}
BDO 2 days+TUDCA	27.5 ± 3.4

The data are expressed as mean ±SD with 5 rats in each group; Experimental groups are descibed in Table 3 and text.
p; $P < 0.05$ vs. BDO 1 day, t; $P < 0.01$ vs. BDO 2 days

Table 12. Effects of taurocholic acid (TCA), and tauroursodeoxycholic acid (TUDCA) infusions after choledochocaval fistula (CCF) on hepatic catalase activities in rats

Experimental groups	Catalase activites (μM H_2O_2 reduced min^{-1} mg $protein^{-1}$)
CCF 1 day	31.4 ± 3.9
CCF 1 day+TCA	23.7 ± 2.7^{k}
CCF 1 day+TUDCA	32.5 ± 4.1
CCF 2 days	29.7 ± 3.5
CCF 2 days+TCA	22.1 ± 2.9^{n}
CCF 2 days+TUDCA	30.7 ± 3.8

The data are expressed as mean ±SD with 5 rats in each group; Experimental groups are descibed in Table 2 and text.
k; $P < 0.05$ vs. CCF 1 day, n; $P < 0.01$ vs. CCF 2 days

Catalase

Catalase activities in the rat liver was found to remain in the same level in either or both models in comparison with those activities from the sham-operated or normal control except on the 2nd day after BDO where the enzyme activity decreased significantly in the cholestatic rat liver after the ligation in comparison with the activities from the sham-operated and normal control ($P \leq 0.05$) (Table 10). Subsequent to the TCA infusion, the catalase activities in the rat liver decreased significantly from the 1st ($P \leq 0.05$) to the 2nd day ($P \leq 0.01$) in comparison with those activities from each control group (Tables 10 and 11). Following the TUDC infusion, catalase activities in the rat liver was not increased to any significant level in either or both models in comparison with those of the BDO or CCF control group (Tables 10 and 11).

Subcellular ALDH activities

The ALDH activities of cytosolic, mitochondrial and microsomal preparations of the rat liver were not increased any significant level in either or both models in comparison with those activities from sham-operated control or normal control (Table 13). Subsequent to the TCA infusion, the cytosolic ALDH activity in the cholestatic rat liver increased significantly on the 2nd day post-ligation in comparison with that activity from the BDO control group ($P \leq 0.01$). The microsomal ALDH activity in the cholestatic rat liver also increased from the 1st ($P \leq 0.05$) to the 2nd day post-ligation

Table 13. Effects of time and model of biliary retention on hepatic subcellular aldehyde dehydrogenase (ALDH) activities in rats

Experimental groups	ALDH activities (nM NADH min^{-1} mg $protein^{-1}$)		
	Cytosol	Mitochondria	Microsome
Normal	58.7 ± 7.2	95.2 ± 11.8	70.2 ± 10.8
Sham 1 day	59.0 ± 6.4	96.3 ± 12.5	71.5 ± 11.5
Sham 2 days	59.4 ± 7.3	97.1 ± 13.2	71.9 ± 10.6
CCF 1 day	59.7 ± 6.5	96.2 ± 13.5	71.7 ± 10.3
CCF 2 days	62.2 ± 7.0	96.8 ± 13.4	72.2 ± 11.2
BDO 1 day	62.8 ± 6.2	97.8 ± 13.7	73.6 ± 11.4
BDO 2 days	64.7 ± 7.5	98.7 ± 14.3	77.8 ± 11.9

The data are expressed as mean ±SD with 5 rats in each group; Experimental groups are descibed in Table 1 and text.

Table 14. Effects of taurocholic acid (TCA), and tauroursodeoxycholic acid (TUDCA) infusions after bile duct obstruction (BDO) on hepatic subcellular aldehyde dehydrogenase (ALDH) activities in rats

Experimental groups	ALDH activities (nM NADH min^{-1} mg $protein^{-1}$)		
	Cytosol	Mitochondria	Microsome
BDO 1 day	62.8 ± 6.2	97.8 ± 13.7	73.6 ± 11.4
BDO 1 day+TCA	78.2 ± 6.6^{q}	102.7 ± 13.3	97.5 ± 12.8^{p}
BDO 1 day+TUDCA	62.2 ± 6.9	95.5 ± 13.1	73.9 ± 10.6
BDO 2 days	64.7 ± 7.5	98.7 ± 14.3	77.8 ± 11.9
BDO 2 days+TCA	81.3 ± 7.3^{t}	108.4 ± 14.6	109.5 ± 14.2^{t}
BDO 2 days+TUDCA	63.0 ± 7.1	96.8 ± 12.7	79.6 ± 10.3

The data are expressed as mean ±SD with 5 rats in each group; Experimental groups are descibed in Table 3 and text.
p; P<0.05 vs. BDO 1 day, q; P<0.01 vs. BDO 1 day, t; P<0.01 vs. BDO 2 days

Table 15. Effects of taurocholic acid (TCA), and tauroursodeoxycholic acid (TUDCA) infusions after choledocho caval fistula (CCF) on hepatic subcellular aldehyde dehydrogenase (ALDH) activities in rats

Experimental groups	ALDH activities (nM NADH min^{-1} $protein^{-1}$)		
	Cytosol	Mitochondria	Microsome
CCF 1 day	59.7 ± 6.5	96.2 ± 13.7	71.7 ± 10.3
CCF 1 day+TCA	72.3 ± 6.7^{j}	97.5 ± 13.1	87.4 ± 10.5^{j}
CCF 1 day+TUDCA	60.8 ± 7.3	96.6 ± 12.9	72.5 ± 11.0
CCF 2 days	62.2 ± 7.0	96.8 ± 13.4	72.2 ± 11.2
CCF 2 days+TCA	76.8 ± 6.2^{n}	99.7 ± 13.5	95.3 ± 12.6^{m}
CCF 2 days+TUDCA	63.8 ± 6.8	97.2 ± 12.6	73.9 ± 10.8

The data are expressed as mean SD with 5 rats in each group; Experimental groups are descibed in Table 2 and text.
j; P<0.05 vs. CCF 1 day, m; P<0.05 vs. CCF 2 days, n; P<0.01 vs. CCF 2 days

BDO control subsequent to the TCA infusion (Table 13). The cytosolic ALDH activity in the CCF rat liver increased from the 1st to the 2nd day after the TCA infusion ($P \leq 0.05$ and $P \leq 0.01$). And the microsomal ALDH activity in the CCF rat liver also increased from the 1st to the 2nd day after the TCA infusion ($P \leq 0.05$) (Table 15). The ALDH activity in the liver mitochondria did not show a significant increase in either or both models in comparison with those activities from the BDO or CCF control group subsequent to the TCA infusion (Tables 14 and 15). Subsequent to the TUDC infusion, the level of ALDH activities in the rat liver did not show a significant change in either or both models in comparison with those activities from the BDO or CCF control group (Tables 14 and 15).

Discussion

Bile acids are planar, surface-active amphipathic molecules. When present in sufficient concentration, conjugated bile acid molecules is capable of solubilizing other lipids (Hofmann, 1999). At abnormally high levels, bile acids become cytotoxic for being able to solubilize membrane lipids into aquous phase. Bile acid cytotoxicity is strongly affected by its structure: the greater the hydrophobicity, the greater the cytotoxicity within the confines of dipole integrity. The simple experimental preparations for studying abnormalities associated with cholestasis have been to induce complete biliary obstruction (BDO) and total biliary fistula (CCF) in the animal system, most often in the rat. The serum ADH, a cytosolic marker enzyme, is a specific reflexion of hepatocellular necrosis (Mezey *et al*., 1968; Skursk *et al*., 1979). We have earlier reported the leakage of ADH into the blood stream and activity changes of the other alcohol metabolizing enzymes in cholestasis induced by BDO in rats (Kwak *et al*., 1988). The cytotoxicity of bile acid *in vivo* was further extended by examining the leakage of ADH and other alcohol metabolizing enzymes into the blood stream as a cytotoxic parameter of the liver from CCF rats and BDO during 2 days period under the influence of the hydrophobic TCA (7α-planar) or hydrophilic TUDCA (7β-nonplanar) (Hardison *et al*., 1981). The activity of ADH in the serum increased significantly soon after BDO or CCF (groups 3 and 6) over the levels of those activities from each control (groups 1 and 2), although the cytosolic ADH activity levels remained about the same in either or both models. However, the level of MEOS activity was increased significantly in group 3B in comparison with those activity from group 2B. Catalase activity level was found to be significantly lower in the group 3B than those levels of activities from groups 1 and 2. The ALDH activities did not show a significant change in all the hepatic preparations in either or both models (groups 3 and 6).

As expected, the activity level of cytosolic ADH and catalase in the rat liver decreased significantly after TCA injection in either or both models (groups 4 and 7) in comparison with each control. Also the level of serum ADH activity and the activity of MEOS as well as the cytosolic and microsomal ALDH activity in the rat liver were increased significantly in group 4 and 7B after the TCA infusion (groups 3 and 6). However, the enzyme activity in the mitochondrial preparation did not show a significant change in either or both models before and after the TCA infusion. After the TUDC infusion, activities of ADH, MEOS, catalase and ALDH in the rat liver as well as ADH activitiy in the rat serum did not change significantly in either or both models. All the results by BDO model were coincident with our previous study (Kwak *et al*., 1988).

These present findings indicate that the rise of ADH activity in serum is a reflection of cholestasis-induced hepatocellular damage in these animal models. Also, BDO is found to be similar to or slightly exceeds CCF in the degree of retention at the early stage of cholestasis. These results suggest that hydrophobic TCA (7α) did induce damage to the plasmalemma lipids to allow leakage of cytosolic ADH into the vascular circulation system. In addition, the hepatic MEOS and ALDH (microsomal and cytosolic) expression was elevated whereas the levels of hepatic ADH and catalase appeared to decrease perhaps due to the spillage to extracellular fluid. And in either or both models when cholestasis is combined with high TCA load, the change in activity of alcohol metabolizing enzymes is significantly amplified. TUDC (7β) induced hepatoprotective effect have likely resulted from its hydrophilic property and ability to neutralize the other toxic bile salts by competition. However, the mechanism of changes in activities of alcohol metabolizing enzymes in cholestatic hepatobiliary disease is still unknown. Further investigations should eventually resolve this issue.

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