

Epoxide Hydratase

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Effects of Extrahepatic Cholestasis on Epoxide Hydratase Activity in Chronic Ethanol Intoxicated Rat Liver

Chun Sik Kwak, Ph.D., Ki Suk Choi, M.D.,
Kyo Cheol Mun, M.D., You Hee Kim, M.D.

*Department of Biochemistry,
Keimyung University School of Medicine, Taegu, Korea*

Abstract : To investigate the adverse effects of alcohol ingestion to the liver under the hepatobiliary disease, the activity of the rat hepatic epoxide hydratase was measured in extrahepatic cholestasis induced by common bile duct (CBD) ligation after chronic ethanol intoxication. The liver microsomal epoxide hydratase activity in CBD ligated rats combined with chronic ethanol intoxication was lower than that in CBD ligation alone. On the other hands, the values of Vmax of the liver microsomal epoxide hydratase in CBD ligated rats combined with chronic ethanol intoxication was lower than that in CBD ligation alone. However, the value of Km of the above hepatic enzyme did not vary in the all experimental groups. This results indicate that the biosynthesis of the hepatic epoxide hydratase decreases in chronic ethanol intoxication combined with cholestasis than in cholestasis alone. Accordingly, the results will be the data supporting that alcoholic ingestion is enzymologically harmful in hepatobiliary disease.

Key words : Alcohol intoxication, Cholestasis, Epoxide hydratase

가 [1] epoxide hydratase

가 [2]. Km Vmax

가 [3,4]. (xenobiotic)

[2], 1.

가 [5]. Trans-stilbene oxide, potassium phosphate monobasic, potassium phosphate dibasic (10 g/100 mL bovine albumin) Sigma ()

Epoxide hydratase(epoxide hydrolase, EC 3.3.2.3) epoxide 가 glycol [6,7]

[8,9], 4

[8,10]. 280 320 g Sprague-Dawley

epoxide hydratase epoxide 가 22 (1),

가 1 , 2 , 3 , 7 14

[7,11] 가 (5),

가 가 [12] 1 , 2 , 3 , 7 14 가

epoxide hydratase (5), Eagon [15]

가 [12], 5%(v/v) 60

(1), 5%(v/v) 60

가 5%(v/v) 가

[13,14]가 1 , 2 , 3 , 7 14

가 5), 5%(v/v) 60

5%(v/v)

1 , 2 , 3 , 7 14

(5)

가
5%(v/v) [15]
가
12
1 cm
가
12
4 0.25 M sucrose
sucrose 가
3.
2 4
7 g 9 0.25 M
sucrose Teflon pestle glass
homognizer(chamber clearance 0.005
-0.007 inches, Thomas) 2 4
400 rpm 5
10%(w/v)
sucrose
density gradient [16]

4.
Epoxide hydratase
5 mg/mL가 0.25
M sucrose
5.
epoxide hydratase
trans-stilbene oxide
37 20
trans-stilbene oxide 229 nm
1,2-diphenyl-1,2-ethanediol
Hasegawa Hammock[17]
1 1 mg
1,2-diphenyl-1,2-
-ethanediol nmol
2
computer
controlled enzyme spectrophotometer(Cary
210, Varian,)
6. Km Vmax
가 14
가
14
epoxide hydratase
1/vi
1/[S] (double
reciprocal plot)

Km Vmax hydratase (Table 1 & 2). epoxide hydratase
 7. 가 (Table 3).
 acid methanol-ether 0.5 M perchloric (3 : 1) epoxide hydratase
 Greenberg Rothstein[18] 가
 biuret 가 1
 62% (P<0.05), 2 85%
 (P<0.05), 3 80% (P<0.05), 7
 8. 74% (P<0.05) 가
 Student's t-test 가
 0.05 14 43%
 (P<0.05)
 1. 7
 43% (P<0.05), 14 51% (P<0.01)
 epoxide hydratase (Table 3).
 epoxide 2.

Table 1. Effect of common bile duct ligation on liver cytosolic epoxide hydratase activity in chronic ethanol intoxicated rats

Day (s) following operation	Epoxide hydratase activity (nmol 1,2-diphenyl-1,2-ethanediol min ⁻¹ mg protein ⁻¹)			
	Sham	CBDL	Ethanol+Sham	Ethanol+CBDL
1	1.91 ± 0.84	2.89 ± 1.14	1.92 ± 0.87	2.62 ± 0.97
2	1.86 ± 0.82	2.58 ± 1.08	1.89 ± 0.85	2.41 ± 0.94
3	1.85 ± 0.83	2.39 ± 0.84	1.91 ± 0.88	2.26 ± 1.06
7	1.83 ± 0.70	2.06 ± 0.81	1.87 ± 0.92	1.78 ± 0.92
14	1.81 ± 0.64	1.85 ± 0.73	1.88 ± 0.86	1.31 ± 0.84

All values are expressed as mean ± SD with 5 rats in each group; Sham: sham operated rats; CBDL: common bile duct ligated rats; Ethanol 60 days: rat were given 5% (v/v) ethanol solution for 60 days.

Table 2. Effect of common bile duct ligation on liver mitochondrial epoxide hydratase activity in chronic ethanol intoxicated rats

Day (s) following operation	Epoxide hydratase activity (nmol 1,2-diphenyl-1,2-ethanediol min ⁻¹ mg protein ⁻¹)			
	(Normal: 0.76 \pm 0.24; Ethanol 60 days: 0.79 \pm 0.22)			
	Sham	CBDL	Ethanol+Sham	Ethanol+CBDL
1	0.78 \pm 0.26	0.74 \pm 0.22	0.81 \pm 0.24	0.70 \pm 0.24
2	0.77 \pm 0.25	0.70 \pm 0.21	0.79 \pm 0.27	0.66 \pm 0.26
3	0.75 \pm 0.22	0.64 \pm 0.17	0.79 \pm 0.25	0.59 \pm 0.20
7	0.76 \pm 0.23	0.57 \pm 0.20	0.80 \pm 0.23	0.53 \pm 0.22
14	0.75 \pm 0.21	0.48 \pm 0.18	0.77 \pm 0.26	0.46 \pm 0.18

All values are expressed as mean \pm SD with 5 rats in each group. Animal groups are described in Table 1.

Table 3. Effect of common bile duct ligation on liver microsomal epoxide hydratase activity in chronic ethanol intoxicated rats

Day (s) following operation	Epoxide hydratase activity (nmol 1,2-diphenyl-1,2-ethanediol min ⁻¹ mg protein ⁻¹)			
	(Normal: 2.20 \pm 0.73; Ethanol 60 days: 2.38 \pm 0.75)			
	Sham	CBDL	Ethanol+Sham	Ethanol+CBDL
1	2.33 \pm 0.81	3.76 \pm 0.94 ^a	2.52 \pm 0.78	3.65 \pm 0.83
2	2.30 \pm 0.78	4.25 \pm 1.16 ^a	2.46 \pm 0.73	3.56 \pm 0.91
3	2.27 \pm 0.75	4.08 \pm 0.96 ^a	2.39 \pm 0.77	3.14 \pm 0.86
7	2.24 \pm 0.74	3.89 \pm 0.85 ^a	2.41 \pm 0.72	2.23 \pm 0.74 ^a
14	2.25 \pm 0.72	2.77 \pm 0.75	2.37 \pm 0.76	1.36 \pm 0.41 ^{d,h}

All values are expressed as mean \pm SD with 5 rats in each group. Animal groups are described in Table 1. a, P<0.05 vs. Sham; d, P<0.05 vs. Ethanol + Sham; g, P<0.05 vs. CBDL; h, P<0.01 vs. CBDL.

14 epoxide hydratase Km
Vmax

14 epoxide hydratase Km
Vmax

14 trans-stilbene oxide
Vmax

14 epoxide hydratase Km
Vmax

14 epoxide hydratase Km
Vmax

Table 4

14 (Table 4).

Table 4. Microsomal epoxide hydratase kinetic parameters from cholestasis with chronic ethanol intoxicated rat liver determined with trans-stilbene oxide

Sham	CBDL	Ethanol+Sham	Ethanol+CBDL
Km (mM)			
1.43 ± 0.21	1.45 ± 0.17	1.41 ± 0.19	1.47 ± 0.18
Vmax (nmol 1,2-diphenyl-1,2-ethanediol min ⁻¹ mg protein ⁻¹)			
2.96 ± 0.73	3.57 ± 0.75	3.09 ± 0.76	1.78 ± 0.40 ^{e,h}

Michaelis-Menten constants for epoxide hydratase were determined using trans-stilbene oxide at 37°... for microsomal fraction 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 Table 1. e, P<0.01 vs. Ethanol + Sham; h, P<0.01 vs. CBDL.

가 [1], 가 가 glutathione S-transferase[25] arylesterase [26] carboxylesterase[27] epoxide hydratase

epoxide hydratase 가 [12]

[19,20]. epoxide hydratase 가

[21] 가 [22,23] 7 14

가 epoxide hydratase 가 가

가 가 14 epoxide hydratase Km

xanthine oxidase[24], glutathione S-transferase, glutathione peroxidase Km 가 monoamine oxidase[13] 가 14

