Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 1753 – 1759
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Phytophenols in whisky lower blood acetaldehyde level by depressing alcohol metabolism through inhibition of alcohol dehydrogenase 1 (class I) in mice

Takeshi Haseba[a,*], Junichi Sugimoto[b], Shigeo Sato[b], Yuko Abe[c], Youkichi Ohno[a]

[a]*Department of Legal Medicine, Nippon Medical School, Bunkyo-ku, Tokyo 113-8602, Japan*
[b]*The Nikka Whisky Distilling Co, Ltd., Kashiwa, Chiba 277-0033, Japan*
[c]*Brewing Research and Development Laboratory, Asahi Breweries, Ltd, Moriya-shi, Ibaraki 302-0106, Japan*

Abstract

We recently reported that the maturation of whisky prolongs the exposure of the body to a given dose of alcohol by reducing the rate of alcohol metabolism and thus lowers the blood acetaldehyde level (*Alcohol Clin Exp Res*. 2007;31:77s-82s). In this study, administration of the nonvolatile fraction of whisky was found to lower the concentration of acetaldehyde in the blood of mice by depressing alcohol metabolism through the inhibition of liver alcohol dehydrogenase (ADH). Four of the 12 phenolic compounds detected in the nonvolatile fraction (caffeic acid, vanillin, syringaldehyde, ellagic acid), the amounts of which increase during the maturation of whisky, were found to strongly inhibit mouse ADH 1 (class I). Their inhibition constant values for ADH 1 were 0.08, 7.9, 15.6, and 22.0 μmol/L, respectively, whereas that for pyrazole, a well-known ADH inhibitor, was 5.1 μmol/L. The 2 phenolic aldehydes and ellagic acid exhibited a mixed type of inhibition, whereas caffeic acid showed the competitive type. When individually administered to mice together with ethanol, each of these phytophenols depressed the elimination of ethanol, thereby lowering the acetaldehyde concentration of blood. Thus, it was demonstrated that the enhanced inhibition of liver ADH 1 due to the increased amounts of these phytophenols in mature whisky caused the depression of alcohol metabolism and a consequent lowering of blood acetaldehyde level. These substances are commonly found in various food plants and act as antioxidants and/or anticarcinogens. Therefore, the intake of foods rich in them together with alcohol may not only diminish the metabolic toxicity of alcohol by reducing both the blood acetaldehyde level and oxidative stress, but also help limit the amount of alcohol a person drinks by depressing alcohol metabolism.

1. Introduction

Alcohol metabolism produces a reactive intermediate of acetaldehyde, which plays a major role in the metabolic toxicity of alcohol. Because this substance is highly pharmacoactive, it produces a strong, unpleasant aversive reaction; and because it is also cytotoxic, it induces various chronic organ diseases and cancers [1]. The blood acetaldehyde level after drinking is regulated by the activities of both alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in the liver [2]: the former oxidizes ethanol to acetaldehyde, and the latter oxidizes acetaldehyde to acetate. Both these alcohol-metabolizing enzymes exhibit genetic polymorphisms, which are different in their activities [3,4]. Individuals who possess an inactive ALDH coded by a mutant *ALDH2*2* allele rather than a normal ALDH enzyme coded by an *ALDH2*1* allele exhibit higher blood acetaldehyde levels during drinking [3] and are more susceptible to various organ diseases caused by chronic drinking [5,6]. Similarly, individuals who possess high-activity ADHs coded by *ADH1B*2* or *3* alleles rather than a low-activity ADH coded by an *ADH1B*1* allele also exhibit an upward tendency of blood acetaldehyde levels [7] and greater susceptibility to such diseases [8]. Thus, for a person who drinks daily, it is important that the blood acetaldehyde level be kept low to prevent alcohol-related organ diseases.

The intake of certain foods affecting the activities of these alcohol-metabolizing enzymes might control the blood

* Corresponding author. Tel.: +81 3 3822 2131; fax: +81 3 5814 5680.
E-mail address: hasebat@nms.ac.jp (T. Haseba).

0026-0495/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2008.09.003

acetaldehyde level during drinking. Several constituents of food have been reported to accelerate the rate of alcohol metabolism, such as fructose [9] and corn peptide [10]. A meal itself accelerates the elimination rate of ethanol, probably by increasing hepatic blood flow and/or the activity of alcohol-metabolizing enzymes [11,12]. However, the acceleration of alcohol metabolism by the intake of food usually raises the blood acetaldehyde level [10,13]. If certain food constituents that activate mammalian ALDH [14] were also taken, the blood acetaldehyde level would become lower. However, a higher ALDH activity easily leads to overdrinking and a susceptibility to alcoholism, as demonstrated in those homozygous for the *ALDH2*1* allele [15]. On the other hand, the intake of compounds that inhibit mammalian ADH might also lower the blood acetaldehyde level by depressing alcohol metabolism and result in a lesser likelihood of overdrinking. Unfortunately, hardly any such ADH-inhibitory compounds have been detected in food, although various chemical reagents have been shown to inhibit liver ADH [16,17].

We recently found that the maturation of whisky prolongs the exposure of the body to a given dose of alcohol by reducing the rate of alcohol metabolism and thus reduces the blood acetaldehyde level. This suggests that the amounts of some ADH-inhibitory constituents increase during the maturation process [18]. In this study, we identified and characterized the phytochemicals in whisky that can depress alcohol metabolism through the inhibition of ADH and thus lower the blood acetaldehyde level.

2. Materials and methods

2.1. Analyses of nonvolatile constituents of whisky

All the whisky samples were single-malt whiskies obtained from Nikka Whisky Distilling (Kashiwa, Japan). They had been stored in oak casks for 0, 5, 10, 15, 16, or 20 years (0- to ~20-year whiskies); and they contained 63.9, 62.8, 61.6, 60.5, 58.0, or 57.4 vol/vol% of ethanol, respectively. The 16-year whisky was concentrated about 10 times by evaporation at 50°C for 30 minutes to extract the nonvolatile fraction (NVF). To analyze the nonvolatile constituents of whisky, each whisky sample (5 μL) was analyzed with a high-performance liquid chromatography (HPLC) (Hitachi L-6000, Hitachi, Tokyo, Japan) equipped with an Inertsil pH column (4.6 × 150 mm, GL Science, Tokyo, Japan) equilibrated with 0.1 mol/L phosphate buffer (pH 7.4), ECD-100 (Eicom, Kyoto, Japan), an AS-200 auto sampler (Hitachi), and a D-2500 integrator (Hitachi). Before the NVF was analyzed, it was diluted by a factor of 10 with an aqueous solution of 60% (vol/vol) ethanol.

2.2. Administration of ethanol to mice

Male ddY mice (9 weeks old) were injected intraperitoneally with 20% (wt/vol) ethanol/saline solution containing the NVF or a pure sample of each compound detected in the NVF. The ethanol dose was 3 g/kg body weight. The concentration of NVF in the ethanol solution was 10% or 20%, which corresponds to a dose of 1.5 or 3.0 mL NVF per kilogram body weight, respectively. The concentration of ethanol in the solution was kept at 20%. As the pure compounds, special- or biochemical-grade chemicals were obtained from a commercial source (Wako Pure Chemical, Osaka, Japan). Each pure compound was dissolved in a 20% ethanol solution and administered to mice at a dose of 1.5 mmol/kg. However, the actual dose was less than that because the compounds did not completely dissolve in the ethanol solution. The effect of the NVF or of each compound on alcohol metabolism was investigated by comparison with a control group (mice to which only 20% ethanol/saline solution was administered).

Blood samples (10 μL) were taken from the tail vein of each mouse at 0.5, 1, 2, 3, 4, and 5 hours after ethanol administration; and the concentration of blood ethanol was measured with a headspace gas chromatograph as previously reported [18]. The rate of ethanol elimination (in millimoles per kilogram per hour) was calculated by dividing the ethanol dose (3 g/kg) by the duration of alcohol metabolism, which was obtained from the x-intercept of a regression line fitted by the linear least squares method to the blood alcohol concentrations from 1 to 4 hours after ethanol administration [19]. The blood acetaldehyde level was measured by the perchloric acid (PCA)/thiourea method [20] as previously reported [18].

The mice were cared for in accordance with *The Regulations of Animal Experimentation of Nippon Medical School*, which are based on *The Guidelines of the International Committee on Laboratory Animals, 1974*.

2.3. In vitro analyses of ADH

Mouse liver extract and purified ADH 1 (class I) were prepared as previously reported [21]. The enzyme samples were divided into small aliquots and stored at −80°C until assay. All procedures for enzyme preparation were performed at 4°C. The ADH activity was assayed with ethanol as a substrate at 37°C in 0.1 mol/L Na-K phosphate buffer (pH 7.4) containing 1.7 mmol/L nicotinamide adenine dinucleotide (NAD) by measuring the rate of reduced NAD (NADH) production using a fluorescence spectrophotometer (excitation at λ = 350 nm, emission at λ = 466 nm; FP-770F, Japan Spectroscopic, Tokyo, Japan) [22]. The effects of the NVF and each compound detected in it on ADH activity were investigated after the incubation of ADH with the NVF or a pure sample of each compound in a reaction mixture for 5 minutes. *One unit of ADH activity* was defined to be the production of 1.0 μmol NADH per minute.

2.4. Statistics

Measurements of blood ethanol were compared by a 2-way analysis of variance (ANOVA) for the experimental mouse groups and times using the standard statistics

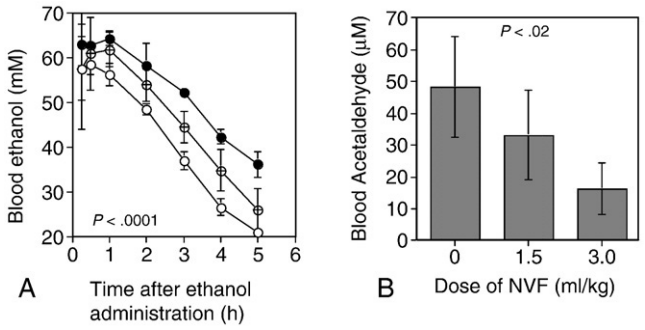

Fig. 1. Effect of NVF of whisky on concentration of (A) blood alcohol and (B) acetaldehyde in mice after ethanol administration. The NVF, which is a 10-times concentrate of mature whisky by evaporation, was added to a 20% ethanol solution. Mice were injected with an ethanol solution containing the NVF at an ethanol dose of 3.0 g/kg. The dose of NVF was 0, 1.5, or 3.0 mL/kg ("Materials and methods"). The concentrations of blood ethanol (n = 5) and acetaldehyde (n = 4) are expressed as mean ± SD. A, Ethanol in tail blood was periodically measured with a headspace gas chromatograph after administration of the NVF in an ethanol solution at a dose of 0 (○), 1.5 (⊕), or 3.0 mL/kg (●). $P < .0001$ for 0 vs 1.5 mL/kg and for 1.5 vs 3.0 mL/kg by ANOVA. B, The acetaldehyde level of heart blood was measured 1 hour after the administration of an ethanol solution containing the NVF. $P < .05$ by ANOVA for dose of the NVF.

software Stat View, version 4.5 (Abacus Concepts, Berkley, CA). Measurements of blood acetaldehyde were compared by a 1-way ANOVA for the mouse groups (Fig. 1B) or compared between the control and phytophenol groups by Student *t* test (Fig. 8). Determinations of the elimination rates of blood ethanol were compared by Fisher protected least significant difference (PLSD).

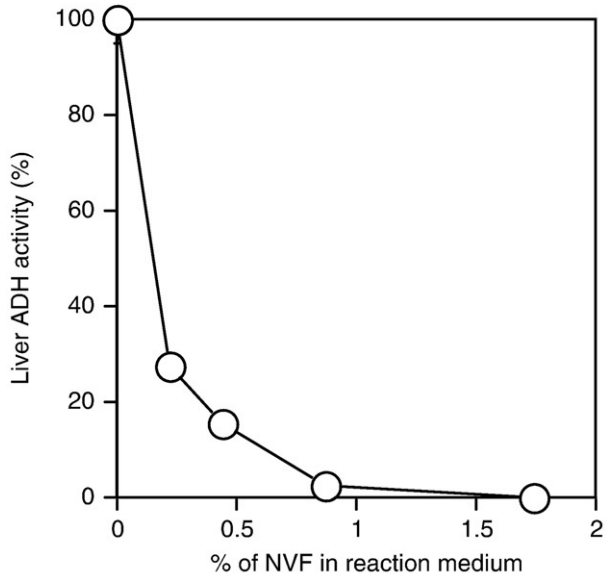

Fig. 2. Effect of NVF of whisky on liver ADH activity. The ADH activity of mouse liver extract was measured with 15 mmol/L ethanol as a substrate after incubation with NAD and various amounts of the NVF in the reaction medium for 5 minutes ("Materials and methods"). Data are expressed as mean ± SE from triplicate reactions.

Fig. 3. Change in concentrations of phytophenols in whisky during maturation. The amounts of the nonvolatile constituents of single-malt whisky were measured by HPLC ("Materials and methods"). EA, ellagic acid; SY, syringaldehyde; VN, vanillin; GA, gallic acid; SA, syringic acid; VA, vanillic acid; CO, coniferyl alcohol; CA, caffeic acid.

Results were considered statistically significant when *P* was less than .05.

3. Results

3.1. Effects of NVF of whisky on alcohol metabolism and liver ADH activity

As shown in Fig. 1A, the NVF increased the concentration of blood ethanol in a dose-dependent manner ($P < .0001$ for control [0 mL/kg] vs 1.5-mL/kg groups and for 1.5-mL/kg vs

Table 1
Constituents of NVF of mature whisky

	(μg/mL)	(μmol/L)
Ellagic acid	263.1	777.9
Syringaldehyde	184.6	1013.3
β-Phenethyl-alcohol	145.5	
Syringic acid	135.2	
(ethyl laurate)	124.4	
Gallic acid	112.8	
Vanillin	103.7	681.6
(ethyl palmitate)	75.6	
Vanillic acid	65.4	
(ethyl caprate)	49.9	
Scopoletin	28.2	
Coniferyl alcohol	25.4	
(ethyl palmitolate)	22.4	
Caffeic acid	21.1	117.1
Others	31.1	

A 16-year-old single-malt whisky was concentrated 10 times by evaporation to obtain the NVF.

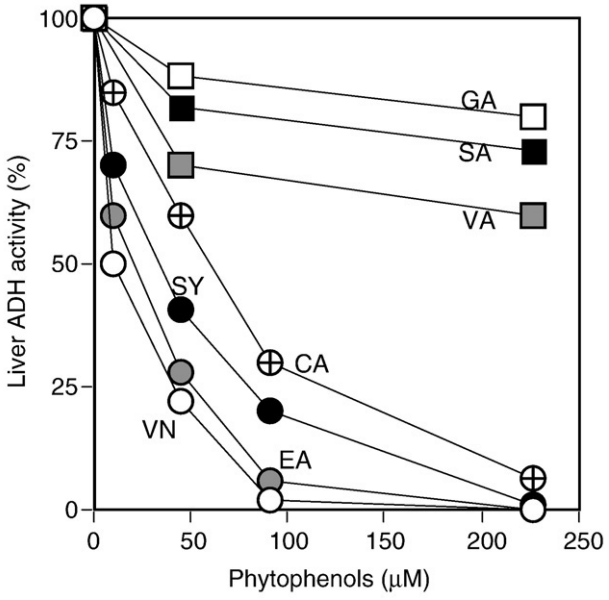

Fig. 4. Effect of phytophenols on liver ADH activity. The ADH activity of mouse liver extract was measured as in Fig. 2 in the presence of various amounts of each phytophenol, which was obtained from a commercial source. GA, gallic acid; SA, syringic acid; VA, vanillic acid; CA, caffeic acid; SY, syringaldehyde; EA, ellagic acid; VN, vanillin.

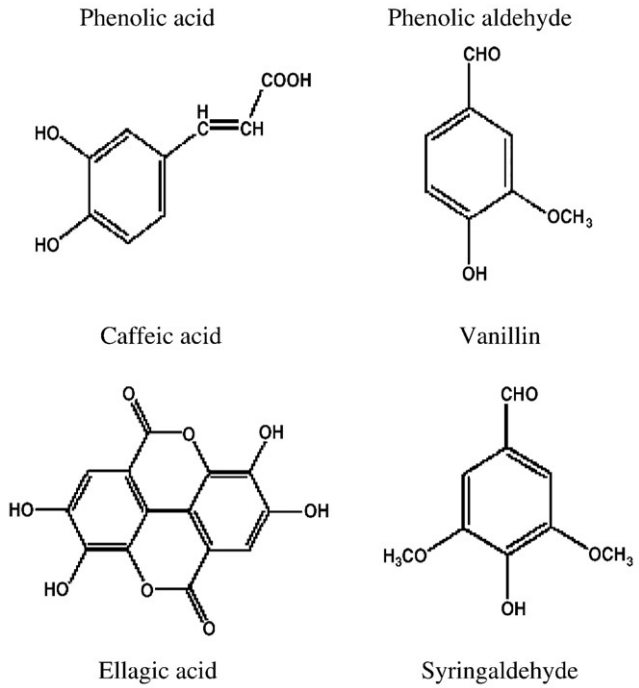

Fig. 6. Chemical structures of 4 phytophenols that inhibit ADH 1.

3.0-mL/kg groups by ANOVA) when administered to mice together with ethanol at a dose of 3.0 g/kg. The NVF also reduced the elimination rate of blood ethanol (in millimoles per kilogram per hour) in a dose-dependent manner: 13.80 ± 1.05, 11.91 ± 0.99, and 9.31 ± 0.95 mmol/kg/h for the control, 1.5-mL/kg, and 3.0-mL/kg groups, respectively ($P < .05$ for

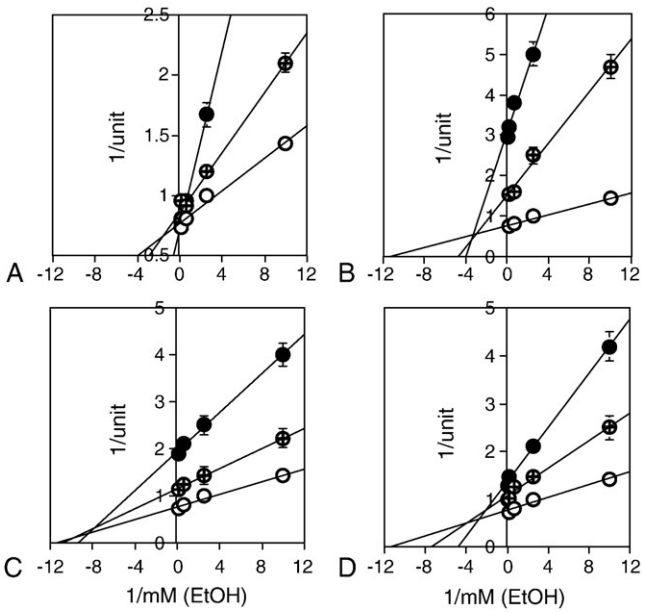

Fig. 5. Kinetic analyses of phytophenol inhibition of mouse ADH 1. Purified ADH 1 (class I) from mouse liver was preincubated with NAD and various concentrations of each phytophenol for 5 minutes in the reaction medium before the addition of the substrate at various concentrations. A, Caffeic acid, (B) vanillin, (C) ellagic acid, (D) syringaldehyde. The Lineweaver-Burk plot was obtained in the absence (○) and presence of 9.0 (⊕) or 45.0 (●) μmol/L of each phytophenol. Data are expressed as mean ± SE from triplicate reactions. The regression line was fitted by the linear least squares method.

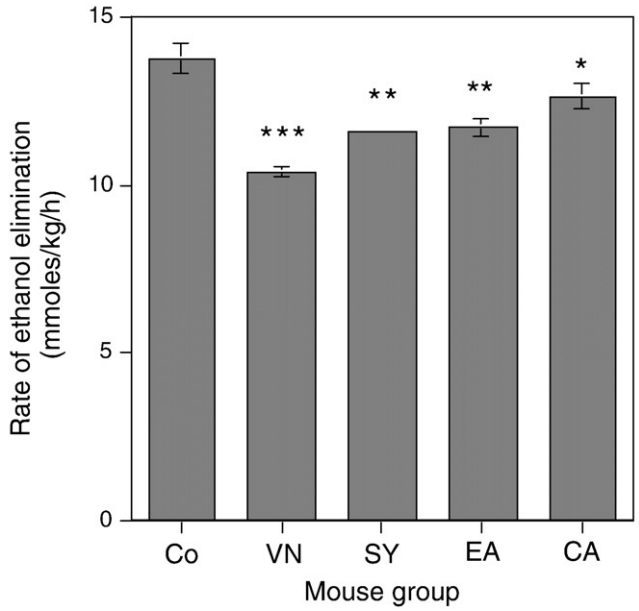

Fig. 7. Effect of phytophenols on alcohol metabolism in mice. Mice (n = 4) were injected with a 20% ethanol solution containing each phytophenol at an ethanol dose of 3.0 g/kg. The elimination rate of blood ethanol was calculated from the concentration of blood ethanol from 1 to 4 hours after administration ("Materials and methods"). The phytophenols had doses of less than 1.5 mmol/kg because they did not dissolve completely in 20% ethanol. Co, control; VN, vanillin; SY, syringaldehyde; EA, ellagic acid; CA, caffeic acid. *, **, and *** mean $P < 01$, .0005, and .0001, respectively, by PLSD.

control vs 1.5-mL/kg groups and for 1.5-mL/kg vs 3.0-mL/kg groups by PLSD).

Fig. 1B shows that administration of the NVF reduced the blood acetaldehyde level 1 hour after ethanol administration in a dose-dependent manner ($P < .05$ by ANOVA).

In vitro, when the NVF was added to the reaction medium, it strongly inhibited the liver ADH activity of the extract in a concentration-dependent manner (Fig. 2).

3.2. Nonvolatile constituents of whisky

The HPLC analyses revealed the nonvolatile constituents of whisky to be as follows (in order of amount): ellagic acid, syringaldehyde, vanillin, gallic acid, syringic acid, vanillic acid, coniferyl alcohol, caffeic acid, and other compounds. The amounts of these phytophenols increase with the age of whisky (Fig. 3) extracted from oak casks. The NVF of 16-year whisky contained all these phenolic compounds and also several fatty acid esters (Table 1).

3.3. Effects of phytophenols on liver ADH activity

Among the phytophenols detected in the NVF, vanillin, syringaldehyde, ellagic acid, and caffeic acid were found to strongly inhibit liver ADH activity in a concentration-dependent manner (10~230 μmol/L) when each of them was added to the reaction medium. Vanillin and syringaldehyde are phenolic aldehydes, whereas ellagic acid and caffeic acid are phenolic acids. However, phenolic acids with a carboxylic acid group (vanillic acid, syringic acid, gallic acid, etc) were found to have a weaker inhibitory effect on ADH (Fig. 4).

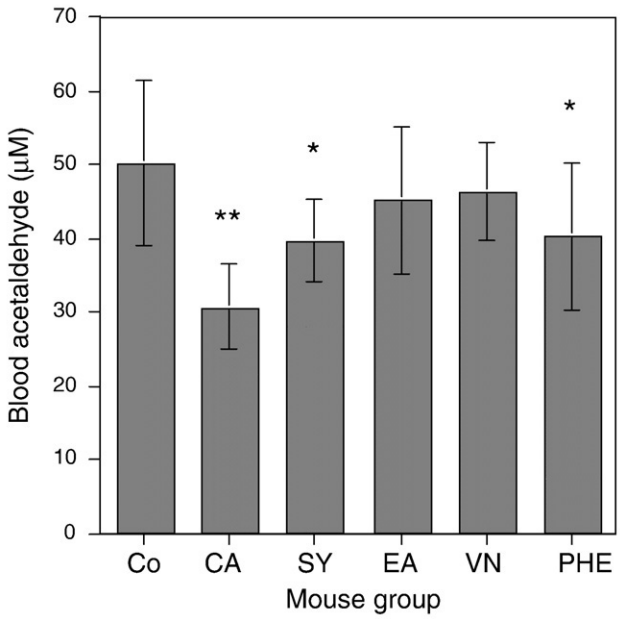

Fig. 8. Effect of phytophenols on blood acetaldehyde after ethanol administration. The acetaldehyde level of heart blood was measured 1 hour after the administration of an ethanol solution containing each phytophenol, as in Fig. 7. Co, control (n = 16); VN, vanillin (n = 4); SY, syringaldehyde (n = 4); EA, ellagic acid (n = 4); CA, caffeic acid (n = 4); PHE, 4 phytophenolic groups (n = 16). * and ** mean $P < 05$ and 0.01, respectively, by t test.

The concentrations of these ADH-inhibitory phytophenols in the ADH reaction medium containing approximately 0.22% to 1.74% NVF (Fig. 2) can be calculated from Table 1: ellagic acid, 1.7 to 13.5 μmol/L; syringaldehyde, 2.2 to 17.6 μmol/L; vanillin, 1.5 to 11.9 μmol/L; and caffeic acid, 0.3 to 2.0 μmol/L.

The inhibitory effects of these phenolic aldehydes and acids on the enzyme kinetics of ADH were investigated using purified mouse ADH 1, a key enzyme in alcohol metabolism. As shown in Fig. 5, mouse ADH 1 was inhibited in a competitive manner by caffeic acid, but in a mixed-type manner by ellagic acid, vanillin, and syringaldehyde. The ADH 1 inhibition constants (K_Is) were 0.08, 7.9, 15.6, and 22.0 μmol/L for caffeic acid, vanillin, syringaldehyde, and ellagic acid, respectively. For reference, the K_Is for the well-known ADH inhibitors pyrazole and capronamide were 5.1 and 29.0 μmol/L, respectively. The chemical structures of the 4 phytophenols are shown in Fig. 6.

3.4. Effects of phytophenols on alcohol metabolism

All of these phytophenolic aldehydes and acids significantly reduced the elimination rate of blood ethanol when they were administered to mice together with a 20% ethanol solution at an ethanol dose of 3.0 g/kg (Fig. 7). The blood acetaldehyde level 1 hour after ethanol administration was significantly lower for the caffeic acid group ($P < .01$) and the syringaldehyde group ($P < .05$) than for the control group. The vanillin and ellagic acid groups also exhibited lower blood acetaldehyde levels than the control group, although the differences were not statistically significant. The average level for the 4 phenolic groups was also significantly lower than that for the control group ($P < .02$) (Fig. 8).

4. Discussion

We recently reported that, the longer whisky matures, the more effective it is in suppressing the elimination of blood ethanol and thus reducing the blood acetaldehyde level [18]. In this study, we found that the NVF of a single-malt whisky inhibited liver ADH in mice, thereby reducing the elimination rate of blood ethanol and lowering the blood acetaldehyde level (Figs. 1 and 2). Among the constituents of NVF, some phytophenols (vanillin, syringaldehyde, caffeic acid, ellagic acid) were found to strongly inhibit the activity of mouse ADH 1 (Figs. 4 and 5). Their amounts increase as whisky matures (Fig. 3). When they were administered to mice together with ethanol, all of them reduced the elimination rate of blood ethanol, which resulted in a lower blood acetaldehyde level (Figs. 7 and 8).

The levels of blood acetaldehyde in the cases of pure phytophenols, however, were not exactly relative to the elimination rates of blood ethanol (Figs. 7 and 8), differing

from those in the cases of NVF (Figs. 1A and B). These phytophenols might have some inhibitory effect on ALDH and not only on ADH because they have aldehyde or carboxylic acid group. Moreover, phenolic polymers increasing with aging in whisky might have some effects on alcohol and/or acetaldehyde metabolism. These expectations will be elucidated in the near future.

In any case, it has been demonstrated that a depression of ethanol elimination resulting in a lower level of blood acetaldehyde, which has been observed by the maturation of whisky [18], is caused by the enhanced inhibition of liver ADH 1 due to the greater amounts of these phytophenols in mature whisky. These results suggest that these phytophenols reduce the aversive reaction and cytotoxicity due to acetaldehyde after the drinking of whisky. Furthermore, they may prolong drunkenness and limit how much a person drinks to some extent by depressing alcohol metabolism. These effects could be related to the reduced likelihood of unpleasant aftereffects when mature whisky is drunk [18], which we empirically know to be true from the experience of those who drink such beverages.

The lower concentration of blood acetaldehyde usually increases the likelihood of overdrinking, as known in individuals with *ALDH2*1/1* [1], because of reductions in aversive reactions due to acetaldehyde during drinking. However, the lower level of blood acetaldehyde when drinking mature whisky may not increase the likelihood of overdrinking because the alcohol metabolism of mature whisky is depressed and, thereby, the drunkenness is prolonged.

Previous studies have reported that liver ADH is inhibited by various kinds of chemical reagents [16,17]. Zinc-chelating reagents (pyrazoles, imidazoles, *o*-phenanthroline) are well-known ADH inhibitors that bind in the pocket and compete with the substrate. Fluorescent aromatic cations (auramine o, tricyclic psychopharmaca, etc) are also substrate-competitive inhibitors of liver ADHs. Another type of aromatic compound with a negative charge in its ring system (anilinonaphthalene sulfonate, rose bengal, etc) inhibits ADH activity by binding to the coenzyme binding site. In this study, we found ADH inhibitors in food constituents. Among them, vanillin, syringaldehyde, and ellagic acid showed a mixed type of inhibition against mouse ADH 1 (Fig. 5) and exhibited smaller K_Is than that of capronamide, a well-known mixed-type inhibitor of mammalian ADH ("Results") [16]. In addition, the K_I of syringaldehyde was larger than that of vanillin ("Results"), suggesting that the methoxy group at the *m*-position of phenolic aldehydes may interfere with ADH inhibition. Because neither vanillic nor syringic acid was found to be effective in inhibiting ADH 1, it seems that the 2 phenolic aldehydes, vanillin and syringaldehyde, inhibit ADH by binding to the substrate binding site on the enzyme with their aldehyde groups. Gallic acid, which has 2 hydroxy groups at the *m*-positions instead of the 2 methoxy groups in syringic acid, was also found to be less inhibitory (Fig. 4). However, ellagic acid, a dimer of gallic acid, strongly inhibited ADH 1 (Figs. 4 and 5). This inhibitory effect is probably due to its chelating ability [23]. As mentioned before, pyrazoles and *o*-phenanthroline are also well-known zinc-chelating ADH inhibitors.

Caffeic acid, a hydroxycinnamic acid, was found to inhibit ADH 1 in a competitive manner and had the smallest K_I (0.08 μmol/L) among the 4 phytophenols ("Results"). Its K_I was smaller than that of pyrazole, a well-known competitive inhibitor of mammalian ADHs ("Results") [17]. Unlike other phenolic acids with a carboxylic acid group, the potent competitive inhibition of caffeic acid is probably due to its propenoic acid group because ADH 1 exhibits a very low Km of 0.7 μmol/L for hydroxycinnamic alcohol as a substrate, which has a propenyl alcohol group [24]. Thus, our inhibition studies demonstrate that these phytophenolic aldehydes and acids in whisky are potent inhibitors of mouse ADH 1. It has already been reported that caffeic acid inhibits lipooxygenase, whereas ellagic acid inhibits aldose reductase, xanthine oxidase, and angiotensin-converting enzyme [25].

These phytophenols are found in various plant foods and in alcoholic beverages stored in wooden casks and have an antioxidative effect and an inhibitory effect on carcinogenesis [26]. Vanillin is present in vanilla beans and soy sauce; syringaldehyde is present in walnuts; caffeic acid is present in coffee beans, soy beans, cereals, walnuts, and some fruits; and ellagic acid is present in some fruits (red raspberries, strawberries, grapes pomegranates) and in certain nuts. The intake of foods rich in these phytophenols together with alcohol may diminish the metabolic toxicity of alcohol due to acetaldehyde and oxidative stress and help reduce the likelihood of overdrinking by depressing alcohol metabolism.

5. Conclusions

The phytophenols (such as vanillin, syringaldehyde, caffeic acid, ellagic acid) detected in whisky strongly inhibited liver ADH 1, thus lowering the blood acetaldehyde level by depressing ethanol elimination. Therefore, the intake of these phytophenols together with alcohol could have a preventive effect on alcohol-related diseases by diminishing the metabolic toxicity of alcohol due to acetaldehyde and oxidative stress and by reducing the likelihood of overdrinking through the depression of alcohol metabolism.

Acknowledgment

This work was financially supported by Asahi Breweries, Ibaraki, Japan.

References

[1] Eriksson CJP. The role of acetaldehyde in the actions of alcohol (update 2000). Alcohol Clin Exp Res 2001;25:15s-32s.

[2] Braggins TJ, Crow KE, Batt RD. Acetaldehyde and acetate production during ethanol metabolism in perfused rat liver. Adv Exp Med Biol 1980;132:441-9.

[3] Harada S, Agarwal DP, Goedde HW. Acetaldehyde dehydrogenase deficiency as cause of facial flushing reaction to alcohol in Japanese. Lancet 1981;ii:982.
[4] Bosron WF, Li TK. Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases, and their relationship to alcohol metabolism and alcoholism. Hepatology 1986;6:502-10.
[5] Enomoto N, Takase S, Takada N, Takada A. Alcoholic liver disease in heterozygotes of mutant and normal aldehyde dehydrogenase-2 genes. Hepatology 1991;13:1071-5.
[6] Yokoyama A, Muramatsu T, Ohmori T, Higuchi S, Hayashida M, Ishii H. Esophageal cancer and acetaldehyde dehydrogenase-2 genotypes in Japanese males. Cancer Epidemiol Biomarkers Prev 1996;5:99-102.
[7] Thomasson HR, Crabb DW, Edenberg HJ, Li TK. Alcohol and aldehyde dehydrogenase polymorphisms and alcoholism. Behav Genet 1993;23:131-6.
[8] Yamauchi M, Maezawa Y, Mizuhara Y, Ohata M, Hirakawa H, Nakajima J, Toda G. Polymorphisms in alcohol metabolizing enzyme genes and alcoholic cirrhosis in Japanese patients: a multivariate analysis. Hepatology 1995;22:1136-42.
[9] Sprandel U, Troger HD, Liebherdt EW, Zollner N. Acceleration of ethanol elimination with fructose in man. Nutr Metab 1980;24:324-30.
[10] Yamaguchi M, Takada M, Nozaki O, Ito M, Furukawa Y. Preparation of corn peptide from corn gluten meal and its administration effect on alcohol metabolism in stroke-prone spontaneously hypertension rats. J Nutr Sci Vitaminol 1996;42:219-31.
[11] Ramchandani VA, Kwo PY, Li TK. Effect of food and food composition on alcohol elimination rates in healthy men and women. J Clin Pharmacol 2001;41:1345-50.
[12] Jones AW, Jonsson KA, Kechagias S. Effect of high-fat, high-protein, and high-carbohydrate meals on the pharmacokinetics of a small dose of ethanol. Br J Clin Pharmacol 1997;44:521-6.
[13] Uchima H, Funabashi M, Shimizu Y, Abe T. Effects of meals on ethanol metabolism. Jpn J Legal Med 1984;38:582.
[14] Pietruszko R. Aldehyde dehydrogenase. In: Watson RR, editor. Biochemistry and physiology of substance abuse, vol. 1. Boca Raton (Fla): CRC Press; 1989. p. 89-127.
[15] Harada S, Agarwal DP, Goedde HW, Takagi S, Ishikawa B. Possible protective role against alcoholism for aldehyde dehydrogenase isozyme deficiency in Japan. Lancet 1982;ii:827.
[16] Sund H, Theorell H. Alcohol dehydrogenases. In: Boyer PD, editor. The enzymes, 2nd ed., vol. 7. New York: Academic Press; 1963. p. 25-83.
[17] Branden CI, Jornvall H, Eklund H, Furugren B. Alcohol dehydrogenases. In: Boyer PD, editor. The enzymes, 3rd ed., vol. 11. New York: Academic Press; 1975. p. 103-90.
[18] Haseba T, Mashimo K, Sugimoto J, Sato S, Ohno Y. Maturation of whisky changes ethanol elimination kinetics and neural effects by increasing nonvolatile congeners. Alcohol Clin Exp Res 2007;31: 77s-82s.
[19] Widmark EMP. Verteilung und umwandlung des aethyalkohols im organismus des hundes. Biochem Z 1933;267:128-34.
[20] Tsukamoto S, Kanegae T, Isobe E, Hirose M, Shimamura M, Nagoya T. Effects of bezafibrate on ethanol oxidation in rats. Alcohol Clin Exp Res 1996;20:1599-603.
[21] Haseba T, Sato S, Ishizaki M, Yamamoto I, Kurosu M, Watanabe T. Intralobular and intracellular localization of alcohol dehydrogenase (ADH) isozymes in mouse liver—basic ADH (class I) and acidic ADH (class III). Biomed Res 1991;12:199-209.
[22] Yamamoto I, Haseba T, Kurosu M, Watanabe T. Allosterism of acidic alcohol dehydrogenase (class III ADH) of mouse liver and its role in alcohol metabolism. J Nippon Med Sch 1992;59:136-44.
[23] Shahidi F. Phenolic compounds of *Brassica* oilseeds. In: Ho C-T, Lee CY, Huang M-T, editors. Phenolic compounds in food and their effects on health I. Washington (DC): American Chemical Society; 1992. p. 130-42.
[24] Pietruszko R. Mammalian liver alcohol dehydrogenases. Adv Exp Med Biol 1975;56:1-31.
[25] Duke JA. Handbook of biologically active phytochemical and their activities. Boca Raton (Fla): CRC Press; 1992. p. 21-157.
[26] Ho CT. Phenolic compounds in food—an overview. In: Ho CT, Lee CY, Huang MT, editors. Phenolic compounds in food and their effects on health I. Washington (DC): American Chemical Society; 1992. p. 2-7.