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Effects of Common Bile Duct Ligation on Serum and Hepatic Carboxylesterase Activity in Ethanol-Intoxicated Rats

Kwan Wook Ahn and You Hee Kim*
Department of Biochemistry, Keimyung University School of Medicine, Taegu, 700-712, Korea

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Ethanol catabolism is thought to produce metabolic disorders resulting in alcoholic liver disease. To investigate the mutual effects of ethanol catabolism and cholestasis induced by common bile duct ligation on the activities of carboxylesterase, we have determined the enzyme activities in rat hepatic (cytosolic, mitochondrial, and microsomal) preparations as well as in rat serum using ten animal models: normal rats (group 1), sham-operated rats (group 2), common bile duct-ligated rats (group 3), ethanol-intoxicated rats (group 4), sham-operation plus chronic ethanol-intoxicated rats (group 5), common bile duct-ligated plus chronic ethanol-intoxicated rats (group 6), acute ethanol-intoxicated rats at 1.5 h and 24 h (groups 7A and 7B), and duct-ligated and acute ethanol intoxicated rats at 1.5 h and 24 h (groups 8A and 8B). The K_m and V_{max} values of carboxylesterase from these hepatic preparations of cholestatic rat liver combined with chronic ethanol intoxication were also measured by using ethyl valerate as the substrate from the 14th day post-ligation. Carboxylesterase activities of all hepatic preparations and rat serum (group 3) showed significant decreases compared to the activities from the sham-operated control (group 2). Enzyme kinetic parameters indicated that V_{max} of carboxylesterase from all the hepatic preparations in cholestatic rats (group 3) decreased significantly, although the K_m values were about the same as in the sham-operated control (group 2). When cholestasis was combined with chronic ethanol intoxication (group 6), carboxylesterase activities showed further decrease in all the hepatic preparations and serum compared to the control activity (group 5). The V_{max} also decreased significantly, although K_m values did not change. When common bile duct ligation was combined with acute ethanol intoxication (group 8), the enzyme activities in the rat liver and serum showed significant decrease compared to the activity from acute ethanol-intoxicated rats (group 7). However, quite contrary to this, the activities of serum from acute ethanol intoxication 1.5 h (group 7A) increased significantly compared to the activities in the normal control (group 1). These results, therefore, suggest that the biosynthesis of hepatic carboxylesterase seems to decrease when cholestasis is combined with chronic and acute ethanol intoxication, and the decrease in activity is more significant than from cholestasis alone.

Keywords: Carboxylesterase, Cholestatic rat liver, Ethanol intoxication.

Introduction

Ethanol catabolism produces striking metabolic imbalances in the liver, and chronic ethanol consumption is associated with the progression of alcoholic liver disease which includes fatty liver, alcoholic hepatitis, and cirrhosis (Chang, 1985; 1987; Hall, 1985; Lieber, 1985). The turnover rate of ethanol is remarkably decreased by procedures damaging the liver such as common bile duct ligation (Lieber, 1985). Cholestatic liver disease includes inflammation, necrosis, fatty changes, biliary hyperplasia, fibrosis, and cirrhosis (Kountouras *et al.*, 1984; Chang, 1987). Common bile duct-ligated rats have been widely used as an experimental model in human extrahepatic cholestasis (Kaplan and Righetti, 1970; Righetti and Kaplan, 1971).

Non-specific carboxylesterase (EC 3.1.1.1) is one of the phase II xenobiotic biotransforming enzymes which hydrolyze carboxyl esters, thioesters, and arylamides to less toxic compounds (Hashinotsume, 1978; Heymann, 1980; Webb, 1992). The enzyme mainly presents in the endoplasmic reticulum (Junge and Krisch, 1975), cytosol

* To whom correspondence should be addressed.
Tel: 82-53-250-7462; Fax: 82-53-252-1605
E-mail: noel@dsmc.or.kr

(Nousiainen and Hänninen, 1981), mitochondria (Junge and Krisch, 1975), and lysosomes (Tanaka *et al.*, 1987) of mammalian liver, lung, spleen, intestine, brain, adipose tissues, and blood (Junge and Krisch, 1975; Stoops *et al.*, 1975; Hφring and Svenmark, 1976; 1977; Tsujita *et al.*, 1982; Tsujita and Okuda, 1983; Junge, 1984), and is closely related to cholinesterase (Cain *et al.*, 1983). The microsomal carboxylesterase remains active after liberation from the membrane. It has been detected in high concentration in the sera of patients with necrotizing liver disease (Junge, 1984).

Many studies have been done on the activity changes of hepatic xenobiotic enzymes in cholestatic rat livers (Mun and Kwak, 1989; Park *et al.*, 1994; Ihm *et al.*, 1995; Ihm and Kim, 1997; Kim and Kim, 1999) and also in cholestatic rat livers combined with ethanol intoxication (Kwak *et al.*, 1990; Chung and Kwak, 1992; Byun *et al.*, 1995; Ra *et al.*, 1995; Kwon and Kim, 1997; No and Kim, 1997). The activities of two carboxylic ester hydrolases, carboxylesterase and cholinesterase, are known to decrease in cholestatic rat livers and serum induced by common bile duct ligation (Kwak and Lee, 1992). Recent studies have demonstrated that liver and serum cholinesterase activities decrease synergistically in cholestatic rats combined with chronic ethanol intoxication (Kwak *et al.*, 1994). Previously, we reported that the activity of carboxylesterase decreased, using two experimental cholestatic models: common bile duct ligation and choledococaval shunt of high taurocholate load in the rat (Han and Kim, 1998). However, the possible changes of carboxylesterase activities in cholestatic rat liver combined with ethanol intoxication were not yet carried out. In the present study, we have estimated carboxylesterase activities with acute and chronic ethanol intoxications to investigate the synergic effects of ethanol catabolism and cholestasis induced by common bile duct ligation in rats. In addition, the values of K_m and V_{max} of carboxylesterase in rat liver from cholestasis alone and from cholestasis combined with chronic ethanol intoxication on the 14th day post-ligation were also measured using ethyl valerate as the substrate.

Materials and Methods

Chemicals Sodium pyrophosphate, semicarbazide-HCl, eserine hemisulfate, ethyl valerate, glycine, NAD^+ (nicotinamide adenine dinucleotide, grade III, disodium salt), alcohol dehydrogenase (from bakers yeast, A-3263), carboxylesterase (from rabbit liver, E-0887), and bovine albumin (10 g/ 100 ml) were purchased from Sigma (St. Louis, USA).

Animals Normal male Sprague-Dawley rats, weighing 280–320 g, were used in the experiments. All the experimental groups, with five rats in each group, were divided as follows: One normal group (group 1); Sham operation group (group 2): the rats were sacrificed at the 1st, 2nd, 3rd, 7th, and 14th days after sham operation; Common bile duct ligation group (group 3): the rats were sacrificed at the 1st, 2nd, 3rd, 7th, and 14th days after common bile duct ligation; Chronic ethanol intoxication group (group 4): the rats were fed 5% (v/v) ethanol instead of water for 60 days according to the method of Eagon *et al.* (1987); Sham operation group combined with chronic ethanol intoxication (group 5): the rats were fed 5% (v/v) ethanol instead of water for 60 days. They were continuously supplied with ethanol, and sham operations were performed. Rats were sacrificed at the 1st, 2nd, 3rd, 7th, and 14th days after sham operations; Common bile duct ligation combined with chronic ethanol intoxication group (group 6): the rats were fed 5% (v/v) ethanol instead of water for 60 days. They were continuously supplied with ethanol, and common bile ligation was done. Rats were sacrificed at the 1st, 2nd, 3rd, 7th, and 14th days after common bile duct ligation; Acute ethanol intoxication group (group 7): the rats were intoxicated with ethanol (4 g/kg) according to the method of Liu *et al.* (1975). They were sacrificed at 1.5 h (group 7A) and 24 h (group 7B) after acute ethanol intoxication. Common bile duct-ligated, acute ethanol intoxication group (group 8): the rats were intoxicated with ethanol (4 g/kg) on the 14th day post-ligation. They were sacrificed at 1.5 h (group 8A) and 24 h (group 8B) after acute ethanol intoxication. All the animals were maintained on a pellet diet obtained commercially (Sam Yang Food Co., Wonju, Korea). Animals of groups 1–3 were supplied with tap water, whereas animals of groups 4–6 and 7–8 were supplied with ethanol instead of water for more than 60 days (above). Rats were fasted for 12 h prior to surgical procedures (common bile duct ligation, sham operation, and sacrifice). They were anesthesized lightly with ether during surgery and sacrifice, and sacrificed by withdrawal of blood from the abdominal aorta while livers were excised following perfusion. The serum from the collected blood was separated by centrifugation and stored at −30°C.

Subcellular fractionation Livers were perfused via the portal vein with cold 0.25 M sucrose, and then excised, blotted dry, weighed, minced with scissors, and homogenized in 9 vol of 0.25 M sucrose. All homogenates were subjected to cell fractionation. Cytosol, mitochondria, and microsomal fractions were obtained by sucrose linear density gradient centrifugation (Kwak and Kwak, 1986) and stored at −80°C. All procedures were performed at 2 to 4°C. The mitochondrial and microsomal fractions were resuspended in 0.25 M sucrose to maintain protein concentration at 5 mg/ml, and then diluted in 1 vol of 1% Triton X-100 and used for enzyme assays. The cytosolic fraction was used for enzyme assays without any processing (Junge, 1984).

Enzyme assays Carboxylesterase activities were determined with hepatic subcellular fractions and sera, according to the method of Junge (1984). The enzyme activities were measured using ethyl valerate as the substrate. Carboxylesterase hydrolyzes ethyl valerate to ethanol, and then ethanol is oxidized by alcohol dehydrogenase at the expense of NAD^+ to acetaldehyde and NADH. Briefly, the reaction mixture consisted of 125 mM phosphate, 35.6 mM glycine, 125 mM semicarbazide, 4.9 mM NAD, 44 kU of alcohol dehydrogenase, and 0.2 ml of sample (hepatic subcellular preparations and sera), in a final volume of 2.2 ml. The mixture was incubated at 25°C for 5 min. Excitation of $NADH^+$ was scanned for 3 min at 339 nm using a Varian Cary 210 spectrophotometer. The enzyme activities were expressed as

nmol of NADH formed per min per mg of protein of hepatic subcellular fractions or as per ml of serum.

The kinetic parameters (K_m and V_{max}) of carboxylesterase in subcellular fractions of cholestatic rat livers combined with chronic ethanol intoxication at the 14th day post-ligation were determined by using ethyl valerate as the substrate at variable concentrations (between 1 and 10 mM). The kinetic contants were calculated using the Lineweaver-Burk plot and compared to the kinetic parameters from two control groups (groups 3 and 5).

Determination of protein The protein concentration was determined by the biuret reaction (Gornal *et 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

The activities of cytosolic and mitochondrial carboxylesterase in cholestatic rat liver (group 3) showed significant decreases between the 7th and 14th day after common bile duct ligation compared to the activities in the sham-operated control (group 2). The microsomal preparation as well as the serum also showed significant decreases in activities between the 3rd and 14th day after ligation (Tables 1, 2, and 3). The V_{max} for all the hepatic preparations from the cholestatic rat liver at the 14th day post-ligation decreased significantly, although the K_m values were about the same as the sham-operated control (Table 6).

The activities of cytosolic carboxylesterase in cholestatic rat liver combined with chronic ethanol intoxication (group 6) showed further decrease compared to the activities in the ethanol plus sham-operated control group (group 5), and in the common bile ligated rats (group 3), between the 2nd and 14th day and 3rd and 14th day, respectively (Table 1). The mitochondrial and microsomal enzyme activities in the cholestatic rat liver combined with chronic ethanol intoxication (group 6) also showed significant decreases compared to the activities in the ethanol plus sham-operated control (group 5) between the 3rd and 14th day, and between the 1st and 14th day post-ligation (Tables 2 and 3). Carboxylesterase activity in serum from cholestatic rats with chronic ethanol intoxication (group 6) showed a significant decrease compared to the activity from cholestasis alone (group 3) between the 2nd and 14th day post-ligation (Table 4). The V_{max} values of all the hepatic preparations analyzed in cholestasis combined with chronic ethanol intoxication decreased significantly, although the K_m values did not change (Table 6).

In the case of acute ethanol intoxication, carboxylesterase activities in the liver cytosol as well as in the serum from cholestatic rats (groups 8A and 8B) showed significant decreases compared to the activities in the ethanol intoxication and common bile duct-ligated controls. The mitochondrial carboxylesterase activity decreased significantly at 1.5 h (group 8A) and 24 h (group 8B) post-intoxication compared to the activity from the acute ethanol intoxication control (group 7A) and common bile duct-ligated control (group 3), respectively. Carboxylesterase activities in liver microsomes from cholestatic rats with acute ethanol intoxication (groups 8A and 8B) decreased significantly compared to the activity in the common bile duct-ligated control. The V_{max} values of all the hepatic preparations analyzed in cholestasis combined with acute ethanol intoxication also showed significant decreases, although the K_m values did not change (Table 7).

Table 1. Effect of common bile duct ligation on liver cytosolic carboxylesterase activities in chronic ethanol intoxicated rats.

Day(s) following operation	Carboxylesterase activities (nmol NADH mg protein^{-1} min^{-1}) (Normal; 204.5 ± 28.3, Ethanol; 182.4 ± 29.5)			
	Sham	CBDL	Ethanol + Sham	Ethanol + CBDL
1	196.5 ± 26.4	196.0 ± 28.3	176.2 ± 24.7	166.9 ± 31.2
2	193.6 ± 27.1	170.4 ± 31.1	171.6 ± 26.3	121.7 ± 37.2[d]
3	192.0 ± 25.7	162.1 ± 26.8	167.5 ± 23.8	95.5 ± 32.3[e,h]
7	194.4 ± 27.8	139.3 ± 27.2[a]	151.3 ± 21.6[a]	72.9 ± 23.7[f,h]
14	192.8 ± 27.5	124.3 ± 26.4[b]	153.2 ± 24.3[a]	62.7 ± 21.6[f,h]

All values are expressed as mean ± SD with 5 rats in each group.
a, $P<0.05$ vs Sham; b, $P<0.01$ vs sham; d, $P<0.05$ vs Ethanol + Sham; e, $P<0.01$ vs Ethanol + Sham; f, $P<0.001$ vs Ethanol + Sham; h, $P<0.01$ vs CBDL.

Table 2. Effect of common bile duct ligation on liver mitochondrial carboxylesterase activities in chronic ethanol intoxicated rats.

Day(s) following operation	Carboxylesterase activities (nmol NADH mg protein^{-1} min^{-1})			
	(Normal; 71.6 ± 17.3, Ethanol; 72.7 ± 16.9)			
	Sham	CBDL	Ethanol + Sham	Ethanol + CBDL
1	70.1 ± 18.2	59.6 ± 19.8	71.1 ± 17.9	61.4 ± 20.3
2	69.6 ± 18.4	51.6 ± 24.6	68.3 ± 17.3	50.3 ± 25.2
3	68.5 ± 17.7	40.4 ± 23.3	65.5 ± 16.6	29.1 ± 16.3[e]
7	68.9 ± 16.9	28.6 ± 12.3[b]	63.2 ± 17.0	18.2 ± 7.6[f]
14	68.6 ± 17.1	15.2 ± 7.3[c]	64.4 ± 16.7	8.6 ± 4.9[f]

All values are expressed as mean ± SD with 5 rats in each group.
b, $P<0.01$ vs sham; c, $P<0.001$ vs Sham; e, $P<0.01$ vs Ethanol + Sham; f, $P<0.001$ vs Ethanol + Sham.

Table 3. Effect of common bile duct ligation on liver microsomal carboxylesterase activities in chronic ethanol intoxicated rats.

Day(s) following operation	Carboxylesterase activities (nmol NADH mg protein^{-1} min^{-1})			
	(Normal; 278.3 ± 36.4, Ethanol; 263.5 ± 33.2)			
	Sham	CBDL	Ethanol + Sham	Ethanol + CBDL
1	277.8 ± 35.2	211.5 ± 29.3[a]	258.2 ± 34.1	172.8 ± 31.2[e]
2	279.5 ± 34.0	152.2 ± 41.6[c]	261.4 ± 32.8	126.4 ± 43.3[f]
3	275.8 ± 36.1	145.7 ± 44.0[c]	252.6 ± 29.6	107.2 ± 37.6[f]
7	278.4 ± 38.6	101.5 ± 31.3[c]	248.5 ± 27.8	82.3 ± 29.7[f]
14	276.5 ± 36.3	39.7 ± 14.7[c]	243.6 ± 28.7	29.5 ± 13.2[f]

All values are expressed as mean ± SD with 5 rats in each group.
a, $P<0.05$ vs Sham; c, $P<0.001$ vs sham; e, $P<0.01$ vs Ethanol + Sham; f, $P<0.001$ vs Ethanol + Sham.

Table 4. Effect of common bile duct ligation on serum carboxylesterase activities in chronic ethanol intoxicated rats.

Day(s) following operation	Carboxylesterase activities (nmol NADH ml^{-1} min^{-1})			
	(Normal; 87.3 ± 23.4, Ethanol; 78.4 ± 20.6)			
	Sham	CBDL	Ethanol + Sham	Ethanol + CBDL
1	85.9 ± 19.8	72.8 ± 16.9	76.2 ± 21.2	56.4 ± 16.3
2	85.0 ± 21.4	63.9 ± 17.1	73.1 ± 19.6	42.7 ± 17.5[d]
3	86.3 ± 22.1	50.9 ± 14.8[a]	70.5 ± 18.7	38.1 ± 15.2[d]
7	85.4 ± 22.9	42.3 ± 15.5[b]	64.6 ± 19.1	28.6 ± 12.8[e]
14	86.8 ± 20.3	37.9 ± 14.1[b]	61.4 ± 18.2	26.5 ± 10.2[e]

All values are expressed as mean ± SD with 5 rats in each group.
a, $P<0.05$ vs Sham; b, $P<0.01$ vs Sham; d, $P<0.05$ vs Ethanol + Sham; e, $P<0.01$ vs Ethanol + Sham.

Table 5. Effect of common bile duct ligation on serum and liver cytosolic, mitochondrial, and microsomal carboxylesterase activities in acute ethanol intoxicated rats.

Carboxylesterase activities (Liver carboxylesterase; nmol NADH mg protein^{-1} min^{-1}, serum carboxylesterase; nmol NADH ml^{-1} min^{-1})					
Normal	CBDL 14 days	Ethanol 1.5 h	Ethanol 1.5 h + CBDL	Ethanol 24 h	Ethanol 24 h + CBDL
			(Cytosol)		
204.5 ± 28.3	124.3 ± 26.4[k]	184.7 ± 26.8	33.9 ± 20.8[o,u]	139.1 ± 25.5[k]	64.9 ± 15.1[r,t]
			(Mitochondria)		
71.6 ± 17.3	15.2 ± 7.3l	65.8 ± 14.9	6.9 ± 4.3[o]	56.9 ± 17.7	9.8 ± 6.2[r,t]
			(Microsome)		
278.3 ± 36.4	39.7 ± 14.7[l]	194.3 ± 41.4[k]	26.7 ± 17.8[o]	177.0 ± 38.6[k]	34.1 ± 16.3[r]
			(serum)		
87.3 ± 28.4	37.9 ± 14.1[k]	153.4 ± 37.4[k]	17.9 ± 6.2[o,s]	107.2 ± 26.7	18.3 ± 7.8[r,s]

All values are expressed as mean ± SD with 5 rats in each group.
k, $P<0.01$ vs Normal; l, $P<0.001$ vs. Normal; o, $P<0.001$ vs Ethanol 1.5 h; r, $P<0.001$ vs Ethanol 24 h; s, $P<0.05$ vs CBDL; t, $P<0.01$ vs CBDL 14 days; u, $P<0.001$ vs CBDL 14 days.

Table 6. Carboxylesterase kinetic parameters from cholestasis with chronic ethanol intoxicated rat liver determined with ethyl valerate.

Cell fractions	Sham	CBDL	Ethanol + Sham	Ethanol + CBDL
	K_m (mM)			
Cytosol	1.56 ± 0.25	1.49 ± 0.21	1.58 ± 0.23	1.52 ± 0.31
Mitochondria	6.78 ± 1.23	6.48 ± 1.16	6.74 ± 1.32	6.57 ± 1.38
Microsome	1.43 ± 0.17	1.40 ± 0.19	1.45 ± 0.21	1.43 ± 0.24
	V_{max} (nmol NADH mg protein^{-1} min^{-1})			
Cytosol	358.4 ± 35.5	135.1 ± 27.1[c]	287.2 ± 34.1	68.6 ± 21.3[f,h]
Mitochondria	52.1 ± 9.4	28.5 ± 7.2[b]	48.4 ± 8.3	16.5 ± 5.6[f,g]
Microsome	1,049.6 ± 158.3	409.9 ± 54.3[c]	967.7 ± 142.2	306.4 ± 48.7[f,g]

Michaelis-Menten constants for carboxylesterase were determined using ethyl valerate and NAD^+ at 25°C for cytosolic, mitochondrial, and microsomal fractions of male rat livers on the 14th day after operation.
The data are expressed as mean ± SD with 5 rats in each group.
b, $P<0.01$ vs Sham; c, $P<0.001$ vs Sham; f, $P<0.001$ vs Ethanol + Sham; g, $P<0.05$ vs CBDL; h, $P<0.01$ vs CBDL.

Table 7. Carboxylesterase kinetic parameters from cholestasis with acute ethanol intoxicated rat liver determined with ethyl valerate.

Animals groups	Cytosol		Mitochondria		Microsome	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
	(mM)		(nmol NADH mg protein^{-1} min^{-1})			
Normal	1.54 ± 0.27	347.8 ± 34.7	6.73 ± 1.26	50.6 ± 8.8	1.40 ± 0.16	1,016.2 ± 149.8
CBDL 14 days	1.49 ± 0.21	135.1 ± 27.1[l]	6.48 ± 1.16	28.5 ± 7.2[k]	1.40 ± 0.19	409.9 ± 54.3[l]
Ethanol 1.5 h	1.57 ± 0.24	308.3 ± 29.3	6.62 ± 1.19	44.3 ± 7.6	1.42 ± 0.17	733.8 ± 126.5[j]
Ethanol 1.5 h + CBDL	1.55 ± 0.26	40.5 ± 18.3[o,i]	6.54 ± 1.22	16.2 ± 4.7[o,g]	1.45 ± 0.20	269.0 ± 33.8[o,h]
Ethanol 24 h	1.56 ± 0.28	242.9 ± 25.3[l]	6.68 ± 1.15	41.7 ± 6.7	1.44 ± 0.24	662.2 ± 108.7[k]
Ethanol 24 h + CBDL	1.53 ± 0.24	71.3 ± 22.3[r,h]	6.59 ± 1.17	19.3 ± 5.6[r]	1.47 ± 0.22	348.7 ± 42.4[r]

Michaelis-Menten constants for carboxylesterase were determined using ethyl valerate and NAD^+ at 25°C for cytosolic, mitochondrial and microsomal fractions in male rat livers of acute intoxication with ethanol done after 14 days of the common bile duct ligation. The data are expressed as mean ± SD with 5 rats in each group.
g, $P<0.05$ vs CBDL 14 days; h, $P<0.01$ vs CBDL 14 days; i, $P<0.001$ vs CBDL 14 days; j, $P<0.05$ vs Normal; k, $P<0.01$ vs Normal; l, $P<0.001$ vs Normal; o, $P<0.001$ vs Ethanol 1.5 hrs; r, $P<0.001$ vs Ethanol 24 hrs.

Discussion

Ethanol easily crosses cell membranes (Ellenhorn and Barceloux, 1988), and is metabolized mainly in the liver (Lieber, 1985) to form acetaldehyde and eventually acetic acid (Borson and Li, 1980; Lieber, 1985). Acetaldehyde impairs microtubular formation and decreases mitochondrial function (Ellenhorn and Barceloux, 1988) with morphological changes (Chang, 1985; 1987): chronic acetaldehyde levels may also promote peroxidation of cellular membrane (Ellenhorn and Barceloux, 1988; Sherlock and Dooley, 1993). Chronic ethanol consumption results in peroxidation of the smooth endoplastic reticulum in animals and humans (Lieber, 1985). Ethanol disposal produces striking metabolic imbalances in the liver by alteration in the cellular redox state (Lieber, 1985; Ellenhorn and Barceloux, 1988). The earliest hepatic damages produced by ethanol are the deposition of fat and the enlargement of the liver (Lieber, 1985), which progresses to fatty liver, alcoholic hepatitis and may finally lead to cirrhosis (Wooddel, 1980; Lieber, 1985; Chang, 1985; 1987; Sherlock and Dooley, 1993).

The effects of cholestasis on the activities of several xenobiotic biotransforming enzymes, such as monoamine oxidase (Mun and Kwak, 1989), α-D-mannosidase, β-D-mannosidase (Park *et al.*,1994), arylsulfotransferase (Ihm *et al.*, 1995), thiosulfate sulfurtransferase, UDP-glucuronosyltransferase (Ihm and Kim, 1997), benzoyltransferase, and phenylacetyltransferase (Kim and Kim, 1999) have been previously studied in cholestatic rat liver induced by common bile duct ligation. In addition, many studies have been done on the activity changes of biotransforming enzymes such as glutathione S-transferase, glutathione peroxidase, glutathione reductase (Kwak *et al.*, 1990), monoamine oxidase (Chung and Kwak, 1992), 5′-nucleotidase (Ra *et al.*, 1995), arylsulfotransferase, UDP-glucuronosyltransferase (Kwon and Kim, 1997), glyoxalase I (Byun *et al.*, 1997), and glyoxalase II (No and Kim, 1997) in cholestatic rat liver combined with ethanol intoxication. In particular, carboxylesterase is one of the xenobiotic biotransforming enzymes and two closely related carboxyl ester hydrolases, carboxylesterase and cholinesterase, reside in the same enzyme molecules (Tsujita and Okuda, 1983). Some studies have reported the decreased activities of these liver esterases in cholestatic rat liver (Kwak and Lee, 1992). In a previous study, we demonstrated that the activity of this enzyme in choledochocaval fistula rats was decreased by high taurocholate load (Han and Kim, 1998). Kwak *et al.* (1994) reported the synergic effect on activity decrease in liver and serum cholinesterases in cholestatic rats combined with chronic ethanol intoxication.

In order to investigate the mutual effects of ethanol catabolism and cholestasis, we determined the activities of cytosolic, mitochondrial, and microsomal carboxylesterase in cholestatic rat liver combined with acute and chronic ethanol intoxication for a period of 14 days post-ligation, as well as in the serum. The activities of this enzyme in sera were also measured. Values of K_m and V_{max} for this enzyme at the 14th day after common bile duct ligation were determined in the liver of sham operation, common bile duct ligation, sham and common bile duct ligation plus chronic ethanol intoxication, using ethyl valerate as the substrate.

The activities of cytosolic, mitochondrial, and microsomal preparations from cholestatic rat liver as well

as the serum showed significant decrease compared to the activities from the sham-operated control. The V_{max} for all the hepatic preparations decreased significantly, although the K_m values remained about the same as the sham-operated control. These results are consistent with the previous studies on carboxylesterase activities in cholestatic rat liver (Kwak and Lee, 1992) and in high taurocholate load in choledococaval fistula rats (Han and Kim, 1998) as well as on cholinesterase, the other hepatic esterase, in cholestatic rat liver combined with chronic ethanol intoxication (Kwak *et al.*, 1994). These results may reflect the decreased biosynthetic capability of carboxylesterase in the cholestatic liver.

The synergic effects of cholestasis and ethanol intoxication on the decrease in carboxylase were significant in the cytosolic preparation from the cholestatic rat liver combined with chronic and acute ethanol intoxication (groups 6, 8A, and 8B). The synergicity was also significant in the mitochondrial preparation from cholestatic rat liver combined with acute ethanol intoxication (group 8B), as well as in the serum from cholestatic rat with acute ethanol intoxication (groups 8A and 8B). The V_{max} values of all the hepatic preparations analyzed in cholestasis combined with chronic and acute ethanol intoxication decreased significantly, although the K_m values did not change.

These results, therefore, suggest that the biosynthesis of hepatic carboxylesterase seems to decrease when cholestasis is combined with chronic and acute ethanol intoxication, and the decrease in activity is more significant compared to the decrease from cholestasis alone.

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