Amino Acids (2012) 43:2545–2551
DOI 10.1007/s00726-012-1406-8

ORIGINAL ARTICLE

Leucine accelerates blood ethanol oxidation by enhancing the activity of ethanol metabolic enzymes in the livers of SHRSP rats

Hitoshi Murakami · Michiko Ito · Yuji Furukawa · Michio Komai

Received: 16 June 2012 / Accepted: 21 September 2012 / Published online: 30 September 2012

Abstract Chronic ethanol consumption induces liver diseases, such as alcoholic hepatitis and cirrhosis. The enhancement of alcohol oxidation is important in the prevention of these liver diseases. Chronic supplementation with branched chain amino acids (BCAAs) prevents liver cirrhosis. Therefore, BCAAs may be associated with enhanced ethanol oxidation. To evaluate this hypothesis, we investigated the effect of the administration of individual BCAAs on ethanol oxidation and changes in alcohol-metabolizing enzyme activities following acute alcohol intake in rats. Blood ethanol concentrations and the activities of alcohol-metabolizing enzymes, such as alcohol dehydrogenase (ADH) and low and high Km aldehyde dehydrogenase (ALDH), were measured in the liver following acute ethanol administration in rats; the ethanol was administered 30 min after the treatment with amino acids [such as leucine (Leu), isoleucine (Ile), valine (Val) or alanine (Ala)]. Leu significantly decreased the blood ethanol concentration 1 h after ethanol administration compared to the water-treated control (C) [C 0.46 ± 0.09, Leu 0.18 ± 0.04, Ile 0.27 ± 0.09, Val 0.46 ± 0.1, Ala 0.43 ± 0.06, mean ± SEM (g/l), $P < 0.05$]. In addition, leucine significantly stimulated ADH activity 30 min after ethanol intake [C 0.042 ± 0.014, Leu 0.090 ± 0.016, Ile 0.042 ± 0.008, Val 0.022 ± 0.010, Ala 0.070 ± 0.016, mean ± SEM (unit/mg protein), $P < 0.05$] and low Km ALDH activity 15 min after ethanol intake [C 0.51 ± 0.63, Leu 3.72 ± 0.66, Ile 1.26 ± 0.89, Val: ND, Ala 1.86 ± 1.57, mean ± SEM (unit/mg protein), $P < 0.05$]. However, leucine and its metabolite α-keto-isocaproic acid did not enhance ethanol clearance in isolated rat hepatocytes. These results indicate that leucine accelerates ethanol oxidation by indirectly enhancing ADH and low Km ALDH activities in the liver.

Keywords Leucine · Ethanol oxidation · Alcohol metabolic enzymes

Abbreviations

BCAAs	Branched-chain amino acids
Leu	Leucine
Ile	Isoleucine
Val	Valine
Ala	Alanine
KIC	α-Keto-isocaproic acid
KMV	α-Keto-β-methyl valeric acid
ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
GOT	Glutamate oxaloacetate transaminase
IL-6	Interleukin-6
TNF-α	Tumor necrosis factor-α
mTOR	Mammalian target of rapamycin
SHRSP	Spontaneously hypertensive stroke prone
NAD	Nicotinamide adenine dinucleotide

Introduction

Ethanol is metabolized to acetaldehyde by catalase and the microsomal ethanol-oxidizing system, but it is primarily metabolized by alcohol dehydrogenase (ADH). Acetaldehyde

H. Murakami · M. Ito · Y. Furukawa · M. Komai (✉)
Laboratory of Nutrition, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan
e-mail: mkomai@biochem.tohoku.ac.jp

Present Address:
H. Murakami
Frontier Research Laboratories, Institute for Innovation, Ajinomoto Co., Inc., 1-1 Suzuki-cho, Kawasaki 210-8681, Japan

causes hangovers and flush, and it is detoxified by aldehyde dehydrogenase (ALDH) in the liver. Ethanol exposure induces alcoholic liver diseases, such as hepatitis and liver cirrhosis. Ingested ethanol is a potent inhibitor of hepatic regeneration (Wands et al. 1979) and growth hormone-mediated insulin-like growth factor 1 (IGF-1) expression (Xu et al. 1995). Acute ethanol administration induces increased oxidative stress (Koch et al. 2004), impairs protein metabolism by affecting the anabolic signaling pathway in cardiac muscle (Vary et al. 2005) and affects energy and hormone metabolism (Deaciuc et al. 1992). Therefore, the acceleration of ethanol oxidation is very important to prevent liver disease and the impairment of metabolic anabolism, which are induced by alcohol intake.

The development of ethanol-induced fatty liver, alcoholic hepatitis and cirrhosis has been partially attributed to nutritional deficiencies in compounds such as amino acids, lipids, and vitamins (Leevy and Moroianu 2005). Some studies have indicated that amino acids reduce liver damage and oxidative stress caused by chronic ethanol consumption. Yang et al. (1993) demonstrated that a diet supplemented with amino acids reduces plasma glutamate oxaloacetate transaminase (GOT) levels in rats that chronically consume ethanol. Glutamine pretreatment suppresses plasma inflammation in chronic ethanol-fed rats (Peng et al. 2011). Histidine or carnosine post-treatment increases catalase mRNA expression level and downregulates IL-6 and TNF-α mRNA expression levels (Liu et al. 2008). Aspartate attenuates ethanol-induced oxidative stress (Oh et al. 2002). Torii (1997) indicated that preference for both L-alanine and L-glutamine was observed in test preferences test when alcoholic rats fell in the hepatic disorder, and supplementation with these amino acids prevents the ethanol-induced inhibition of liver regeneration (Tanaka et al. 1993, 1994).

Ethanol consumption alters plasma amino acid concentrations. Specifically, the concentrations of essential amino acids decrease in fasting alcoholics, but they increase with ethanol loading (Siegel et al. 1964). Chronic ethanol consumption also increases the plasma and tissue concentrations of branched chain amino acids (BCAAs), which may be associated with protein turnover impairment (Bernal et al. 1993). Plasma BCAAs are well known to reduce liver cirrhosis (Morgan et al. 1982). Holecek et al. (1996) indicated that the predominant mechanism that causes decreased plasma leucine levels in cirrhotic rats is an increase in the oxidized leucine fraction that is associated with decreased leucine turnover. BCAA supplementation improved the survival of rats with CCl_4-induced cirrhosis by preventing hypoalbuminemia and hyperammonemia (Kajiwara et al. 1998). The oral administration of BCAAs activates anabolic signaling pathways, such as mTOR, in cirrhotic rat livers (Matsumura et al. 2005). Therefore, BCAAs may contribute to the enhancement of ethanol oxidation, resulting in reduced liver damage induced by ethanol intake.

However, few studies have focused on the ability of BCAAs to oxidize ethanol. This study was performed to investigate whether BCAAs can accelerate ethanol elimination following acute ethanol intake in rats and in isolated rat hepatocytes.

Materials and methods

Animals

This study was approved by the Animal Research-Animal Care Committee of the Graduate School of Agricultural Science, Tohoku University. Alcohol preference differs among different rat strains, such as Spontaneously Hypertensive Stroke Prone (SHRSP), Spontaneously Hypertensive (SHR), Wistar-Kyoto, Sprague-Dawley or Wistar-slc rats. Of these, an alcohol preference was high in the SHRSP strain (Yang et al. 1994; Kimura et al. 1991); therefore, SHRSP rats were used. The rats were received from Shimane Medical University (Prof. Yukio Yamori) and bred in our animal facility. The rats were housed in a temperature-controlled room with a 12-h light and dark cycle. The animals were 10 weeks old at the end of the experiment. The rats were provided water ad libitum and a 15 % purified egg protein diet for 2 weeks prior to the experiment.

Experimental design

The effects of orally administered amino acids, such as leucine, isoleucine, valine and alanine, on ethanol oxidation were investigated in SHRSP rats (Experiment 1). After the rats fasted overnight, each amino acid (95 umol/100 g BW) was orally administered via a gastric tube to groups of 4–6 rats; ethanol (100 mg/100 g BW anhydro-ethanol) was administered 30 min later. Distilled water was also administered to the control group. Blood was collected from the tail vein under anesthesia at 0, 0.5, 1, 3, 6, and 8 h after ethanol administration. The plasma was separated by centrifugation at $3{,}000 \times g$ for 15 min at 4 °C, and the plasma was used for the ethanol measurements.

The effects of infused amino acids on blood ethanol clearance were studied in groups of 5 rats (Experiment 2). Each amino acid was infused for 15 min in the tail veins of rats that were anesthetized with sodium pentobarbital (the amino acid infusion rate was 4 umol/h). At the end of the infusion, ethanol was orally administered via a gastric tube, and blood was collected at 0, 0.5, 1, 2, 3, 4 and 5 h following ethanol administration. The plasma was separated

by centrifugation at 3,000×*g* for 15 min at 4 °C and was used for the ethanol measurements.

The effects of orally administered amino acids on alcohol-oxidative enzyme activities were investigated in the rat livers (Experiment 3). The appropriate amino acid and ethanol were administered under the same conditions as in Experiment 1 to groups of 4–6 rats. The rats were euthanized by decapitation at 0, 15, 30 and 60 min following ethanol administration, and the livers were immediately removed, frozen in liquid nitrogen and stored at −80 °C until the assay was performed.

The effects of leucine and its metabolite α-keto-isocaproic acid (KIC) on ethanol metabolism were investigated in isolated rat hepatocytes (Experiment 4). SHRSP rats were anesthetized with sodium pentobarbital, and hepatocytes were isolated by the method of Cronholm (1993). The isolated hepatocytes were diluted in a preincubation medium composed of Krebes–Henseleit bicarbonate buffer, 10^{-9} mol insulin, 5,000 KIU/l aprotinin, 10^{-9} mol dexamethasone and 1% albumin (Matsuzaki et al. 1981; Cronholm 1993). The hepatocytes were diluted to 5×10^6 cells/ml, and 1.5 ml of the cell suspension was placed in a 20-ml conical flask with a silicon cap (Iwaki Glass Co., Ltd., Japan). Each flask was saturated in advance with 95:5 of O_2:CO_2 gas. The BCAAs, KIC, α-Keto-β-methyl valeric acid (KMV) and alanine were added to each flask (each at a final concentration of 1 mM). Following 10 min of incubation, 0.5 ml of 24 mM ethanol (final concentration of 4 mM) was injected into each flask. A 100-ul sample of the medium was taken 90 min after the ethanol injection and used for the ethanol measurement.

To determine whether leucine stimulates insulin secretion under acute ethanol exposure, Experiment 5 was performed. Leucine or alanine was administered with ethanol to groups of 5 rats under the same conditions that were described in Experiment 1. Blood was taken 0, 15, 30, 60, 90 and 120 min after ethanol was administered and used to measure the plasma insulin concentrations. All of the amino acids were purchased from Ajinomoto Co., Inc., Japan, and the other materials were provided by Wako Pure Chemical Industry., Ltd., Japan.

Sample preparation

Samples of 1 g of frozen liver were powdered using a Cryo-Press (Microtec Co., LTD., Japan) and homogenized in a 10 mM sodium phosphate buffer that included 2 mM sucrose, 2 mM mercaptoethanol and 0.1 mM EDTA for the measurements of ADH and ALDH activities. The homogenate was centrifuged at 1,000×*g* for 10 min at +4 °C, and the supernatant was centrifuged at 1,000×*g* for 30 min at +4 °C. The supernatant was centrifuged at 18,000×*g* for 30 min at +4 °C and then used for the ADH enzyme activity measurements. The pellet was suspended in the homogenized buffer containing 1% deoxycholate, and the suspension incubated on ice for 1 h before it was centrifuged at 18,000×*g* for 30 min at +4 °C. The supernatant was used to perform low or high Km ALDH activity measurements.

Analysis

The plasma and medium ethanol concentrations were measured using a commercial spectrophotometric kit (F-kit ethanol, Boehringer Mannheim, Germany). Plasma insulin level was also measured using a commercial radioimmunoassay kit (Shionogi insulin kit, Shionogi & Co., Ltd., Japan).

Liver ADH and ALDH activities were measured using a modification of Koivisto's method (Koivisto and Eriksson 1994). The enzyme assays were performed spectrophotometrically by monitoring the formation of NADH or NAD^+ at 340 nm at 37 °C. ADH activity was assayed in a mixture containing 0.1 ml of the homogenate supernatant and 2.8 ml of 0.5 mM NAD^+ in 0.1 M potassium diphosphate buffer (pH 9.0) with 1 mM rotenone; 0.1 ml of 40% ethanol was used as the substrate. ALDH activity was assayed using a mixture including the homogenate sample and 2.8 ml of reaction buffer (0.5 mM NAD^+ in 0.1 M potassium diphosphate buffer (pH 9.0) with 1 mM rotenone and 30 mM pyrazole); 0.1 ml of 1.5 mM acetaldehyde was used as the substrate for the low Km ALDH enzyme activity assay, and 150 mM acetaldehyde was used for the high Km ALDH assay. After suspending the reaction substrate, the spectrum was monitored. The results were corrected based on the reaction lacking the homogenate sample. The protein concentrations of the supernatants for the ADH and ALDH measurements were determined by the Lowry method.

Statistics

The values are presented as the mean ± SEM. The statistical significance among each diet group was evaluated with Bonferroni's post-test to compare with the control group following a two-way ANOVA for multiple comparisons or Dunnett's test following a one-way ANOVA for multiple comparisons (Graph Pad Prism, Graph Pad Software Inc., CA, USA). Values of $P < 0.05$ were considered to be significant.

Results

The effects of orally administered amino acids on ethanol oxidation are shown in Fig. 1. Leucine significantly decreased the blood ethanol levels 1 h after ethanol was

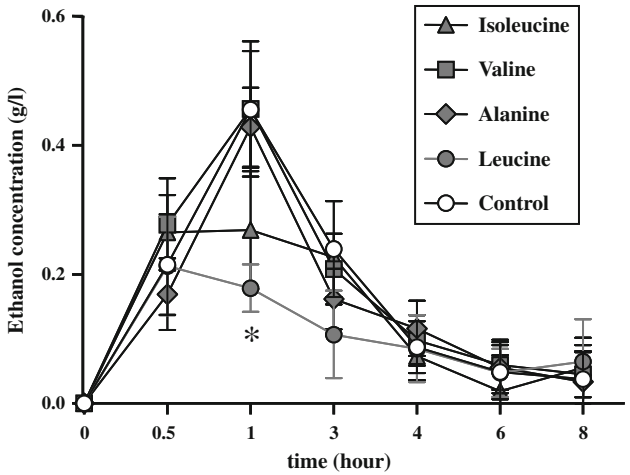

Fig. 1 The effects of orally administered amino acids on blood ethanol clearance in rats. Leucine significantly decreased the blood ethanol concentration 1 h after ethanol administration, but the other amino acids did not significantly decrease the blood ethanol values. The values are presented as the mean ± SEM. Comparisons with the control group at each time point were performed with a Bonferroni's post-test following a two-way ANOVA for multiple comparisons (*$P < 0.05$)

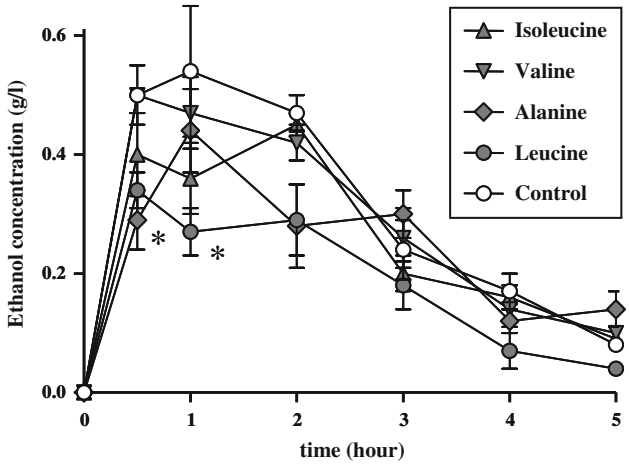

Fig. 2 The effects of amino acids infused into the tail vein on ethanol oxidation in rats. Leucine significantly decreased the blood ethanol concentration 1 h after ethanol administration, and the ethanol concentration in the leucine group was lower for 5 h after the ethanol administration. In addition, alanine significantly decreased the blood ethanol level 0.5 h after acute ethanol administration. However, isoleucine and valine did not decrease the blood ethanol concentrations. The values are presented as the mean ± SEM. Comparisons with the control group at each time point were performed with a Bonferroni's post-test after a two-way ANOVA for multiple comparisons (*$P < 0.05$)

administered ($P < 0.05$), and the isoleucine group tended to have a reduced ethanol level ($P = 0.056$). In contrast, alanine slightly decreased the ethanol concentrations 0.5 h after administration, but this reduction was not significant. Valine did not affect blood ethanol oxidation (Fig. 1).

Leucine also significantly decreased blood ethanol concentration 1 h after ethanol intake in rats-infused amino acid into tail vein ($P < 0.05$), and ethanol concentration in leucine group was lower for 5 h after ethanol administration (Fig. 2). In addition, alanine significantly decreased the blood ethanol levels 0.5 h after ethanol administration ($P < 0.05$, Fig. 2). However, isoleucine and valine did not decrease the blood ethanol concentrations (Fig. 2).

The changes in alcohol-metabolizing enzyme activities following the administration of each amino acid and ethanol are shown in Table 1. Alanine significantly increased ADH activity 15 min after ethanol administration ($P < 0.05$), and ADH activity in the leucine-treated rats increased 30 min after ethanol intake ($P < 0.05$). In contrast, ADH activity was lower in the valine group for 60 min after ethanol administration. The low Km ALDH activity was significantly increased in the leucine group 15 min after ethanol administration ($P < 0.05$) and was higher for 60 min after the leucine treatment. The high Km ALDH activity levels did not differ between the groups (Table 1).

Figure 3 shows the ethanol levels in the media of isolated rat hepatocytes 90 min after incubation with ethanol. Alanine significantly decreased the ethanol levels in the media compared to the other amino acids and BCAA metabolites. Meanwhile, the branched amino acids and their metabolites did not affect ethanol clearance in the isolated rat hepatocytes (Fig. 3). Changes in insulin levels following leucine or alanine and ethanol intake are shown in Fig. 4. Leucine significantly increased the insulin level 30 min after leucine administration, but alanine did not display this effect.

Discussion

The objective of this study was to investigate the effects of branched amino acids on ethanol clearance following acute ethanol intake in rats. Leucine significantly decreased the blood ethanol level after acute ethanol intake by enhancing the liver enzymatic activities of ADH and low Km ALDH. In contrast, leucine and its metabolite KIC did not decrease the ethanol levels in the media of isolated rat hepatocytes. This result suggests that leucine accelerates ethanol oxidation by indirectly enhancing alcohol-metabolizing enzyme activities in the liver.

Chronic ethanol ingestion impairs several metabolic pathways, such as amino acid metabolism, liver regeneration, and muscle protein metabolism, leading to severe liver failure or muscle loss (Wands et al. 1979; Xu et al. 1995; Vary et al. 2005; Deaciuc et al. 1992). Therefore, it is important to accelerate ethanol oxidation to prevent alcohol-induced tissue damage. First-pass ethanol metabolism

Table 1 Change in alcoholic enzyme activities after each amino acid and ethanol administration

Unit/mg protein	Control	Leucine	Isoleucine	Valine	Alanine
ADH					
15 min	0.041 ± 0.015	0.095 ± 0.006	0.086 ± 0.013	0.038 ± 0.026	0.120 ± 0.027*
30 min	0.042 ± 0.014	0.090 ± 0.016*	0.042 ± 0.008	0.022 ± 0.010	0.070 ± 0.016
60 min	0.090 ± 0.016	0.125 ± 0.014	0.045 ± 0.017	0.035 ± 0.010	0.075 ± 0.030
Low Km ALDH					
15 min	0.511 ± 0.626	3.719 ± 0.657*	1.255 ± 0.887	ND	1.859 ± 1.573
30 min	0.945 ± 0.672	5.249 ± 3.903	0.223 ± 0.249	2.154 ± 1.544	2.283 ± 2.184
60 min	0.772 ± 0.485	4.057 ± 2.584	1.435 ± 1.284	ND	0.801 ± 0.576
High Km ALDH					
15 min	1.899 ± 0.414	2.820 ± 0.651	2.215 ± 0.410	2.186 ± 0.472	2.215 ± 0.338
30 min	0.732 ± 0.372	0.766 ± 0.360	0.828 ± 0.413	0.603 ± 0.204	0.831 ± 0.391
60 min	1.000 ± 0.380	0.926 ± 0.264	0.978 ± 0.357	0.923 ± 0.404	0.942 ± 0.351

Alanine significantly increased ADH activity 15 min after ethanol administration, and leucine increased 30 min after ethanol intake. Low Km ALDH activity was significantly increased in the leucine group 15 min after ethanol administration and was higher for 60 min. High Km ALDH activity was not different between the groups. The values are the mean ± SEM. The comparisons with the DW group were determined by Dunnet's test after ANOVA for multiple comparisons ($*P < 0.05$)

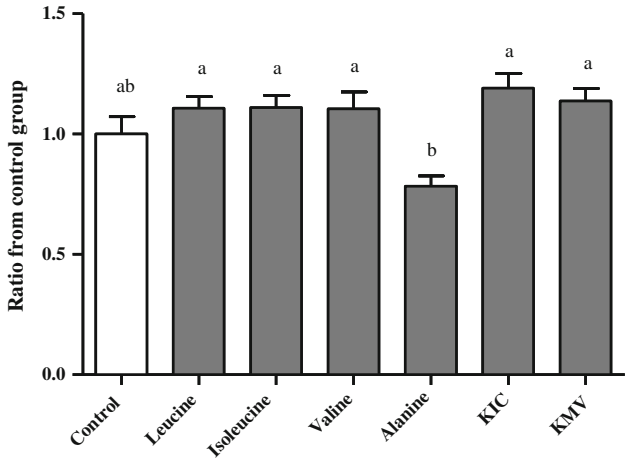

Fig. 3 The effects of amino acids on ethanol oxidation in isolated rat hepatocytes. Alanine significantly decreased the ethanol concentration in the medium 90 min after ethanol injection. However, the BCAAs and their metabolites did not decrease the ethanol concentrations in the isolated rat hepatocytes. The values are presented as the mean ± SEM. Comparisons of each group were performed with a Bonferroni's post-test after a two-way ANOVA for multiple comparisons ($*P < 0.05$)

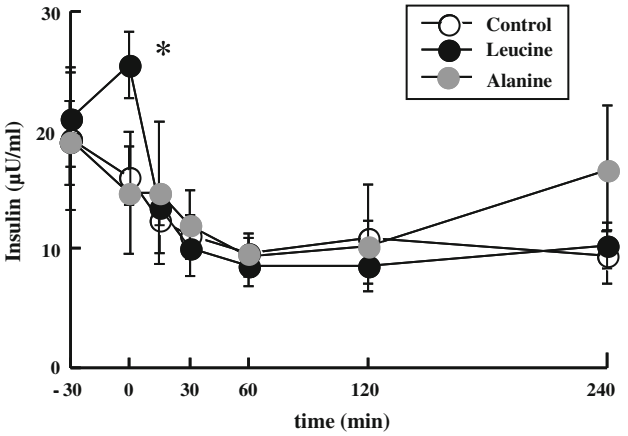

Fig. 4 The effects of leucine or alanine on insulin secretion after following the administration of each amino acid and ethanol. Leucine significantly increased the insulin concentrations 30 min after leucine administration, but alanine did not confer this effect. The values are presented as the mean ± SEM. Comparisons with the control group at each time point were performed with a Bonferroni's post-test after a two-way ANOVA for multiple comparisons ($*P < 0.05$)

in the stomach and the liver is important for alcohol metabolism (Oneta et al. 1998). Ethanol absorption is primarily controlled by gastric emptying because the primary region of ethanol absorption is the small intestine, and ethanol is absorbed via simple diffusion (Holt 1981). To reduce the effects of amino acids in the digestive tract on ethanol absorption, each amino acid (BCAAs or alanine) was administered orally at the constant molar concentration in the fasted state (Experiment 1). Leucine significantly accelerated ethanol clearance in the blood, but the other amino acids did not confer this effect (Fig. 1). In addition, the ethanol metabolic enzyme ADH is found both in the liver and in the stomach (Lieber et al. 1994). Thus, each amino acid was infused into the tail vein to eliminate the effect of digestive passage on alcohol metabolism (Experiment 2). In this experiment, leucine and alanine, but especially leucine, significantly accelerated ethanol clearance, but isoleucine and valine did not confer this effect (Fig. 2). Alanine has been reported to accelerate ethanol oxidation (Tanaka et al. 1993, 1994). The amount of orally administered alanine in Experiment 1 was extremely low compared to the levels of previous studies (0.084 g vs. 1 g/kg BW). Therefore, the effect of orally administered

alanine on ethanol clearance in rats may be weak in the present study. Leucine was the only amino acid that accelerated ethanol oxidation in both experiments (Figs. 1, 2).

Acute leucine intake also enhanced the liver activities of ADH and low Km ALDH (Table 1). Liver ADH is the primary enzyme responsible for ethanol oxidation, and low Km ALDH encodes the mitochondrial enzyme that is primarily responsible for oxidizing the ethanol-derived acetaldehyde (Deitrich et al. 2007; Yin 1994; Ehrig et al. 1990). Impairment of the low Km ALDH results in the impairment of the capacity for acetaldehyde metabolism, which is one of the primary causes of the alcohol-flush reactions that have been observed in Asian individuals (Ehrig et al. 1990). Therefore, these results indicate that leucine intake accelerates ethanol clearance and supports acetaldehyde oxidation in the liver.

BCAAs are primarily metabolized in skeletal muscles and the brain by BCAA aminotransferase; this process produces branched chain α-keto acids, such as KIC and KMV. Branched chain α-keto acids are metabolized in the liver. Some studies have demonstrated that leucine directly affects hepatocytes. Leucine has been shown to inhibit proteolysis in isolated rate hepatocytes (Venerando et al. 1994; Lardeux and Mortimore 1987) and stimulate hepatic growth factor production in hepatic stellate cells (Tomiya et al. 2007). Therefore, leucine or its metabolite KIC may directly affect ethanol clearance in the liver. Experiment 4 was performed using isolated rat hepatocytes to investigate this hypothesis. Interestingly, leucine, other branched amino acids and α-keto acids did not accelerate ethanol oxidation, but alanine increased ethanol oxidation (Fig. 3). This result indicates that leucine and its metabolite do not directly affect ethanol oxidation in the liver. Some articles have reported that alanine can accelerate ethanol oxidation and prevent the inhibition of liver regeneration by ethanol consumption (Tanaka et al. 1993, 1994; Torii 1997). NADH re-oxidation is believed to be the primary rate-determining step in hepatic ethanol oxidation, and the malate–aspartate shuttle is an important rate determinant in hepatic ethanol oxidation (Sugano et al. 1990). Precursors of malate–aspartate shuttle components, such as alanine, increased ethanol metabolism in hepatocytes (Beauge et al. 1980). In addition, it has been suggested that alanine stimulates the glucose–alanine cycle, improving the NADH redox state, thus resulting in increased ethanol elimination (Cunningham et al. 2001). Therefore, this result of Experiment 4 suggests that the effect of leucine on ethanol clearance may be not attributable to changes in the redox state through leucine metabolism in the liver.

Alcohol-metabolizing enzymes are activated by hormonal and nutritional factors. ADH has been shown to be increased by insulin in vivo and by IGF-1 and growth hormone in hepatocytes (Lakshman et al. 1988; Mezey et al. 1986, 1990). Insulin also enhanced the effect of IGF-1 on ADH activation (Mezey et al. 1990). In addition, insulin secretion has been shown to be stimulated by amino acids, such as leucine (Milner 1969), but ethanol has been shown to inhibit insulin secretion (Singh et al. 1980). In Experiment 5, leucine significantly increased insulin secretion, but alanine did not confer this effect (Fig. 4). This result suggests that the stimulation of insulin secretion by leucine contributes to enhanced alcohol-metabolizing enzyme activities. The mechanism of the effect of leucine on ethanol clearance may be associated with the change in liquid factors (such as insulin) induced by leucine administration.

In conclusion, leucine accelerated ethanol clearance following acute ethanol administration by enhancing alcohol-metabolizing enzyme activities such as alcohol and ALDH in rats. The effect of leucine on these enzymatic activities was not exerted directly in the liver. The increase in ADH activity by leucine may be associated with the induction of insulin secretion.

However, the mechanism of action of leucine on the acceleration of ethanol clearance is unclear. Further studies are required to investigate this mechanism.

Acknowledgments We thank Dr. Hideyuki Sone, Mr. Osamu Fukaya and Mrs. Yumi Murakami for their assistance with the experimental trials.

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

References

Beauge F, Mangeney M, Nordmann J, Nordmann R (1980) Comparative study of the effect of amino acids on ethanol oxidation in isolated hepatocytes from starved and fed rats. Adv Exp Med Biol 132:393–402

Bernal CA, Vazquez JA, Adibi SA (1993) Leucine metabolism during chronic ethanol consumption. Metabolism 42(9):1084–1086

Cronholm T (1993) Ethanol metabolism in isolated hepatocytes. Effects of methylene blue, cyanamide and penicillamine on the redox state of the bound coenzyme and on the substrate exchange at alcohol dehydrogenase. Biochem Pharmacol 45(3): 553–558

Cunningham CC, Preedy VR, Paice AG, Hesketh JE, Peters TJ, Patel VB, Volpi E, Mawatari K, Masaki H, Mori M, Torii K (2001) Ethanol and protein metabolism. Alcohol Clin Exp Res 25(5 Suppl ISBRA):262S–268S

Deaciuc IV, D'Souza NB, Lang CH, Spitzer JJ (1992) Effects of acute alcohol intoxication on gluconeogenesis and its hormonal responsiveness in isolated, perfused rat liver. Biochem Pharmacol 44(8):1617–1624

Deitrich RA, Petersen D, Vasiliou V (2007) Removal of acetaldehyde from the body. Novartis Found Symp 285:23–40 discussion 40–51, 198–199

Ehrig T, Bosron WF, Li TK (1990) Alcohol and aldehyde dehydrogenase. Alcohol Alcohol 25(2–3):105–116

Holecek M, Tilser I, Skopec F, Sprongl L (1996) Leucine metabolism in rats with cirrhosis. J Hepatol 24(2):209–216

Holt S (1981) Observations on the relation between alcohol absorption and the rate of gastric emptying. Can Med Assoc J 124(3):267–277, 297

Kajiwara K, Okuno M, Kobayashi T, Honma N, Maki T, Kato M, Ohnishi H, Muto Y, Moriwaki H (1998) Oral supplementation with branched-chain amino acids improves survival rate of rats with carbon tetrachloride-induced liver cirrhosis. Dig Dis Sci 43(7):1572–1579

Kimura S, Kim CH, Ohtomo IM, Yokomukai Y, Komai M, Morimatsu F (1991) Nutritional studies of the roles of dietary protein levels and umami in the preference response to sodium chloride for experimental animals. Physiol Behav 49(5): 997–1002

Koch OR, Pani G, Borrello S, Colavitti R, Cravero A, Farre S, Galeotti T (2004) Oxidative stress and antioxidant defenses in ethanol-induced cell injury. Mol Aspects Med 25(1–2):191–198. doi:10.1016/j.mam.2004.02.019

Koivisto T, Eriksson CJ (1994) Hepatic aldehyde and alcohol dehydrogenases in alcohol-preferring and alcohol-avoiding rat lines. Biochem Pharmacol 48(8):1551–1558

Lakshman MR, Chambers LL, Chirtel SJ, Ekarohita N (1988) Roles of hormonal and nutritional factors in the regulation of rat liver alcohol dehydrogenase activity and ethanol elimination rate in vivo. Alcohol Clin Exp Res 12(3):407–411

Lardeux BR, Mortimore GE (1987) Amino acid and hormonal control of macromolecular turnover in perfused rat liver. Evidence for selective autophagy. J Biol Chem 262(30):14514–14519

Leevy CM, Moroianu SA (2005) Nutritional aspects of alcoholic liver disease. Clin Liver Dis 9(1):67–81. doi:10.1016/j.cld.2004.11.003

Lieber CS, Gentry RT, Baraona E (1994) First pass metabolism of ethanol. Alcohol Alcohol Suppl 2:163–169

Liu WH, Liu TC, Yin MC (2008) Beneficial effects of histidine and carnosine on ethanol-induced chronic liver injury. Food Chem Toxicol 46(5):1503–1509. doi:10.1016/j.fct.2007.12.013

Matsumura T, Morinaga Y, Fujitani S, Takehana K, Nishitani S, Sonaka I (2005) Oral administration of branched-chain amino acids activates the mTOR signal in cirrhotic rat liver. Hepatol Res 33(1):27–32. doi:10.1016/j.hepres.2005.07.001

Matsuzaki S, Gordon E, Lieber CS (1981) Increased alcohol dehydrogenase independent ethanol oxidation at high ethanol concentrations in isolated rat hepatocytes: the effect of chronic ethanol feeding. J Pharmacol Exp Ther 217(1):133–137

Mezey E, Potter JJ, Rhodes DL (1986) Effect of growth hormone on alcohol dehydrogenase activity in hepatocyte culture. Hepatology 6(6):1386–1390

Mezey E, Potter JJ, Mishra L, Sharma S, Janicot M (1990) Effect of insulin-like growth factor I on rat alcohol dehydrogenase in primary hepatocyte culture. Arch Biochem Biophys 280(2):390–396

Milner RD (1969) Stimulation of insulin secretion in vitro by essential aminoacids. Lancet 1(7605):1075–1076

Morgan MY, Marshall AW, Milsom JP, Sherlock S (1982) Plasma amino-acid patterns in liver disease. Gut 23(5):362–370

Oh SI, Lee MS, Kim CI, Song KY, Park SC (2002) Aspartate modulates the ethanol-induced oxidative stress and glutathione utilizing enzymes in rat testes. Exp Mol Med 34(1):47–52

Oneta CM, Simanowski UA, Martinez M, Allali-Hassani A, Pares X, Homann N, Conradt C, Waldherr R, Fiehn W, Coutelle C, Seitz HK (1998) First pass metabolism of ethanol is strikingly influenced by the speed of gastric emptying. Gut 43(5):612–619

Peng HC, Chen YL, Chen JR, Yang SS, Huang KH, Wu YC, Lin YH, Yang SC (2011) Effects of glutamine administration on inflammatory responses in chronic ethanol-fed rats. J Nutr Biochem 22(3):282–288. doi:10.1016/j.jnutbio.2010.02.006

Siegel FL, Roach MK, Pomeroy LR (1964) Plasma amino acid patterns in alcoholism: the effects of ethanol loading. Proc Natl Acad Sci USA 51:605–611

Singh SP, Patel DG, Snyder AK (1980) Ethanol inhibition of insulin secretion by perfused rat islets. Acta Endocrinol 93(1):61–66

Sugano T, Handler JA, Yoshihara H, Kizaki Z, Thurman RG (1990) Acute and chronic ethanol treatment in vivo increases malate-aspartate shuttle capacity in perfused rat liver. J Biol Chem 265(35):21549–21553

Tanaka T, Ando M, Yamashita T, Toda T, Monna T, Nishiguchi S, Matsui T, Kuroki T, Otani S, Maezono K et al (1993) Effects of alanine and glutamine administration on the inhibition of liver regeneration by acute ethanol treatment. Alcohol Alcohol Suppl 1B:41–45

Tanaka T, Imano M, Yamashita T, Monna T, Nishiguchi S, Kuroki T, Otani S, Maezono K, Mawatari K (1994) Effect of combined alanine and glutamine administration on the inhibition of liver regeneration caused by long-term administration of alcohol. Alcohol Alcohol Suppl 29(1):125–132

Tomiya T, Nishikawa T, Inoue Y, Ohtomo N, Ikeda H, Tejima K, Watanabe N, Tanoue Y, Omata M, Fujiwara K (2007) Leucine stimulates HGF production by hepatic stellate cells through mTOR pathway. Biochem Biophys Res Commun 358(1): 176–180. doi:10.1016/j.bbrc.2007.04.093

Torii K (1997) A new pharmacological and physiological aspects of L-amino acids. Nihon Yakurigaku Zasshi 110(Suppl 1):28P–32P

Vary TC, Deiter G, Goodman SA (2005) Acute alcohol intoxication enhances myocardial eIF4G phosphorylation despite reducing mTOR signaling. Am J Phys Heart Circ Phys 288(1):H121–H128. doi:10.1152/ajpheart.00440.2004

Venerando R, Miotto G, Kadowaki M, Siliprandi N, Mortimore GE (1994) Multiphasic control of proteolysis by leucine and alanine in the isolated rat hepatocyte. Am J Phys 266(2 Pt 1):C455–C461

Wands JR, Carter EA, Bucher NL, Isselbacher KJ (1979) Inhibition of hepatic regeneration in rats by acute and chronic ethanol intoxication. Gastroenterology 77(3):528–531

Xu X, Ingram RL, Sonntag WE (1995) Ethanol suppresses growth hormone-mediated cellular responses in liver slices. Alcohol Clin Exp Res 19(5):1246–1251

Yang SC, Ito M, Morimatsu F, Furukawa Y, Kimura S (1993) Effects of amino acids on alcohol intake in stroke-prone spontaneously hypertensive rats. J Nutr Sci Vitaminol 39(1):55–61

Yang SC, Ito M, Furukawa Y, Kimura S (1994) Comparative study of alcohol metabolism in stroke-prone spontaneously hypertensive rats and Wistar-Kyoto rats fed normal or low levels of dietary protein. J Nutr Sci Vitaminol 40(6):547–555

Yin SJ (1994) Alcohol dehydrogenase: enzymology and metabolism. Alcohol Alcohol Suppl 2:113–119