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Effect of β-alanine administration on carbon tetrachloride-induced acute hepatotoxicity

Short Communication

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Summary. Mice were supplemented with β-alanine (3%) in drinking water for one week. β-Alanine intake reduced hepatic taurine levels, but elevated cysteine levels significantly. Hepatotoxicity of CCl_4 in mice fed with β-alanine was decreased as determined by changes in serum enzyme activities. Hepatic glutathione and taurine concentrations after CCl_4 challenge were increased markedly by β-alanine intake. The enhanced availability of cysteine for synthesis of glutathione and/or taurine appears to account for the hepatoprotective effects of β-alanine against CCl_4-induced liver injury.

Keywords: β-Alanine – Carbon tetrachloride – Cysteine – Glutathione – Taurine

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CYP, cytochrome P450; GES, guanidinoethane sulfinate; GSH, glutathione; MAT, methionine adenosyltransferase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SDH, sorbitol dehydrogenase.

Introduction

Taurine or 2-aminoethanesulfonic acid is suggested to have various physiological functions including antioxidation, detoxification, osmoregulation, membrane stabilization and neuromodulation (Huxtable, 1992). This β-amino acid is both ingested directly from foodstuffs and synthesized mainly in liver as an end product of metabolism of sulfur-containing amino acids via the transsulfuration pathway. The metabolic precursor of taurine, cysteine, is also an essential substrate for synthesis of glutathione (GSH). Therefore, utilization of cysteine for generation of GSH and taurine is competitive with each other, and suggested to be regulated by the need for GSH in biological systems (Stipanuk et al., 1992; Kwon and Stipanuk, 2001).

Recently we have observed that acute ethanol administration to rats increased the synthesis of hypotaurine and taurine rapidly while GSH synthesis was depressed (Kim et al., 2003). This is contrary to the expectation that GSH, a primary antioxidant in animal tissues, would be preferentially synthesized for utilization under oxidative stress induced by ethanol intake. This finding suggests a potential role of cysteine catabolism to taurine in animal tissues challenged with oxidants. Beneficial effects of taurine in biological systems have been frequently attributed to its antioxidant potential, but direct oxygen radical scavenging activity of this substance is minimal (Aruoma et al., 1988). On the other hand cysteine sulfinic acid and hypotaurine, metabolic precursors of taurine, have been shown to be excellent scavengers of reactive oxygen species including hydroxyl radical, peroxyl radical, hypochlorous acid, peroxynitrite and singlet oxygen (Arouma et al., 1988; Shi et al., 1997; Pecci et al., 1999; unpublished observation in this laboratory). Thus, we have hypothesized that induction of taurine synthesis, rather than taurine itself, would have a significant role in the defense against oxidants in animal tissues via enhanced generation of metabolic intermediates from cysteine to taurine. In this study we examined the effect of taurine depletion on CCl_4-induced acute liver injury by feeding mice with β-alanine. It was postulated that a rapid decrease in taurine levels would result in an impact on the metabolic

balance in the transsulfuration pathway, leading to acceleration of cysteine metabolism to taurine.

Materials and methods

Animals and treatments

Male ICR mice, weighing 20–25 g, were obtained from Dae-Han Laboratory Animal (Seoul, Korea). The use of animals was in compliance with the guidelines established by the Animal Care Committee of this institute. Animals were acclimated to temperature ($22 \pm 2\,^{\circ}C$) and humidity ($55 \pm 5\%$) controlled rooms with a 12-h light/dark cycle (light: 0700–1900, dark 1900–0700). Laboratory chow was allowed *ad libitum*. β-Alanine (3%) dissolved water replaced regular tap water for one week before CCl_4 (50 µl/kg, ip) treatment. CCl_4 was dissolved in corn oil. Mice were sacrificed 24 h following CCl_4 treatment.

Measurement of hepatotoxicity

Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum were determined using the method of Reitman and Frankel (1957). The method of Gerlach (1983) was used for measurement of sorbitol dehydrogenase (SDH) activity.

Measurement of sulfur-containing metabolites and enzyme activities

Livers were homogenized in a four-fold volume of cold 1 M perchloric acid with 2 mM EDTA. Denatured protein was removed by centrifugation at 10,000 *g* for 10 min. Total GSH concentration was determined using the method of Neuschwander-Tetri and Roll (1989). A HPLC system equipped with a fluorescence detector and a 3.5 µm Symmetry C18 column (4.6 × 75 mm; Waters, Milford, MA, U.S.A.) was employed. Cysteine and cystine were quantified by the acid-ninhydrin method (Gaitonde, 1967). A HPLC method was used for determination of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) (She et al., 1994). The supernatant was directly applied to a HPLC equipped with a UV detector and a TSK-GEL ODS-80TM column (4.6 × 250 mm; Tosoh, Tokyo, Japan). The method of Ide (1997) was used to quantify hypotaurine and taurine. Hypotaurine and taurine were derivatized with *O*-phthalaldehyde/2-mercaptoethanol before injection into a HPLC equipped with a fluorescence detector and a 3.5 µm Kromasil C18 column (4.6 × 100 mm; Eka Chemicals, Bohus, Sweden).

Livers were homogenized in a three-fold volume of a buffer consisting of 0.154 M KCl/50 mM Tris-HCl and 1 mM EDTA (pH 7.4). The homogenate was centrifuged at 10,000 *g* for 20 min. The supernatant fraction was further centrifuged at 104,000 *g* for 60 min. The 104,000 *g* supernatant fraction was used to determine the enzyme activities. Activity of methionine adenosyltransferase (MAT) was estimated by quantifying SAM and SAH production (Kim et al., 2003). Two different methionine concentrations, 0.1 and 5.0 mM, were utilized in the incubation mixture. Activity of NADPH-dependent GSH reductase was determined employing an enzymatic recycling method (Smith et al., 1988). GSH peroxidase activity was assayed by using hydrogen peroxide as a substrate (Lawrence and Burk, 1976).

Results and discussion

β-Alanine administration decreased hepatic taurine level to approximately 60% of normal control, but elevated cysteine concentration significantly (Table 1). It has been accepted that depletion of taurine by β-alanine administration is associated with enhancement of taurine elimination through urine. Since this amino acid utilizes the same β-amino acid uptake system in kidney as taurine, it competitively inhibits taurine reuptake from the tubular fluid into the proximal tubular cells (Shaffer and Kocsis, 1981). The present results suggest that the need for replenishment of taurine provoked by reduction of its body pool leads to an elevation of hepatic cysteine in β-alanine-fed mice. Cysteine is both synthesized in the transsulfuration pathway and uptaken from the systemic circulation. In this study the activity of MAT isozymes or SAM level in liver was not changed by β-alanine administration, but

Table 1. Effect of β-alanine administration on major sulfur-containing metabolites and enzyme activities in liver

		Control	β-Alanine (3%)
SAM (nmol/g liver)		112.3 ± 7.8	125.2 ± 9.3
SAH (nmol/g liver)		32.3 ± 2.5	27.9 ± 2.1
Cysteine (nmol/g liver)		78.7 ± 3.3	$121.6 \pm 9.2^{**}$
GSH (µmol/g liver)		7.4 ± 0.3	7.9 ± 0.8
Taurine (µmol/g liver)		12.2 ± 1.7	$7.6 \pm 0.8^{*}$
Hypotaurine (nmol/g liver)		97.6 ± 8.6	71.5 ± 9.5
GSSG reductase (units/mg protein)		0.09 ± 0.01	0.10 ± 0.004
GSH peroxidase (units/mg protein)		0.49 ± 0.03	0.54 ± 0.04
MAT	(5 mM)	0.18 ± 0.02	0.24 ± 0.04
(nmol/min/mg protein)	(0.1 mM)	0.04 ± 0.001	0.04 ± 0.01

Mice were provided with drinking water containing 3% β-alanine for one week. Each value represents the mean ± S.E.M. for five mice
*,** Statistically different from control at $P < 0.05$, 0.01, respectively (Student's *t*-test)

Table 2. Effect of β-alanine administration on changes in serum enzyme activities and sulfur-containing amino acid metabolites in mice treated with CCl_4

	Control	CCl_4	β-Alanine + CCl_4
AST (units/ml)	72 ± 11	$5226 \pm 773^{\#\#\#}$	$3281 \pm 119^{*}$
ALT (units/ml)	39 ± 8	$12888 \pm 1182^{\#\#\#}$	$5964 \pm 409^{***}$
SDH (units/ml)	12 ± 4	$11869 \pm 588^{\#\#\#}$	$7747 \pm 731^{**}$
Cysteine (nmol/g liver)	78.7 ± 3.3	$115.0 \pm 2.4^{\#\#}$	$96.5 \pm 3.8^{**}$
GSH (µmol/g liver)	7.4 ± 0.3	7.9 ± 1.2	$11.4 \pm 0.4^{*}$
Taurine (µmol/g liver)	12.2 ± 1.7	$3.8 \pm 0.5^{\#\#}$	$6.7 \pm 0.5^{**}$

Mice were provided with drinking water containing 3% β-alanine for one week before CCl_4 treatment. Each value represents the mean ± S.E.M. for five mice. ##, ### Significantly different from control at $P < 0.01$, 0.001, respectively (Student's *t*-test)
*, **, *** Statistically different from mice treated with CCl_4 only at $P < 0.05$, 0.01, 0.001, respectively (Student's *t*-test)

the immediate precursors of cysteine, homocysteine and cystathionine, were not determined. The mechanism responsible for the elevation of hepatic cysteine by β-alanine remains to be examined.

An acute dose of CCl_4 elevated serum AST, ALT and SDH activities significantly (Table 2). Hepatic GSH concentration was not affected, but taurine level reduced to approximately 30% of normal control. In contrast hepatic cysteine was elevated markedly. β-Alanine administration inhibited the induction of CCl_4 hepatotoxicity as determined by a decrease in elevation of serum enzyme activities. Both GSH and taurine levels in liver were increased by β-alanine intake after CCl_4 treatment. It is suggested that the β-alanine-induced increase in the availability of cysteine (Table 1) is responsible for the elevation of hepatic GSH and taurine levels in mice challenged with CCl_4.

CCl_4 is a model hepatotoxicant acting via generation of a reactive free radical metabolite mostly by CYP2E1 activity in liver. Several studies have shown that taurine may modulate the hepatotoxicity of CCl_4 (Nakashima et al., 1982; Vohra and Hui, 2001; Dincer et al., 2002; Erman et al., 2004; Miyazaki et al., 2005). Since taurine has minimal effects on hepatic CYP activities (Matsuda et al., 2002; unpublished observations in this laboratory), most investigators attributed the protective effects of taurine against CCl_4 hepatotoxicity to its antioxidant activity. However, it was shown that taurine intake actually increased lipid peroxidative damage induced by CCl_4 (Nakashima et al., 1983). Also oxygen radical scavenging activity of taurine per se is negligible (Aruoma et al., 1988; unpublished observation in this laboratory). Therefore, the mechanism of protection against CCl_4 hepatotoxicity provided by taurine remains unclear.

Conversely it has been also shown that administration of a taurine-deleting agent alleviates the toxicity of xenobiotics in various tissues. Guanidinoethane sulfonate (GES), an amidino analog of taurine, decreased the hepatotoxicity of methylene dianiline (Seabra and Timbrell, 1997), and the toxicological changes in liver and heart induced by monocrotaline (Yan and Huxtable, 1996). The mechanism of protection provided by GES was not studied, but in both studies an elevation of hepatic GSH was demonstrated. Also β-alanine was shown to provide cardioprotection from reperfusion injury (Allo et al., 1997), and to decrease the toxic effects of β-amyloid in rat brain endothelial cells (Preston et al., 1998), suggesting that this substance might reduce oxidative damage in biological systems. We also observed a reduction of bacterial lipopolysaccharide-induced hepatotoxicity in rats fed with β-alanine (Kim and Kim, 2002).

Taurine is a major product in the transsulfuration pathway, and its depletion in the body is suspected to result in significant changes in metabolism of sulfur-containing substances in liver. In fact an increased methionine level in various tissues was demonstrated in rats treated with GES (Marnela and Kondro, 1984; Marnela et al., 1984). β-Alanine was also shown to raise the urinary excretion of cysteine and homocysteine (Kerai et al., 2001), and the hepatic GSH and taurine concentrations after CCl_4 treatment (Waterfield et al., 1993), which are compatible with the present results. In this study the hepatoprotective effect of β-alanine against CCl_4 could be accounted for by the increased supply of cysteine for production of taurine and/or GSH, both known to have an important role in the maintenance of normal physiology/biochemistry of liver. These findings also suggest that the consequence of taurine depletion is not specific to taurine, but may induce significant changes in metabolism of various biologically important sulfur-containing substances. The extent of alteration in the transsulfuration reactions induced by taurine depletion and its physiological significance remain to be investigated.

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