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

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= Abstract =

Arylesterase is a polymorphic aromatic esterase and organopohosphatase. Determination of the enzyme activities in human plasma is applied in the diagnosis of cirrhosis and carcinoma of the liver Ethanol disposal is thought to produce metabolic disorders resulting in alcoholic liver disease To investigate the mutual effects of ethanol disposal and cholestasis induced by common bile duct (CBD) ligation on the activities of arylesterase, we have determined the enzyme activities in the rat hepatic (cytosolic, mitochondrial and microsomal) preparations as well as in the rat serum by using ten animal models. normal rats (group 1), sham operated rats (group 2), CBD-ligated rats (group 3), chronic ethanol-intoxicated rats (group 4), sham operation with chronic ethanol intoxication (group 5), CBD ligation with chronic ethanol intoxication (group 6), acute ethanol intoxication 1.5 hr and 24 hr (group 7A and 7B), and acute ethanol intoxication 1.5 h and 24 h with CBD ligation (group 8A and 8B). We have estimated the enzyme activities for 14 d (group 2-6), 1.5 h (group 1, 3, 7A and 8A), and 24 h (group 1, 3, 7B and 8B) post-ligation. The values of *Km* and *Vmax* for these hepatic preparations of cholestatic rat liver combined with chronic ethanol intoxication were also measured by using phenyl acetate as the substrate from the 14 d post-ligation. Arylesterase activities of hepatic preparations and in the cholestatic rat serum (group 3) showed significant decrease compared to the activities from sham-operated control (group 2) Enzyme kinetic parameters indicated that *Vmax* for all the hepatic preparations in the cholestatic rat (group 3) decreased significantly, although the *Km* values were about the same as in sham-operated control (group 2). The synergic effects of cholestasis and ethanol intoxication on the decrease in the arylesterase activity were significant in the microsomal preparation from the cholest-

atic rat liver as well as in the serum combined with chronic ethanol intoxication (group 6). The synergic reduction of enzymaic kinetic parameters was significant in the *Vmax* values of the cytosolic and microsomal preparation, as well as in the serum analyzed in group 6, and of the cytosolc preparation analyzed in group 8A and 8B, although the *Km* values did not change These results, therefore, suggest that the biosynthesis of hepatic arylesterase seems to have decreased when cholestasis was combined with chronic ethanol intoxication. And the synergic reduction in the enzyme activity analyzed in the cholestatsis with ethanol intoxication may reflects that preexisting cellular damages with cholestasis were exacerbated by ethanol-induced oxidative stress

Key Words: Arylesterase, Ethanol intoxication, Cholestatic rat liver

Introduction

Ethanol disposal 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 (Lieber,1985 , Hall, 1994, Chang 1985 & 1987) The turnover rate of ethanol is remarkably decreased by procedures damaging the liver such as common bile duct ligations (Lieber, 1985) Cholestatic liver disease includes inflammations, necrosis, fatty changes, biliary hyperplasia, fibrosis and cirrhosis (Kountouras *et al*, 1984) The common bile duct-ligated rats have been widely used as an experimental model in human extrahepatic cholestasis (Kaplan & Righetti, 1970, Righetti & Kaplan, 1971)

Vertebrate plasma contains three types of esterases [EC 3 .1 1] cholinesterase [EC 3. 1 1 8, substrate acetylcholine], carboxylesterase [EC 3 1 1 1, substrate aliphatic esters] and arylesterase [EC 3 1 1 2, substrate aromatic esters] (Augustinsson, 1961 & 1964;Webb, 1992) Human arylesterase is an high density lipoprotein (HDL)-associated lipolytic and polymorphic aromatic esterase and organophosphatase that protects low density lipoprotein from copper ion-induced lipid peroxidation (Aviram *et al*, 1998). In mammalian systems, arylesterase play an important role in the detoxification of paraoxon (diethyl 4-nitrophenyl phosphate), the neurotoxic active metabolite of insecticide parathion (Augustinsson, 1961, Webb, 1992, Shaw *et al*, 1994) The enzyme is mainly present in the endoplasmic

reticulum (Haugen & Suttie, 1974) of mammalian liver (Burlina *et al*, 1977), kidney (von Deimling & Taylor, 1987), placenta, umbilical cord, amniotic fluid (Vincent *et al*, 1977) and erythrocyte (Mekhtiev *et al*, 1975), and in the blood (Dixon & Webb, 1978, Lorentz *et al*, 1979; Mackness & Walker, 1983) It is detected in high concentration in sera of pregnancy (Carpentero *et al*, 1996) and in the high cholesterol-fed rabbit (Beynen *et al*, 1984 a and b) The enzyme activity is absent from the serum of the patient with Tangier disease (Mackness *et al*, 1989) The activity of arylesterase has been shown to be low in the dietary disposure of hyperlipidemic agents (Butler *et al*, 1988), in patients with myocardial infarction (Secchiero *et al*, 1989), diabetes mellitus, chronic renal failure (Dantonie *et al*, 1998) and impaired liver function (Lorentz *et al*, 1979, Lio-Injo *et al*, 1980) Genetically determined serum HDL-arylesterase is well suited for quantify HDL in postmenopausal women without and with coronary artery disease (Chemnticus *et al*, 1998) The serum arylesterase assay is readily applied in the diagnosis of liver cirrhosis (Ikeda, 1989; Kawai *et al*, 1991).

The activities of carboxylesterase, cholinesterase and arylesterase are known to be decreased in the cholestatic rat livers and serum induced by common bile duct ligation (Kwak & Lee, 1992). Previously, we have reported the liver and serum cholinesterase and carboxylesterase activities decrease synergistically in the cholestatic rat combined with chronic ethanol intoxication (Kwak *et al*, 1994, Ahn & Kim, 1999). However, the possible changes of arylesterase activities in the cholestatic rat liver combined with ethanol intoxication have not yet been reported. In the present study, we have estimated arylesterase activities with acute and chronic ethanol intoxication to investigate the synergic effects of ethanol disposal and cholestasis induced by CBD ligation in the rat. In addition, the values of *Km* and *Vmax* for the cytosolic, mitochondrial and microsomal preparations of the rat liver from cholestasis alone and from cholestasis combined with ethanol intoxication were also measured by using phenyl acetate as the substrate on the 14thd post-ligation

Materials and Methods

Chemicals

Tris (hydroxymethyl) aminomethane, phenyl acetate, diethyl-ρ-nitrophenyl phosphate, Triton X 100 and bovine albumin (10 g/ 100 ml) were purchased from Sigma (St. Louis, USA). Calcium chloride and the other chemicals were of analytical reagent grade

Animals

Normal male Sprague-Dawley rats, weighing 280-320 g, were used in the experiments. All the experimental groups, with 5 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 d after sham operation CBD ligation group (group 3)· the rats were sacrificed at the 1st, 2nd, 3rd, 7th and 14th d after CBD ligation Chronic ethanol intoxication group (group 4)· the rats were fed 5% (v/v) ethanol instead of water for 60 d 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 d They were continuously supplied with ethanol and sham operations were performed Rats were sacrificed at the 1st, 2nd, 3rd, 7th and 14th d after sham operations CBD ligation combined with chronic ethanol intoxication group (group 6) · the rats were fed 5% (v/v) ethanol instead of water for 60 d They were continuously supplied with ethanol and CBD ligation was done Rats were sacrificed at the 1st, 2nd, 3rd, 7th and 14th d after CBD ligation. Acute ethanol intoxication group (group 7): the rats were intoxicated with ethanol (4g/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. CBD-ligated, acute ethanol-intoxication group (group 8) · the rats were intoxicated with ethanol (4 g/ kg) on the 14th d 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; however, animals of group 4-6 and 7-8 were supplied with ethanol instead of water for more than 60 d (above) They were famished for 12 h prior to the surgical procedures (CBD ligation, sham operation and sacrifice) They were anesthesized lightly with ether during surgery and sacrifice They were sacrificed by withdrawal of blood from the abdominal aorta while liver was excised following perfusion (below) The serum from the collected blood was separated by centrifugation and stored at -30℃

Subcellular fractionation

The livers were perfused via the portal vein with cold 0 25 M sucrose The livers were then excised, blotted to dry, weighed, minced with scissors and homogenized in 9 vol of 0 25 M sucrose Each homogenate was subjected to cell fractionation. Cytosol, mitochondria and microsomal fractions (hepatic subcellular fractions) were obtained by the

method of sucrose linear density gradient centrifugation (Kwak & Kwak, 1986) and stored at -80℃. All the prodedures were performed at 2 to 4 ℃ The mitochondrial and microsomal fractions were resuspended in 0.25 M sucrose to keep protein concentration at 5 mg/ml, then were diluted in 1 volume of 1% Triton X 100 and were used for enzyme assay The cytosolic fraction was used for enzyme assay without any processing (Junge & Klees, 1984).

Enzyme assays

Arylesterase activities were determined with hepatic subcellular fractions and in sera, according to the method of Junge & Klees (1984). The enzyme activities were measured using phenyl acetate as the substrate Briefly, the reaction mixture consisted of 1.0 ml of 0.49 M Tris-acetate buffer, pH 7 4, containing 10 mM calcium chloride, 1.0 ml of 4 mM phenyl acetate and 0 02 ml of sample (hepatic subcellular preparations and sera), in final volume 2.02 ml. The mixture was incubated at 25℃ for 3 min Then the liberated phenol was detected at at wave length 270 nm spectrophotometically by using Varian, Cary 210 (ε = 1480 $M^{-1}cm^{-1}$). The enzyme activities were expressed at nmol of phenol formed per min per mg of protein of hepatic subcellular fractions and as per ml of serum.

For determination of kinetic parameters (*Km* and *Vmax*) of enzymes with subcellular fractions of cholestatic rat livers combined with chronic ethanol intoxication at the 14th d post-ligation by using phenyl acetate as the substrate at variable concentratio (between 0.2 and 2 mM). The kinetic contants were calculated using Lineweaver-Burk plot and compared to the kinetic parameters from two control groups (group 3 and 5)

Determination of protein

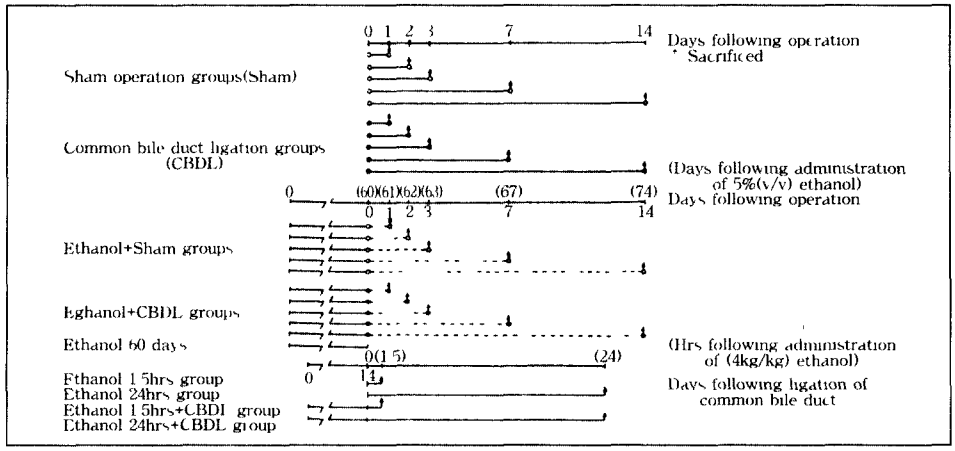

Figure 1. Experimental design.

The protein concentration was determined by the biuret reaction (Gornal *et al*, 1949), using bovine albumin as the calibrator

Statistical analysis

Values were expressed as mean ± S D Statistical significance was calculated using Student's t-test at $P \leq 0.05$

Results

The activities of cytosolic and mitochondrial arylesterase in the cholestatic rat liver (group 3) showed significant decrease on the 14th d after common bile duct ligation compared to the activities from the sham operated control (group 2) ($P \leq 0.05$ and $P \leq 0.01$) The microsomal preparation as well as in the serum also showed significant decrease in their activities between the 1st through the 14th d after ligation compared to the activities from the control ($P \leq 0.01$ to 0.001) (Table 1, 2 and 3). Enzyme kinetic parameters indicated that *Vmax* for all the hepatic preparations from the cholestatic rat liver at the 14th d post-ligation decreased significantly compared to the *Vmax* of the group 2 control ($P \leq 0.01$ to 0.001), although the *Km* values were about the same as the control (Table 6)

The activities of cytosolic arylesterase in the group 6 (ethanol + CBD ligation) showed further significant decrease ($P < 0.05$) compared to the activities from the group 5 control (ethanol + sham operation), between the 3rd through the 14th d (Table 1) The mitochondrial activities in the group 6 also showed further significant decrease ($P \leq 0.01$) compared to the activities from the group 5 control on the 14th d post-ligation (Table 2). Arylesterase activitities of the microsomal preparation as well as in the serum from the group 6 showed further significant decreases between the 1st through the 14th d post-ligation compared to the activity from cholestasis alone (group 3) and from group 5 (ethanol + sham operation) (Table 3 and 4) The *Vmax* values of all the hepatic preparations analyzed in group 6 (cholestasis + chronic ethanol intoxication) decreased significantly compared to the *Vmax* from the group 5 control (ethanol + sham operation), and *Vmax* of cytosolic and microsomal preparations showed further significant decerases compared to the *Vmax* from cholestasis alone (group 3), although the *Km* values did not change (Table 6)

In the case of acute ethanol intoxication, arylesterase activities in the hepatic preparations, as well as in the serum from the cholestatic rats (group 8) showed significant decreases compared to the activities from the group 7 control (ethanol intoxication alone) (Table 5) The *Vmax* values of all the hepatic prep-

arations analyzed in group 8 also decreased significantly compared to the *Vmax* from group 7, although the *Km* values did not change In addition, *Vmax* values of cytosolic and microsomal preparations from group 8, showed further significant decerase copmatred to the Vmax from cholestasis alone (group 3),

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

Day(s) following operation	Arylesterase activities (nmol phenol mg protein^{-1} min^{-1}) (Normal; 625.4±174 6, Ethanol; 582.7±159.3)			
	Sham	CBDL	Ethanol+Sham	Ethanol+CBDL
1	627 3 ±170 9	612.0±167 4	576 6±163.5	504.7±137.8
2	625 5 ±181.8	597.1±158.5	575.2±166.7	453.3±128.6
3	628.8 ±172 7	552.6±142.4	573 5±160.4	367.7±115 1[d]
7	626 7 ±178 1	424.8±112.2	566 3±161 9	352 4±118.3[d]
14	624.1 ±169 9	412.6± 92.3[a]	560.7 ±158.6	321.2±108 5[d]

All values are expressed as mean ± SD with 5 rats in each group.
Animal groups are described in Fig.1
a; P<0 05 vs Sham, b; P<0.01 vs. Sham, c; P<0.001 vs. Sham,
d; P<0 05 vs Ethanol + Sham

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

Day(s) following operation	Arylesterase activities (nmol phenol mg protein^{-1} min^{-1}) (Normal; 222.1±68.3, Ethanol; 221.7±71.4)			
	Sham	CBDL	Ethanol+Sham	Ethanol+CBDL
1	223.6±70.1	219.1±66 6	223.3±72.5	218.6±73.7
2	222.9±69.5	212.0±66.2	220 2±73 7	211 7±71.4
3	221.2 ±70 9	166.9±55.2	218.4±72.1	172.3±58.2
7	219 1 ±70 5	147.6±48.3	216 2±71.4	145.4±46.9
14	218.3±68.2	84.5±33.6[b]	213 8±69 7	91.3±37.1[e]

All values are expressed as mean ± SD with 5 rats in each group
Animal groups are described in Fig.1.
a; P<0.05 vs. Sham, b; P<0.01 vs. Sham, c; P<0.001 vs. Sham,
d; P<0.05 vs Ethanol + Sham, e; P<0 01 vs Ethanol + Sham

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

Day(s) following operation	Arylesterase activities (nmol phenol mg protein^{-1} min^{-1}) (Normal; 5,746±872, Ethanol, 4,992±793)			
	Sham	CBDL	Ethanol+Sham	Ethanol+CBDL
1	5,776±845	3,975±463[b]	4,963±817	2,287±442[f,i]
2	5,739±889	2,130±388[c]	4,986±831	1,545±393[f,g]
3	5,721±867	2,110±428[c]	3,062±805	1,462±387[f,g]
7	5,765±858	1,961±476[c]	5,052±808	1,298±374[f,g]
14	5,734±862	1,876±417[c]	5,017±788	1,253±368[f,g]

All values are expressed as mean ± SD with 5 rats in each group
Animal groups are described in Fig.1
a; P<0 05 vs Sham, b, P<0 01 vs. Sham, c, P<0.001 vs Sham,
d; P<0 05 vs Ethanol + Sham, e, P<0 01 vs Ethanol + Sham,
f; P<0 001 vs Ethanol + Sham, g, P<0 05 vs CBDL, i, P<0 001 vs CBDL

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

Day(s) following operation	Arylesterase activities (nmol phenol mg protein^{-1} min^{-1}) (Normal; 166 9±20 7, Ethanol; 144 6±17 3)			
	Sham	CBDL	Ethanol+Sham	Ethanol+CBDL
1	164 4±20 2	113 1±22 0[b]	145 3±18 5	71 5±23 2[f,g]
2	165 5±21 3	97 6±21 6[b]	142 5±19 2	61 2±21 3[f,g]
3	167 9±21.5	96 3±24 5[b]	140 2±18 3	57 3±22 0[f,g]
7	165 0±20 6	84 2±25.4[c]	133 4±17 6[a]	49 4±20 2[f,g]
14	164 6±21 8	57 8±22 9[c]	130 8±16 6[a]	38 3±15 4[f,g]

All values are expressed as mean ± SD with 5 rats in each group
Animal groups are described in Fig 1
a, P<0 05 vs Sham, b, P<0 01 vs Sham, c, P<0.001 vs Sham,
d, P<0 05 vs. Ethanol + Sham, e, P<0 01 vs. Ethanol + Sham,
f; P<0 001 vs Ethanol + Sham, g; P<0 05 vs CBDL

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

Normal	CBDL 14 days	Ethanol 1.5 hrs	Ethanol 1.5 hrs +CBDL	Ethanol 24 hrs	Ethanol 24 hrs +CBDL
Arylesterase activities (Liver arylesterase; nmol phenol mg protein^{-1} min^{-1}, serum arylesterase; nmol phenol ml^{-1} min^{-1})					
			(Cytosol)		
625 4 ±74.6	412.6 ± 92.3^{j}	560 6 ±139.2	319 2 ± 51.8^{n}	588 7 ±147.3	349.4 ±112.3^{p}
			(Mitochondria)		
221.1 ± 68.3	84.5 ± 33.6^{k}	213.6 ± 56.8	95.6 ± 39.5^{n}	197.7 ± 45.1	89.6 ± 43.7^{q}
			(Microsome)		
5,746 ±872	1,876 ±4171	5,873 ±783	1,622 ±554^{o}	5,397 ±936	1,656 ±429^{r}
			(serum)		
166 9 ± 20 7	57.8 ±22.9^{l}	157.3 ± 26.1	46 8 ± 17.9^{o}	148.8 ± 20.3	49.0 ± 18.4^{r}

All values are expressed as mean ± SD with 5 rats in each group.
Animal groups are described in Fig. 1.
h; P<0.01 vs.CBDL, i; P<0.001 vs.CBDL, j; P<0.05 vs. Normal, k; P<0.01 vs. Normal, l; P<0.001 vs Normal, m; P<0.05 vs. Ethanol 1.5 hrs, n; P<0.01 vs.Ethanol 1 5 hrs, o; P<0.001 vs. Ethanol 1.5 hrs, p; P<0.05 vs. Ethanol 24 hrs, q; P<0.01 vs Ethanol 24 hrs, r; P<0.001 vs Ethanol 24 hrs

Table 6. Arylesterase kinetic parameters from cholestasis with chronic ethanol intoxicated rat liver determined with phenyl acetate

Cell fractions	Sham	CBDL	Ethanol+Sham	Ethanol+CBDL
		Km(mM)		
Cytosol	0.52±0.03	0.51±0.04	0.54±0 04	0.56±0.03
Mitochondria	7 17±0.67	7.26±0.43	7.23±0.58	7.31±0 61
Microsome	1 28±0.16	1.32±0.14	1.30±0.18	1.34±0.17
		Vmax(nmol phenol mg protein^{-1} min^{-1})		
Cytosol	750.5± 51.7	526.8± 40 5^{c}	675.4± 47.6	408.7± 39.3^{f,g}
Mitochondria	95.9± 25.1	27 3± 12 8^{c}	97 3± 22.7	28.9± 11.2^{f}
Microsome	2,523.3±276.4	1,751.7±251.6^{b}	2,176.7±263 4	1,226.7±201.8^{f,h}

Michaelis-Menten constants for arylesterase were determined using phenylacetate at 25℃ for cytosolic, mitochondrial and microsomal fractions of male rat livers at the 14th day after operation.
The data are expressed as mean ± SD with 5 rats in each group
Animal groups are described in Fig 1.
a; P<0 05 vs. Sham, b; P<0 01 vs. Sham, c; P<0.001 vs. Sham,
d; P<0 05 vs. Ethanol + Sham, e; P<0 01 vs. Ethanol + Sham,
f; P<0 001 vs Ethanol + Sham, g; P <0 05 vs. CBDL, h, P<0.01 vs. CBDL

Table 7 Arylesterase kinetic parameters from cholestasis with acute ethanol intoxicated rat liver determined with phenyl acetate

Normal	0.51±0.03	747.3±49.8	7.15±0.64	96.2±24.3	1.29±0.15	2,498.2±267.3
CBDL 14 days	0.51±0.04	526.8±40.5[l]	7.26±0.43	27.3±12.8[l]	1.32±0.14	1,751.7±251.6[k]
Ethanol 1.5 hrs	0.53±0.05	659.3±46.3[j]	7.31±0.49	91.5±28.2	1.30±0.17	2,576.8±271.4
Ethanol 1.5 hrs + CBDL	0.55±0.03	318.6±35.8[o,l]	7.37±0.46	33.2±14.3[n]	1.34±0.15	1,603.3±236.3[o]
Ethanol 24 hrs	0.52±0.04	552.4±43.7[l]	7.28±0.54	85.3±26.6	1.32±0.16	2,389.4±263.5
Ethanol 24 hrs + CBDL	0.54±0.03	348.4±37.1[r,l]	7.34±0.50	30.8±13.7[q]	1.35±0.17	1,647.6±247.2[q]

Michaelis-Menten constants for arylesterase were determined using phenylacetate at 25℃ 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

Animal groups are described in Fig 1.

g, P<0.05 vs. CBDL 14 days, h; P<0.01 vs CBDL 14days,

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, m, P<0.05 vs Ethanol 1.5 hrs, n, P<0.01 vs Ethanol 1.5 hrs,

o, P<0.001 vs Ethanol 1.5 hrs, q, P<0.01 vs. Ethanol 24 hrs,

r, P<0.001 vs Ethanol 24 hrs

although the *Km* values did not change (Table 7).

Discussion

Ethanol easily crosses cell membranes (Ellenhorn & Barceloux, 1988) and is metabolized mainly in the liver (Lieber, 1985) to form acetaldehyde and eventually to form acetic acid (Borson & Li, 1980, Lieber, 1985) Acetaldehyde impairs microtubular formation and decreases mitochondrial function (Ellenhorn & Barceloux, 1988) with morphological changes (Chang, 1985, Chang, 1987) and chronic acetaldehyde levels may promote peroxidation of cellular membrane (Ellenhorn & Barceloux, 1988; Sherlock & Dooley, 1993) Acute and chronic ethanol consumption results in peroxidation of smooth endoplastic reticulum in animals and men (Sippel, 1983, Lieber,

1985; Savolainen *et al*, 1986; Yamada *et al*, 1988). Ethanol disposal produces striking metabolic imbalances in the liver by alteration in the cellular redox state (Lieber, 1985; Ellenhorn & Barceloux, 1988). The earliest hepatic damages produced by ethanol are the deposition of fat and the enlargement of the liver (Lieber, 1985) which progress in fatty liver, alcoholic hepatitis and finally lead to cirrhosis (Wooddel, 1980; Lieber, 1985 ; Chang, 1985; Chang, 1987, Sherlock & Dooley, 1993).

Human arylesterase is a thiol esterase hydrolyzing aromatic esters and organophosphates The enzyme actvities are mainly detected in the hepatic microsomal fractions and in the serum. Serum arylsterase is bound to high-density lipoprotein (HDL) and prevent oxidation of low-density lipoprotein (LDL) by hydrolyzing lipid peroxides (Kao *et al*, 1989). Oxidative stress leads to an HDL -associated reduction in arylesterase activity (Aviram *et al*, 1998). Kwak & Lee (1992) have reported the decreased activities of liver and serum esterases, carboxylesterase, cholinesterase and arylesterase in the cholestatic rat. In the previous studies, we have reported the synergic reduction in activities of serine esterase, cholinesterase (Kwak *et al*, 1994) and carboxylesterase (Ahn & Kim 1999) in the cholestatic rat combined with ethanol intoxication Nevertheless, the change of arylesterase activity in the cholestatic rat liver combined with chronic and acute ethanol intoxication have not yet been investigated.

In order to investigate the mutual effects of ethanol disposal and cholestasis, we have determined the activities of cytosolic, mitochondrial and microsomal arylesterase in the cholestatic rat liver combined with acute and chronic ethanol intoxication for a period of 14 d post-ligation The activities of this enzyme in sera were also measured Values of *Km* and *Vmax* for this enzyme at the 14th d 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, as well as in the serum, using phenyl acetate as the substrate.

The activities of cytosolic, mitochondrial and microsomal preparations from the cholestatic rat liver as well as in the serum showed significant decrease compared to the activities from the sham operated control. The *Vmax* for all the hepatic preparations decreased significantly, although the *Km* values remained about the same as the sham operated control

These results were consistent with the previous studies on arylesterase activities in the cholestatic rat liver (Kwak & Lee, 1992) as well as on cholinesterase, the other hepatic serine esterase, in the cholestatic rat liver combin-

ed with chronic ethanol intoxication (Kwak *et al*, 1994) These results may reflect the decreased biosynthetic capability of arylesterase in the cholestatic liver.

The synergic effects of cholestasis and ethanol intoxication on the decrease in the arylesterase activity were significant in the microsomal preparation from the cholestatic rat liver as well as in the serum combined with chronic ethanol intoxication (group 6) The synergic reduction of enzymaic kinetic parameters was significant in the *Vmax* values of the cytosolic and microsomal preparation, as well as in the serum analyzed in cholestasis combined with chronic ethanol intoxication (group 6), and of the cytosolic preparation analyzed in the cholestatic rat liver with acute ethanol intoxication (group 8A and 8B), although the *Km* values did not change

These results, therefore, suggest that the biosynthesis of hepatic arylesterase seems to have decreased when cholestasis was combined with chronic ethanol intoxication And the synergic reduction in the enzyme activity analyzed in the cholestatsis with ethanol intoxication may reflet preexisting cellular damages with cholestasis was exacerbated by ethanol-induced oxidative stress.

Summary

To investigate the mutual effects of ethanol disposal and cholestasis induced by common bile duct ligation on the activities of arylesterase, we have determined the enzyme activities in the rat hepatic (cytosolic, mitochondrial and microsomal) preparations as well as in the rat serum in ten animal models' normal (group 1), sham operation (group 2), CBD-ligated rats (group 3), chronic ethanol-intoxicated rats (group 4), sham operation plus chronic ethanol intoxication (group 5), CBD-ligation plus chronic ethanol intoxication (group 6), acute ethanol intoxication 1 5 hr and 24 hr (group 7A and 7B), and acute ethanol intoxication 1 5 h and 24 h plus CBD ligation (group 8A and 8B). We have estimated the enzyme activities for 14 d (group 2-6), 1 5 h (group 1, 3, 7A and 8A), and 24 h (group 1, 3, 7B and 8B) post-ligation. The values of *Km* and *Vmax* for these hepatic preparations of cholestatic rat liver combined with chronic ethanol intoxication were also measured by using phenylacetate as the substrate from the 14 d post-ligation

The synergic effects of cholestasis and ethanol intoxication on the decrease in the arylesterase activity were significant in the microsomal preparation from the cholestatic rat liver as well as in the serum combined with chronic ethanol intoxication (group 6) The synergic reduction of enzymaic kinetic parameters was significant in the *Vmax* values of the cytosolic and microsomal prepar-

ations, as well as in the serum analyzed in cholestasis combined with chronic ethanol intoxication (group 6), and of the cytosolc preparation analyzed in the CBD-ligated rat liver with acute ethanol intoxication (group 8A and 8B), although the *Km* values did not change.

These results, therefore, suggest that the biosynthesis of hepatic arylesterase seems to have decreased when cholestasis was combined with chronic ethanol intoxication. And the synergic reduction in the enzyme activity analyzed in the cholestatsis with ethanol intoxication may reflect preexisting cellular damages with cholestasis are exacerbated by ethanol-induced oxidative stress.

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