

Rhodanese

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Effect of Extrahepatic Cholestasis on Hepatic Rhodanese Activity in Chronic Ethanol Intoxicated Rats

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Abstract : To investigate the adverse effects of alcohol ingestion on the liver of hepatobiliary disease, the activity of rat serum and hepatic rhodanese was measured in extrahepatic cholestasis induced by common bile duct (CBD) ligation after chronic ethanol intoxication. Liver cytosolic, mitochondrial and microsomal rhodanese activities in CBD ligated rats with chronic ethanol intoxication were found to be decreased much more than that in CBD ligation alone. V_{max} values of the liver cytosolic and mitochondrial rhodanese in these animals were lower than in the control, whereas K_m values of the above hepatic enzymes did not vary in the all experimental groups. On the other hand, serum rhodanese activity in CBD ligated rats with chronic ethanol intoxication was greater increased more than that in CBD ligation alone. These results indicate that the biosynthesis of the hepatic rhodanese decreases and the serum rhodanese activity increases in cholestasis combined with chronic ethanol intoxication, reflecting damages of aggravated hepatocytic membrane.

Key Words : Alcohol intoxication, Cholestasis, Rhodanese

[7]가
가

가

가
가

rhodanese

14

Km Vmax

[1].

(xenobiotic)

1.

가 [2,3].

Potassium cyanide, sodium thiosulfate pentahydrate, formaldehyde, ferric nitrate, ferric thiocyanate, potassium phosphate monobasic, rhodanese(thiosulfate sulfurtransferase, type , from bovine liver) (10 g/100 mL bovine serum albumin) Sigma ()

biotransformtaion)

(xenobiotic

Rhodanese(thiosulfate: cyanide sulfurtransferase, EC 2. 8. 1. 1) cyanide, sulfite, organic sulfinat dithiol

2.

[4,5]

[4].

4

[6]

280 320 g Sprague-Dawley

[3,4].

5

22

가

(1),

가

가 가

1 , 2 , 3 , 7 14

[3]

(5),

1 , 2

rhodanese

, 3 , 7 14

가

가 [3],

(5), Eagon [8]

5%

(v/v) 60

(1), 5%(v/v) 60
5%(v/v) 1, 2, 3, 7 14

(5), 5%(v/v) 60
5%(v/v) 1, 2, 3, 7 14
가

(5) .
가
5%(v/v) [8]
가

12

1 cm

가

12

4 0.25 M sucrose

sucrose 가

3.

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 [9]
2 4

Du Pont
Sorvall () RC-5B refrigerated
superspeed centrifuge OTD-65B ultracentrifuge
rotor Du Pont Sorvall
SS-34 T865 rotor, sucrose linear
density gradient gradient
former(model 570, ISCO,)

4.

Rhodanese

5 mg/mL가 0.25 M
sucrose

5.

rhodanese
potassium cynaide sodium thiosulfate
25 20
thiocyanate ferric nitrate
ferric thiocyanate 460

nm

Westley [5]

1 1 mL 1 mg

ferric thiocyanate

nmol

Sigma ()

가 (Table 1

2

computer controlled enzyme spectrophotometer (Cary 210, Varian ,) .

6. Km Vmax

, 가 14

, 가 14

rhodanese 1/vi , 1/[S] (double reciprocal Km

plot) Vmax

7.

0.5 M perchloric acid methanol-ether (3:1) Greenberg Rothstein [10] biuret

rhodanese

가 7

8. 43%(P<0.01), 14 49%(P<0.01)

Student's t-test 0.05

가 2 51%(P<0.01), 3 54%(P<0.01), 7 64%(P<0.001), 14 71%(P<0.001)

1. rhodanese ,

rhodanese , 2 47%(P<0.05), 3 42% (P<0.05), 7 40%(P<0.05), 14

가

16

20%(P<0.05), 3

27% (P<0.01), 7 41%

(P<0.001), 14 64%(P<0.001)

가

19%(P<0.05), 2

46%(P<0.001), 3 48%(P<0.001), 7

62%(P<0.001), 14 76% (P<0.001)

가

34%

30%(P<0.01), 7

36%(P<0.05)

(Table 1).

rhodanese

47% (P<0.05) (Table 2). rhodanese 가 2 38%
 (P<0.01) 가 50% (P<0.05), 3 42% (P<0.05), 7

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

Day (s) following operation	Rhodanese activity (nmol ferric thiocyanate min ⁻¹ mg protein ⁻¹)			
	Sham	CBDL	Ethanol+Sham	Ethanol+CBDL
1	3,108 ± 329	2,604 ± 285 ^a	3,023 ± 342	2,437 ± 263 ^d
2	3,101 ± 316	2,483 ± 322 ^a	3,046 ± 356	1,638 ± 231 th
3	3,097 ± 311	2,261 ± 310 ^b	3,067 ± 378	1,587 ± 289 th
7	3,089 ± 306	1,816 ± 318 ^c	3,054 ± 327	1,165 ± 322 ^{fg}
14	3,079 ± 284	1,106 ± 285 ^c	2,996 ± 345	710 ± 231 ^{fg}

The data are expressed as mean ± SD with 5 rats in each group; Sham: Sham operated rats, CBDL: Common bile duct ligated rats, Ethanol: Rats were given 5% (v/v) ethanol solution for 60 days. 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; f, P<0.001 vs. Ethanol + Sham; g, P<0.05 vs. CBDL; h, P<0.01 vs. CBDL.

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

Day (s) following operation	Rhodanese activity (nmol ferric thiocyanate min ⁻¹ mg protein ⁻¹)			
	Sham	CBDL	Ethanol+Sham	Ethanol+CBDL
1	4,316 ± 914	4,023 ± 762	4,162 ± 942	3,526 ± 819
2	4,313 ± 925	3,746 ± 813	4,106 ± 928	1,997 ± 915 ^{eg}
3	4,298 ± 916	3,242 ± 941	4,098 ± 917	1,891 ± 728 ^{eg}
7	4,257 ± 898	2,429 ± 750 ^b	3,987 ± 883	1,454 ± 459 ^{fg}
14	4,268 ± 853	2,166 ± 718 ^b	3,993 ± 866	1,141 ± 567 ^{fg}

The data are expressed as mean ± SD with 5 rats in each group. Experimental groups are described in Table 1. b, P<0.01 vs. Sham; e, P<0.01 vs. Ethanol+Sham; f, P<0.001 vs. Ethanol + Sham; g, P<0.05 vs. CBDL.

48% (P<0.01), 14 70% (P<0.001) 가
 1 452% (P<0.001), 2
 407% (P<0.001), 3 398%
 (P<0.001), 7 308% (P<0.001), 14
 266% (P<0.001) 가
 3 38%
 (P<0.05), 7 32% (P<0.01)
 (Table 3).

2. rhodanese
 rhodanese 7 45% (P<0.05), 14
 48% (P<0.05) 가 (Table
 4).
 3. 14 rhodanese
 Km Vmax
 rhodanese 가
 1 463% 14
 (P<0.001), 2 366% (P<0.001), 3
 342% (P<0.001), 7 206% rhodanese sodium
 (P<0.001), 14 170% (P<0.001) thiosulfate Km
 가 가

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

Day (s) following operation	Rhodanese activity (nmol ferric thiocyanate min ⁻¹ mg protein ⁻¹)			
	(Normal, 235 ^o ±57; Ethanol, 318 ^o ±60)			
	Sham	CBDL	Ethanol+Sham	Ethanol+CBDL
1	255 ^o ± 70	319 ^o ± 95	312 ^o ± 83	306 ^o ± 74
2	248 ^o ± 72	306 ^o ± 93	317 ^o ± 86	195 ^o ± 64 ^d
3	252 ^o ± 67	297 ^o ± 73	321 ^o ± 94	185 ^o ± 50 ^{d,g}
7	246 ^o ± 61	241 ^o ± 55	315 ^o ± 84	165 ^o ± 47 ^{e,h}
14	244 ^o ± 56	122 ^o ± 46 ^b	318 ^o ± 80	96 ^o ± 44 ^f

The data are expressed as mean ^o± SD with 5 rats in each group. Experimental groups are described in Table 1. 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; g, P<0.05 vs. CBDL; h, P<0.01 vs. CBDL.

Vmax rhodanese 가 (Table 5).

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

Day (s) following operation	Rhodanese activity (nmol ferric thiocyanate min ⁻¹ mg protein ⁻¹)			
	(Normal, 204 ^a ±47; Ethanol, 225 ^a ±51)			
	Sham	CBDL	Ethanol + Sham	Ethanol + CBDL
1	211 ± 52	1,187 ± 368 ^c	233 ± 58	1,286 ± 358 ^f
2	208 ± 51	969 ± 252 ^c	235 ± 60	1,192 ± 286 ^f
3	210 ± 50	928 ± 202 ^c	232 ± 61	1,156 ± 253 ^f
7	209 ± 51	639 ± 115 ^c	227 ± 59	926 ± 217 ^{f,g}
14	207 ± 48	558 ± 121 ^c	225 ± 58	824 ± 172 ^{f,g}

The data are expressed as mean ± SD with 5 rats in each group. Experimental groups are described in Table 1. c, P<0.001 vs. Sham; f, P<0.001 vs. Ethanol + Sham; g, P<0.05 vs. CBDL.

Table 5. Rhodanese kinetic parameters from cholestasis with chronic ethanol intoxicated rat liver

Cell fractions	Sham	CBDL	Ethanol + Sham	Ethanol + CBDL
	Km (mM)			
Cytosol	248 ± 43	239 ± 52	242 ± 38	238 ± 45
Mitochondria	267 ± 48	255 ± 57	263 ± 51	255 ± 48
Microsome	608 ± 124	592 ± 133	596 ± 118	587 ± 126
Vmax (nmol ferric thiocyanate min ⁻¹ mg protein ⁻¹)				
Cytosol	3,636 ± 343	1,418 ± 253 ^c	3,544 ± 406	1,026 ± 189 ^{f,g}
Mitochondria	4,658 ± 971	3,225 ± 722 ^a	4,361 ± 992	1,692 ± 536 ^{f,h}
Microsome	238 ± 62	191 ± 56 ^a	374 ± 86	152 ± 51 ^c

Michaelis-Menten constants for rhodanese were determined using sodium thiosulfate and potassium cyanide at 25°... for cytosolic, mitochondrial and microsomal fractions of experimental rat livers at the 14th day after operation. The data are expressed as mean ± SD with 5 rats in each group. Experimental group are described in Table 1. 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; g, P<0.05 vs. CBDL; h, P<0.01 vs. CBDL.

[17] .

[18] 가 [12]

[11].

[1]

[1,12]. 가

[1] 가

[1,12] 가 가

xanthine oxidase[19],
glutathione S-transferase, glutathione
peroxidase[20], aldehyde
dehydrogenase[21]

가

glutathione S-transferase[20]
arylesterase[22], carboxylesterase[23] aryl
sulfotransferase[24], alcohol dehydrogenase
aldehyde dehydrogenase[21]

가

가 가 aryl
sulfotransferase[24], alcohol dehydrogenase[21]
xanthine oxidase[19] 가
arylesterase[21]
carboxylesterase[23]
rhodanese

[15] 가 가

[16] 가 가 [3]

가

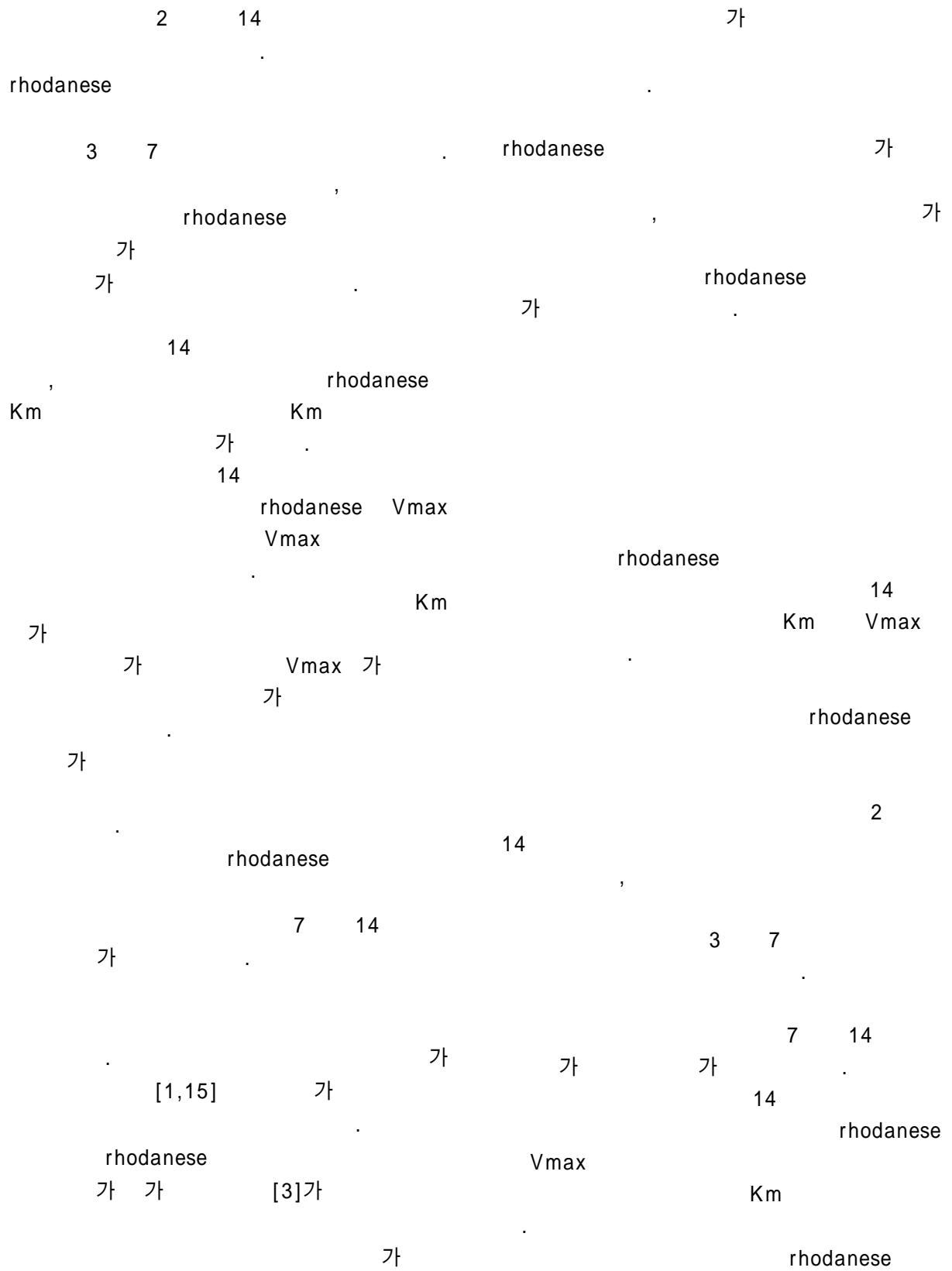
가

rhodanese

rhodanese 가

[3].

rhodanese



- 가
가
rhodanese
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