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SHORT COMMUNICATION

Enhancement of cysteine catabolism into taurine impacts glutathione homeostasis in rats challenged with ethanol

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Abstract We determined the alterations in metabolic conversion of cysteine into glutathione and taurine in liver of rats treated with ethanol acutely. Ethanol treatment reduced cysteine as well as glutathione levels in liver for 24 h. However, cysteine dioxygenase was up-regulated rapidly, and hypotaurine/taurine levels were significantly higher than those found in the saline-treated rats. It is therefore suggested that enhancement of cysteine catabolism into taurine contributes to the depletion of hepatic glutathione, which could exacerbate the ethanol-induced oxidative liver injury.

Keywords Ethanol · Transsulfuration · Cysteine · Glutathione · Taurine

Introduction

It is well known that both acute and chronic ethanol intake result in reduction of hepatic glutathione (GSH) contents, which may have an important role in the development of oxidative liver injury (Nordmann 1994). Various mechanisms have been proposed to explain the ethanol-induced GSH depletion in liver. Since ethanol biotransformation is associated with generation of acetaldehyde and reactive oxygen species (ROS), an increased consumption of GSH to counteract the oxidative substances has been suggested to be responsible for its depletion (Kera et al. 1985; Videla et al. 1980). Other proposed mechanisms of the ethanol-induced GSH depletion include an increase in GSH efflux from the liver (Fernandez-Checa et al. 1987; Speisky et al. 1988; Choi et al. 2000) and/or an inhibition of its biosynthesis (Lauterburg et al. 1984). More recently, we observed that the GSH depletion in rats fed ethanol was accompanied with a decrease in cysteine, an essential substrate for the synthesis of this tripeptide (Kim et al. 2003, 2008). In that study, however, taurine, the other major metabolite of cysteine, was elevated markedly. This appears to be a paradox when considering the pivotal role of GSH in the defense against various oxidative substances including those generated during metabolic breakdown of ethanol. It was therefore of interest to investigate the effects of ethanol on cysteine and glutathione homeostasis in liver by determining expression and activity of the critical enzymes involved in the metabolism of these sulfur-containing substances.

Methods

Animals and treatments

Male Sprague–Dawley rats, weighing 300–350 g, were purchased from Dae-Han Experimental Animal (Seoul, Korea). The use of these animals was in compliance with the guidelines established by the Animal Care Committee in College of Pharmacy, Seoul National University, and approved by the Ethical Animal Care and Use Committee of Seoul National University (No. SNU-120601-2). Rats were acclimated to temperature (22 ± 2 °C)- and humidity (55 ± 5 %)-controlled rooms with a 12-h light/dark cycle (light 0700–1900, dark 1900–0700) for 1 week before use.

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Rats were treated acutely with ethanol (40 %; 3 g/kg, ip) at 10:00 am and killed 6 h and 24 h later. Regular rat chow (Cargill Agri Purina, Seongnam, Korea) and tap water were allowed ad libitum until killing.

Determination of sulfur-containing substances

The liver was homogenized in a fourfold volume of 1.15 % KCl and centrifuged with 1 M $HClO_4$ for detection of cysteine, GSH, and GSH disulfide (GSSG), or in a fivefold volume of methanol for detection of hypotaurine, taurine and cysteinesulfinic acid. GSH and GSSG were determined using the method of Griffith (1980). Cysteine was measured by the acid-ninhydrin method (Gaitonde 1976). Taurine, hypotaurine, and cysteinesulfinic acid were derivatized with *O*-phthaldialdehyde/2-mercaptoethanol prior to quantification using the HPLC method of Rajendra (1987).

Enzyme activity and expression

The liver was homogenized in a threefold volume of ice-cold buffer containing 0.154 M KCl/50 mM Tris–HCl with 1 mM EDTA (pH 7.4). The 104,000 g supernatant fraction (cytosol) was used to measure the enzyme activity and expression. CDO activity was measured by quantification of cysteinesulfinic acid using the method described above. Cytosol was incubated in 0.5 mM $Fe(NH_4)_2(SO_4)_2$, 5 mM $NH_2OH{\cdot}HCl$, 2 mM NAD^+, and 5 mM cysteine for 16 min at 37 °C.

For Western blotting analysis, liver protein was loaded, separated by gel electrophoresis, and transferred to nitrocellulose membranes by electroblotting. The membranes were blocked in 5 % nonfat dry milk in 0.1 % Tween 20 in Tris-buffered saline. The blots were incubated overnight with primary antibodies followed by incubation with secondary antibodies conjugated to horseradish peoxidase. Polyclonal antibodies against rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Santa Cruz Biotechnology, Santa Cruz, CA), γ-glutamylcysteine ligase catalytic unit (GCLC) (NeoMarkers, Fremont, CA), and cysteine dioxygenase (CDO) were used as probes. CDO antibody was a kind gift from Dr. Yu Hosokawa (Faculty of Human Sciences, Jissen Women's University, Tokyo, Japan). Proteins were detected by enhanced chemiluminescence. Signal intensity was normalized relative to that of GAPDH.

Gene expression was examined by quantitative real-time PCR. Total hepatic RNA was isolated using TRIzol® reagent (Invitrogen, Carlsbad, CA) and purified by an RNeasy Mini Kit (Qiagen, Hilden, Germany). cDNA was obtained from isolated RNA template by reverse transcription. Gene expression was measured using cDNA with corresponding gene-specific primers and a Light-Cycler System (Roche Diagnostic, Mannheim, Germany) following the manufacturer's standard protocol. The gene-specific primers applied were as follows: CDO sense, 5′-GCTGGAAGCCTACGAGAGCAA-3′, anti-sense, 5′-AGCAGTGGGAGTCCGTGTGA-3′; GCLC sense, 5′-CTGCACATCTACCACGCAGTCA-3′, anti-sense, 5′-ATCGCCGCCATTCAGTAACAA-3′; GAPDH sense, 5′-GGCACAGTCAAGGCTGAGAATG-3′, anti-sense, 5′-ATGGTGGTGAAGACGCCAGTA-3′.

Results and discussion

The GSH contents in liver of the saline-treated rats were reduced in 6 h and recovered 24 h later (Table 1). The circadian variation in hepatic GSH has been well documented (Schnell et al. 1983; Kim and Lee 1998). An acute dose of ethanol decreased the GSH contents further, which persisted for 24 h. GSSG, the oxidized form of GSH, was elevated at 6 h after ethanol treatment, but returned to normal in 24 h. Hepatic cysteine concentrations were decreased by ethanol for 24 h. Contrary to the depletion of GSH and

Table 1 Effects of ethanol treatment on sulfur-containing substances in rat liver

	0 h	6 h		24 h	
	Control	Saline	EtOH	Saline	EtOH
tGSH (μmol/g)	7.1 ± 0.3	5.3 ± 0.1$^{\#\#}$	4.7 ± 0.2$^{\#\#\#,*}$	6.3 ± 0.2	5.2 ± 0.4$^{\#\#,*}$
GSSG (nmol/g)	160 ± 5	111 ± 7$^{\#\#\#}$	150 ± 5**	161 ± 7	158 ± 6
GSSG/GSH (%)	2.66 ± 0.07	2.76 ± 0.11	3.73 ± 0.12$^{\#\#\#,***}$	2.85 ± 0.17	3.20 ± 0.28
Cysteine (nmol/g)	190 ± 8	178 ± 4	150 ± 10$^{\#,*}$	185 ± 7	160 ± 8$^{\#,*}$
Hypotaurine (nmol/g)	222 ± 68	100.0 ± 13	342 ± 100*	180 ± 26	149 ± 59
Taurine (μmol/g)	4.14 ± 1.41	1.53 ± 0.65	4.27 ± 0.43**	1.20 ± 0.39	2.67 ± 0.61

Each value represents the mean ± SEM for six rats

$^{\#, \#\#, \#\#\#}$ Significantly different from the normal control measured at 0 h (Student's *t*-test, $P < 0.05$, 0.01, 0.001, respectively)

*, **, *** Significantly different from the saline-treated rats (Student's *t*-test, $P < 0.05$, 0.01, 0.001, respectively)

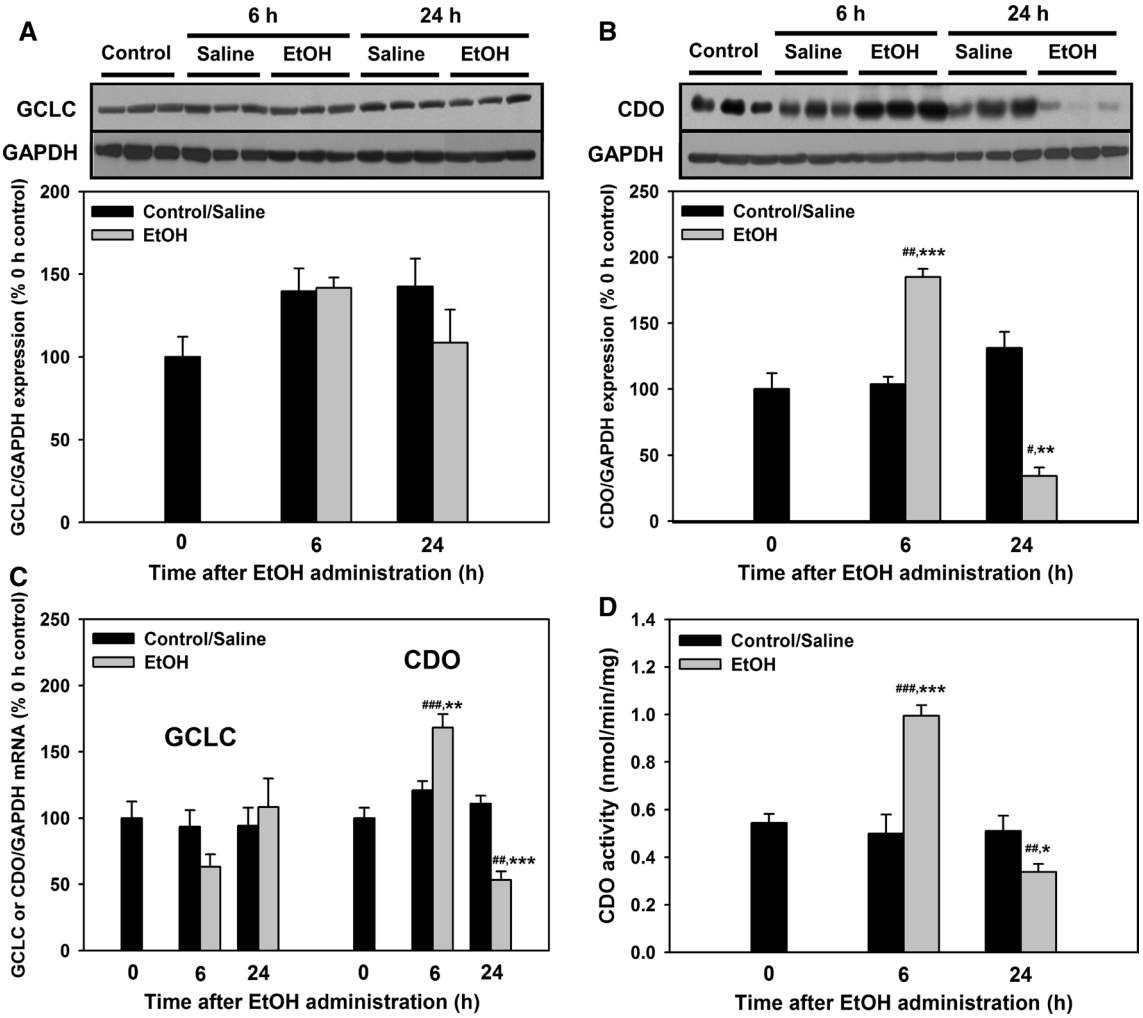

Fig. 1 Effects of ethanol treatment on GCLC and CDO in rat liver. **a** GCLC protein expression, **b** CDO protein expression, **c** GCLC and CDO mRNA expression, **d** CDO activity; **a** and **b** mean ± SE for three rats, **c** and **d** mean ± SE for six rats. $^{\#, \#\#, \#\#\#}$Significantly different from the normal control measured at 0 h (Student's *t*-test, $P < 0.05$, 0.01, 0.001, respectively). $^{*, **, ***}$Significantly different from the saline-treated rats (Student's *t*-test, $P < 0.05$, 0.01, 0.001, respectively)

cysteine, hepatic hypotaurine and taurine levels in the ethanol-treated rats were significantly higher than those found in the saline-treated rats. There appeared to be no difference in hypotaurine or taurine levels between the two groups at 24 h after the treatment. CDO was up-regulated at 6 h after the treatment, but reversed in 24 h, whereas GCL appeared to be unaffected (Fig. 1). It is therefore suggested that the enhancement of cysteine catabolism into taurine has a significant role in the ethanol-induced GSH depletion in liver via reduction in the essential substrate for GSH synthesis.

In liver, the cysteine concentration is regulated by a balance between the rates of its synthesis, hepatic uptake from blood, and its metabolic conversion into GSH, inorganic sulfate, and taurine. CDO and GCL are the rate-limiting enzymes in the metabolic pathways for synthesis of taurine and GSH, respectively, and therefore, the relative dominance between the two enzymes is a major determinant for partitioning of cysteine sulfur into either GSH or taurine in liver. CDO catalyzes the oxidation of cysteine to cysteinesulfinic acid, which is further metabolized to hypotaurine by cysteinesulfinic acid decarboxylase, and subsequently to taurine via enzymatic and/or non-enzymatic reactions (Pecci et al. 1999). Alternatively, cysteinesulfinic acid is transaminated to yield inorganic sulfate (Stipanuk and Ueki 2011). Taurine is also synthesized from the oxidation of cysteamine by cysteamine dioxygenase of which expression is relatively low in liver compared to extrahepatic tissues such as heart, kidney, and brain, whereas CDO expression is highest in liver (Dominy et al. 2007). It has been demonstrated that cysteine itself is an important intracellular signal for the regulation of CDO (Stipanuk et al. 2004). Cysteine availability was shown to directly affect

CDO abundance via changes in the rate of CDO ubiquitination and degradation (Stipanuk et al. 2006). Therefore, a high level of cysteine allows more cysteine to be converted to taurine via up-regulation of CDO activity, whereas GCL is induced when cysteine availability is low, ensuring that more cysteine is conserved as GSH (Kwon and Stipanuk 2001). On the other hand, regulation of CDO mRNA expression has been largely unknown. In this study, ethanol was shown to up-regulate CDO mRNA and protein expression rapidly. CDO activity was also changed similarly. To our knowledge, this is the first study revealing that ethanol may increase the utilization of cysteine for taurine synthesis via induction of CDO expression. The induction of CDO was reversed at 24 h after ethanol challenge, which may be associated with excessive expression of this enzyme shown earlier. Meanwhile, the inhibition of CDO at 24 h did not result in a decrease in hypotaurine and taurine levels in liver. Considering the role of taurine as a major organic osmolyte, there appears to be a compensatory mechanism for maintenance of taurine homeostasis when cellular taurine production is inadequate. In fact hepatic CDO-knockout mice fed a taurine-free diet were shown to maintain a normal taurine level in liver, suggesting that extrahepatic tissues are able to compensate for a lack of hepatic capacity to produce taurine (Ueki et al. 2012). Recently, we also showed that the inhibition of CDO in rat liver did not lead to a reduction in hepatic taurine and hypotaurine levels (Kwon et al. 2013).

Synthesis of GSH in liver is limited mostly by two factors, the availability of cysteine and the activity of GCL (Meister and Anderson 1983). The GCL enzyme consists of a catalytic unit (GCLC) and a modifier unit (GCLM) that is enzymatically inactive but has a regulatory function for GCL. In this study, expression of GCLC was not influenced by ethanol, but hepatic GSH level was reduced significantly for 24 h after the treatment. The reduction of GSH paralleled the change in cysteine concentration, suggesting that the decrease in this sulfur amino acid may be responsible for the decrease in GSH synthesis in the ethanol-treated rats. In contrast, CDO was up-regulated rapidly with elevation of both hypotaurine and taurine levels in liver. The results suggest that cysteine catabolism into taurine is favored over GSH generation, which may play a significant role in the ethanol-induced GSH depletion in liver. Considering the well-known role of GSH in detoxification of ROS and oxidative metabolites, the enhancement of taurine synthesis under the ethanol-induced oxidative stress is unexpected.

Numerous studies have suggested that taurine is associated with cytoprotective activity, which is often attributed to its antioxidant potential (Huxtable 1992). However, other studies have shown that taurine has a minimal direct scavenging activity toward oxygen-derived radicals (Aruoma et al. 1988; Mehta and Dawson 2001). Instead, hypotaurine at a concentration of several hundred micromoles per liter in vitro has been shown to be an excellent scavenger of hydroxyl radical, hypochlorous acid, peroxynitrite, and singlet oxygen (Shi et al. 1997; Pecci et al. 1999). We also measured the oxygen radical scavenging capacity of hypotaurine and its precursor, cysteinesulfinic acid, and found that both substances were more or at least equipotent in counteracting hydroxyl radical and peroxynitrite compared with GSH (unpublished results). This raises a possibility that the cysteine metabolites generated during catabolism of this amino acid into taurine may have a yet unidentified role in the antioxidant defense against an acute episode of oxidative stress in animal tissues.

In conclusion, the results in this study suggest that cysteine is preferentially utilized for the synthesis of taurine rather than GSH in rat liver exposed to ethanol. The increase in cysteine partitioning into the taurine synthetic pathway may contribute to the ethanol-induced GSH depletion, which could exacerbate alcoholic liver injury. The physiological significance of these findings remains to be investigated in future studies.

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Conflict of interest The authors declare that they have no financial or other conflict of interest.

Ethical approval All procedures performed in this study involving animals were in accordance with the ethical standards established by the Ethical Animal Care and Use Committee of Seoul National University (Approval No. SNU-120601-2).

References

Aruoma OI, Halliwell B, Hoey BM, Butler J (1988) The antioxidant action of taurine, hypotaurine and their metabolic precursors. Biochem J 256:251–255

Choi DW, Kim SY, Kim SK, Kim YC (2000) Factors involved in hepatic glutathione depletion induced by acute ethanol administration. J Toxicol Environ Health A 60:459–469

Dominy JE Jr, Simmons CR, Hirschberger LL, Hwang J, Coloso RM, Stipanuk MH (2007) Discovery and characterization of a second mammalian thiol dioxygenase, cysteamine dioxygenase. J Biol Chem 282:25189–25198

Fernandez-Checa JC, Ookhtens M, Kaplowitz N (1987) Effect of chronic ethanol feeding on rat hepatocytic glutathione. Compartmentation, efflux, and response to incubation with ethanol. J Clin Invest 80:57–62

Gaitonde MK (1976) A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. Biochem J 104:627–633

Griffith OW (1980) Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem 106:207–212

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72:101–163

Kera Y, Komura S, Ohbora Y, Kiriyama T, Inoue K (1985) Ethanol induced changes in lipid peroxidation and nonprotein sulfhydryl content. Different sensitivities in rat liver and kidney. Res Commun Chem Pathol Pharmacol 47:203–209

Kim YC, Lee SJ (1998) Temporal variation in hepatotoxicity and metabolism of acetaminophen in mice. Toxicology 128:53–61

Kim SK, Seo JM, Jung YS, Kwak HE, Kim YC (2003) Alterations in hepatic metabolism of sulfur-containing amino acids induced by ethanol in rats. Amino Acids 24:103–110

Kim SJ, Jung YS, Kwon DY, Kim YC (2008) Alleviation of acute ethanol-induced liver injury and impaired metabolomics of S-containing substances by betaine supplementation. Biochem Biophys Res Commun 368:893–898

Kwon YH, Stipanuk MH (2001) Cysteine regulates expression of cysteine dioxygenase and gamma-glutamylcysteine synthetase in cultured rat hepatocytes. Am J Physiol Endocrinol Metab 280:E804–E815

Kwon DY, Jung YS, Kim SJ, Kim YS, Choi DW, Kim YC (2013) Alterations in sulfur amino acid metabolism in mice treated with silymarin: a novel mechanism of its action involved in enhancement of the antioxidant defense in liver. Plant Med 79:997–1002

Lauterburg BH, Davies S, Mitchell JR (1984) Ethanol suppresses hepatic glutathione synthesis in rats in vivo. J Pharmacol Exp Ther 230:7–11

Mehta TR, Dawson R Jr (2001) Taurine is a weak scavenger of peroxynitrite and does not attenuate sodium nitroprusside toxicity to cells in culture. Amino Acids 20:419–433

Meister A, Anderson ME (1983) Glutathione. Annu Rev Biochem 52:711–760

Nordmann R (1994) Alcohol and antioxidant systems. Alcohol Alcohol 29:513–522

Pecci L, Costa M, Montefoschi G, Antonucci A, Cavallini D (1999) Oxidation of hypotaurine to taurine with photochemically generated singlet oxygen: the effect of azide. Biochem Biophys Res Commun 254:661–665

Rajendra W (1987) High performance liquid chromatographic determination of amino acids in biological samples by precolumn derivatization with *O*-phthaldehyde. J Liq Chromatogr 10:941–955

Schnell RC, Bozigian HP, Davies MH, Merrick BA, Johnson KL (1983) Circadian rhythm in acetaminophen toxicity: role of nonprotein sulfhydryls. Toxicol Appl Pharmacol 71:353–361

Shi X, Flynn DC, Porter DW, Leonard SS, Vallyathan V, Castranova V (1997) Efficacy of taurine based compounds as hydroxyl radical scavengers in silica induced peroxidation. Ann Clin Lab Sci 27:365–374

Speisky H, Kera Y, Penttila KE, Israel Y, Lindros KO (1988) Depletion of hepatic glutathione by ethanol occurs independently of ethanol metabolism. Alcohol Clin Exp Res 12:224–228

Stipanuk MH, Ueki I (2011) Dealing with methionine/homocysteine sulfur: cysteine metabolism to taurine and inorganic sulfur. J Inherit Metab Dis 34:17–32

Stipanuk MH, Hirschberger LL, Londono MP, Cresenzi CL, Yu AF (2004) The ubiquitin-proteasome system is responsible for cysteine-responsive regulation of cysteine dioxygenase concentration in liver. Am J Physiol Endocrinol Metab 286:E439–E448

Stipanuk MH, Dominy JE, Lee JI, Coloso RM (2006) Mammalian cysteine metabolism: new insights into regulation of cysteine metabolism. J Nutr 136:1652S–1659S

Ueki I, Roman HB, Hirschberger LL, Junior C, Stipanuk MH (2012) Extrahepatic tissues compensate for loss of hepatic taurine synthesis in mice with liver-specific knockout of cysteine dioxygenase. Am J Physiol Endocrinol Metab 302:E1292–E1299

Videla LA, Fernandez V, Ugarte G, Valenzuela A (1980) Effect of acute ethanol intoxication on the content of reduced glutathione of the liver in relation to its lipoperoxidative capacity in the rat. FEBS Lett 111:6–10