Effect of Chronic Administration of Alcoholic Beverages and Seasoning Containing Alcohol on Hepatic Ethanol Metabolism in Mice

Ritsuko KISHIMOTO,* Miwa UEDA, Masaki KAWAKAMI, Kiyoshi GODA, Song-Shin PARK1 and Yozo NAKATA2

Faculty of Nutrition, Kobe Gakuin University,
Nishi-ku, Kobe 651–21, Japan

1Laboratory of Comparative Carcinogenesis, National Cancer Institute,
NIH, Frederick, Maryland 21702–1201, USA

2School of Medical Science, Osaka University,
Yamadaoka, Suita 565, Japan

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Summary Five-week-old male mice, C3H/HeNCrj (C3H/He), were given a 5% (v/v) ethanol solution, commercial alcoholic beverages (Japanese sake (sake) or red wine) or a Japanese seasoning (mirin [containing ethanol and a large amount of glucose]) ad libitum for 45 d, and were then examined for changes in the hepatic enzymes related to ethanol metabolism 2 h after oral administration of 5 g of ethanol/kg body weight. The specific activity of aniline hydroxylase (ANH) in the hepatic microsome increased significantly in all groups chronically administered ethanol solution, sake, red wine or mirin, and the greatest increase was in the hepatic microsome of mirin-administered mice. The cytochrome P-450 (CYP) 2E1 increased in the hepatic microsome of the mice administered ethanol solution, red wine or mirin where accompanied by high ANH activity. The immunoreactive band for CYP1A1 showed high specificity in the microsome of mice given sake, red wine or mirin. It was assumed that CYP1A1 was induced by unknown component(s) other than ethanol in these solutions. In the cytosolic fraction, following the chronic administration of sake and mirin, the total aldehyde dehydrogenase (AIDH) activity with high-Km decreased significantly. In the mitochondrial fraction, the activity of high-Km AIDH increased significantly in the mirin-administered mice which drank a large amount of ethanol, whereas that in the red wine-administered group tended to decrease. These results indicate that the enzyme activities related to the

*To whom correspondence should be addressed.

Abbreviations: CYP, cytochrome P-450; ADH, alcohol dehydroxylase; AIDH, aldehyde dehydroxylase; MEOS, microsomal ethanol-oxidizing system; ANH, aniline hydroxylase; MAb, monoclonal antibody.
oxidation of both ethanol and acetaldehyde in the cytosolic, mitochondrial and microsomal fractions of the liver were affected by either the action of ethanol or its interaction with other constituents of sake, red wine and mirin.

Key Words C3H/He mice, alcoholic beverages, mirin, CYP2E1, CYP1A1

Worldwide, alcoholic beverages play a major social role in many cultures in which drinking is a social custom and a means of fostering social interactions. In addition, alcohol is used as seasoning when cooking to improve the flavor and food texture. Although heavy alcohol consumption increases cancer risk of the head, neck, esophagus and liver (1), moderate consumption of alcohol has some benefits and may actually contribute to a decreased risk of coronary heart disease (2). It was reported that phenolic components of red wines were demonstrated to possess superoxide radical scavenging potentials (3) and inhibited low-density lipoprotein oxidation (4). Hamilton et al (5) suggested that the modulation of experimental colonic tumorigenesis by chronic beer or ethanol consumption was due to the alcohol itself rather than other beverage constituents. However, Niwa et al (6) suggested an enhancing action of ethanol or sake on methylazoxymethanol-induced carcinogenesis in the rectosigmoidal colon.

The major constituent of commercial alcoholic beverages is ethanol. Chronic or a high consumption of ethanol leads to induction of the microsomal ethanol-oxidizing system (MEOS) by proliferation of smooth endoplasmic reticulum (microsome) (7, 8). It was reported that the bulk of MEOS activity is catalyzed by cytochrome P-450 (CYP) 2E1, which is an isozyme of cytochrome P-450, and induced in mice by ethanol exposure (9, 10). Crankshaw and Hines (11) reported that beer contains at least one unidentified component that apparently blocks the induction of CYP2E1. In addition, CYP1A2 is related to MEOS activity (12). CYP1A1 and CYP1A2 were induced by a chemical carcinogen methylcholanthrene in mice liver.

Oxidation of ethanol in the liver results in the formation of acetaldehyde. Acetaldehyde is known to be almost exclusively oxidized to acetate by liver aldehyde dehydrogenase (AIDH). The activities of AIDH with low- and high-Km have been detected in the mitochondrial, microsomal and cytosolic fractions of rat (13) and mouse livers (14). Mitochondrial AIDH with low-Km seems to play a major role in acetaldehyde metabolism (15). However, the role of AIDH is not understood in detail.

The commercial alcoholic beverages Japanese sake (sake) and red wine are derived from the fermented extracts of rice and grapes, respectively. The commercial Japanese sweetener mirin is a mixture of sake and shochu or alcohol, and contains a large amount of glucose. It is obscure how the interaction between constituents and ethanol in alcoholic beverages or alcoholic seasoning affects hepatic enzyme
activity relating to the ethanol metabolism in the case of chronic ethanol administration.

In this study, we orally administered ethanol solution to mice after chronic administration of sake, red wine or mirin to investigate its effects on various enzymatic activities in the pathways of ethanol metabolism in the liver and on the induction of cytochrome P-450 isozymes in the hepatic microsomal fraction.

EXPERIMENTAL

Animals. Five-week-old male C3H/HeNCrj (C3H/He) mice were obtained from Japan Charles River Co., Ltd. One group of the mice was fed commercial MF food (Oriental Yeast Co., Ltd.) with water (control group), while four other groups received the same food for 45 d and given free access to one of the following alcoholic solutions: 5% (v/v) ethanol solution, commercially available sake (Nihonsakari, 15.2% (v/v) ethanol, specific gravity (Sp.gr.) of 1.001, Nishinomiya Shuzo Co., Ltd.), red wine [Cabernet (red), 11.1% (v/v) ethanol, Sp.gr. of 0.994, Mercian Co., Ltd.] or honmirin (mirin, 14.2% (v/v) ethanol, 42% (w/w) saccharide, Sp.gr. of 1.166, Takara Shuzo Co., Ltd.). The ethanol (purity >99.5% and Sp.gr. of 0.793) was purchased from Nacalai Tesque Co. The ethanol concentrations (v/v) of the sake, red wine and mirin were adjusted to approximately 5% (v/v) with distilled water. Throughout the experimental period, the mice were housed in pairs in stainless-steel wire cages under controlled conditions of temperature (22.5 ± 0.5 ºC), humidity (50–60%) and light (12 h/12 h light/dark cycle). The body weight and intake of food and liquid were measured and recorded at 2:00 p.m. every 3 or 4 d during the experimental period. The intake of total energy (kcal/mouse) and total ethanol (g/mouse) during chronic administration of the above alcoholic solutions were calculated. Mice were food-deprived overnight at the end of this period, given 5 g of ethanol/kg at 9:00 a.m. and then killed by cervical dislocation at 11:00 a.m. The serum was collected immediately and prepared to assay the contents of ethanol and acetaldehyde. The liver was also removed immediately and weighed to determine various enzyme activities. The liver weight was expressed as relative liver weight (g/g of body weight). All samples were stored at −70 ºC until use.

Calculation of intake of total energy and ethanol. The total energy intake (kcal/mouse) was calculated using the values of 358 kcal/100 g of MF food, 105 kcal/100 g of sake, 71 kcal/100 g of red wine, 237 kcal/100 g of mirin and 7 kcal/g of ethanol. The total ethanol intake (g/mouse) was also calculated using the values of ethanol content in the four liquids described before.

Cell fraction. Liver cell fractionation was performed by differential centrifugation as described previously (14). The liver was homogenized in four volumes of cold 10 mm Tris-HCl buffer, pH 7.4, containing 0.25 m sucrose and 1 mm mercaptoethanol, and centrifuged at 750 × g for 10 min. The supernatant was re-centrifuged at 10,000 × g for 20 min, and the resultant supernatant was used for
the preparation of microsomal and cytosolic fractions. The precipitate was washed twice with the same buffer solution by centrifugation at 10,000 × g for 10 min and used as the mitochondrial fraction. The supernatant at this centrifugation step was further spun at 100,000 × g for 60 min, this supernatant was used as the cytosolic fraction. The resultant precipitate was washed with 0.15 M KCl at 100,000 × g for 60 min and used as the microsomal fraction. Each fraction was kept at −70°C until the enzyme assay.

Activity measurements of enzymes. Cytosolic alcohol dehydrogenase (ADH) activity was assayed for NADH production by spectrophotometry as described previously (14). The specific activity was expressed as μmol of NADH/min/mg protein. The activity of the MEOS was assayed as described previously (14), according to the method of Klein et al (16). The specific activity was expressed as nmol of acetaldehyde/min/mg protein. The microsomal aniline hydroxylase (ANH) activity was assayed as described previously (14), according to the method of Brodie and Axelrod (17). The specific activity was expressed as nmol of p-aminophenol/min/mg protein. The activities of AlDH in the mitochondrial, microsomal and cytosolic fractions were assayed as described previously (14), according to the method of Lebsack et al (13), except that the acetaldehyde concentration was 5 mM for total AlDH, 300 μM for high-Km AlDH and 50 μM for low-Km AlDH. Two micromolar rotenone was added to inhibit NADH oxidase for the mitochondrial AlDH assay. 4-Methylypyrazole was added at a concentration of 200 μM to inhibit ADH activity in the microsomal and cytosolic AlDH assays. The specific activity was expressed as nmol of NADH/min/mg protein.

Immunoblot analysis. Samples of liver microsome containing 20 μg protein were subjected to sodium dodecyl sulfate (SDS)/9% polyacrylamide gel electrophoresis according to the method of Laemmli (18), followed by electrophoretic transfer to a nitrocellulose sheet at 200 V for 1 h using a Horizblot (ATTO Co.) and immunoblot analysis. Four monoclonal antibodies (MAbs) were used in the immunoblot analysis for primary antibodies: mouse MAb 1-98-1 to ethanol-induced rat CYP2E1 (19); mouse MAb 1-7-1 to 3-methylcholanthrene-induced rat CYP1A1 and CYP1A2 (20); mouse MAb 1-31-2 to 3-methylcholanthrene-induced rat CYP1A2 (20); and mouse MAb 2-66-3 to the phenobarbital-induced rat CYP2B1 (21). These MAbs were diluted 200-fold. The nitrocellulose membrane was incubated with each MAb for 1 h, followed by incubation for 30 min with goat anti-mouse IgG-conjugated alkaline phosphatase (Promega Co.) at 1:5000 dilution. The blot was then developed using an Immun-Blot® Assay Kit (Bio-Rad Lab.).

Contents of ethanol, acetaldehyde and acetate in serum. The contents of ethanol, acetaldehyde and acetate in the serum were determined separately using F-kits (Behringer Mannheim Biochemica).

Other assay methods. Total microsomal cytochrome P-450 content was determined from the CO-reduced difference spectrum in 0.1 M potassium phosphate buffer (22) and expressed as nmol of cytochrome P-450/mg protein. The protein
contents in the mitochondrial, microsomal and cytosolic fractions were measured by the biuret method (23) with bovine serum albumin (Sigma Chemical Co., Ltd.) as the standard.

Statistical analysis. Data were expressed as means±SE. The differences among values were analyzed by analysis of variance (ANOVA) coupled with Duncan's new multiple range test (24) and considered significant at p<0.05.

RESULTS

Total energy and total ethanol intake during chronic administration of ethanol solution, sake, red wine or mirin

As shown in Table 1, C3H/He male mice showed a preference for mirin and avoided red wine. Compared to that of the control mice, the energy intake (kcal/mouse) from food in each of the four groups given alcoholic solutions during the period of treatment decreased significantly. The energy intake from liquids in the group given mirin during the period of treatment increased markedly, though that from food decreased. Although no difference in the total energy intake was recognized between the intake by control mice and the groups given ethanol solution, sake or red wine, the total energy of the group given mirin increased markedly. The total ethanol intake (g/mouse) by this group was approximately twice that by the group given ethanol solution or sake, whereas the red wine-administered mice showed the lowest intake.

Body weights and relative liver weights after chronic administration of ethanol solution, sake, red wine or mirin

As shown in Table 2, the body weight was significantly higher in the control group than in the mirin-administered mice, while the body weights of the mice administered 5% ethanol solution, sake or red wine were not different from the control group. The relative liver weights (g/g of body weight) of mice after chronic administration of ethanol solution, sake, red wine or mirin were almost equal to that of the control mice.

Specific activities of ADH in the hepatic cytosol

As shown in Table 3, the specific activity of ADH in the hepatic cytosolic fraction, at 2 h after the oral administration of ethanol did not significantly change following the chronic administration of 5% ethanol solution, sake, red wine or mirin.

Specific activities of MEOS and ANH and contents of cytochrome P-450 in the hepatic microsome

Table 4 shows the specific activities of MEOS and ANH and the content of cytochrome P-450 in the hepatic microsome at 2 h after the oral administration of ethanol to mice in each group. Although the activity of MEOS in the hepatic microsome did not change significantly, that in the mice given ethanol solution or
Table 1. Total energy and total ethanol intake of C3H/He male mice during the period of chronic administration of ethanol solution, sake, red wine or mirin.

| Treatment group | Total energy intake (kcal/mouse) | Total ethanol intake (g/mouse) |
|-----------------|----------------------------------|--------------------------------|
|                 | Food                             | Liquid                         | Total                          |
| Control         | 631 ± 17<sup>a</sup>             | —                              | 631 ± 17<sup>bc</sup>         | —                              |
| Ethanol         | 557 ± 7<sup>b</sup>              | 89 ± 3<sup>bc</sup>            | 646 ± 9<sup>b</sup>            | 12.7 ± 0.4<sup>b</sup>         |
| Sake            | 534 ± 6<sup>b</sup>              | 107 ± 3<sup>b</sup>            | 643 ± 7<sup>b</sup>            | 12.3 ± 0.3<sup>b</sup>         |
| Red wine        | 537 ± 10<sup>b</sup>             | 67 ± 4<sup>e</sup>             | 606 ± 9<sup>e</sup>            | 8.4 ± 0.5<sup>c</sup>          |
| Mirin           | 347 ± 11<sup>c</sup>             | 623 ± 15<sup>a</sup>           | 964 ± 14<sup>a</sup>           | 25.4 ± 0.6<sup>a</sup>         |

Water (control), ethanol solution, sake, red wine or mirin containing 5% (v/v) ethanol were given ad libitum for 45 d. Total energy intake was calculated using the values of 358 kcal/100 g for MF food, 105 kcal/100 g for sake, 71 kcal/100 g for red wine, 237 kcal/100 g for mirin and 7 kcal/g for ethanol. Values (means ± SE for 5 mice) in the same column not bearing the same superscript letter are significantly different by Duncan’s new multiple range test at p < 0.05.

Table 2. Body weight and relative liver weight after chronic administration of ethanol solution, sake, red wine or mirin.

| Treatment group | Control | Ethanol | Sake | Red wine | Mirin |
|-----------------|---------|---------|------|----------|-------|
| Body weight (g) | 28.5 ± 0.3<sup>b</sup> | 28.0 ± 0.4<sup>b</sup> | 28.4 ± 0.4<sup>b</sup> | 27.6 ± 0.3<sup>b</sup> | 29.5 ± 0.3<sup>b</sup> |
| Relative liver weight (g/g of body weight) | 0.051 ± 0.001 | 0.052 ± 0.001 | 0.051 ± 0.001 | 0.052 ± 0.001 | 0.052 ± 0.001 |

Water (control), ethanol solution, sake, red wine or mirin containing 5% (v/v) ethanol were given ad libitum for 45 d. Values (means ± SE for 10 mice) not bearing the same superscript letter are significantly different by Duncan’s new multiple range test at p < 0.05.

sake increased slightly. The content of cytochrome P-450 in the hepatic microsome also increased slightly, but it did not change significantly following the chronic administration of ethanol solution, sake or red wine. Following chronic administration of the alcoholic solutions, the specific activity of ANH in the hepatic microsome increased significantly compared to that in the control mice. The activity of ANH in the microsome showed the highest and lowest values in the groups given mirin and ethanol solution, respectively.

**Immunoblot analysis of the hepatic microsomal protein**

The microsomal protein obtained from the liver 2 h after the oral administration of ethanol was subjected to SDS-polyacrylamide gel electrophoresis, followed by blotting and immunochemical procedures in which antibodies to multiple cyto-
Table 3. Specific activities of alcohol dehydrogenase in the hepatic cytosol of mice 2 h after the oral administration of ethanol after chronic administration of ethanol solution, sake, red wine or mirin.

| Treatment group | ADH (NADH μmol/min/mg prot.) |
|-----------------|-------------------------------|
| Control         | 7.11 ± 0.11                   |
| Ethanol         | 7.56 ± 0.25                   |
| Sake            | 6.99 ± 0.13                   |
| Red wine        | 7.64 ± 0.29                   |
| Mirin           | 7.00 ± 0.14                   |

Water (control), ethanol solution, sake, red wine or mirin containing 5% (v/v) ethanol were given ad libitum for 45 d. Values are means ± SE for 8 mice.

Table 4. Specific activities of MEOS and aniline hydroxylase and content of cytochrome P-450 in the hepatic microsome of mice 2 h after the oral administration of ethanol after chronic administration of ethanol solution, sake, red wine or mirin.

| Treatment group | MEOS (nmol/min/mg prot.) | Cytochrome P-450 (nmol/min/mg prot.) | Aniline hydroxylase (nmol/min/mg prot.) |
|-----------------|--------------------------|-------------------------------------|----------------------------------------|
|                 | n = 5                    | n = 6                               | n = 6                                  |
| Control         | 1.63 ± 0.11              | 0.679 ± 0.036                       | 0.382 ± 0.062b                         |
| Ethanol         | 1.88 ± 0.09              | 0.795 ± 0.020                       | 0.543 ± 0.047a                         |
| Sake            | 1.80 ± 0.04              | 0.744 ± 0.031                       | 0.557 ± 0.036a                         |
| Red wine        | 1.67 ± 0.08              | 0.749 ± 0.028                       | 0.573 ± 0.044a                         |
| Mirin           | 1.67 ± 0.05              | 0.673 ± 0.043                       | 0.620 ± 0.043a                         |

Water (control), ethanol solution, sake, red wine or mirin containing 5% (v/v) ethanol were given ad libitum for 45 d. Values (means ± SE) in the same column not bearing the same superscript letter are significantly different by Duncan’s new multiple range test at p < 0.05.

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chrome P-450 were used as probes for immunorelated proteins. The detection of CYP2E1 was carried out using MAb 1-98-1. As shown in Fig. 1 (top), one immunoreactive band of CYP2E1 was observed with the control microsome. The intensity of this band was increased markedly in the hepatic microsomes of mice in the groups given ethanol solution, red wine or mirin. The detection of CYP1A1 and CYP1A2 was carried out using MAb 1-7-1 (Fig. 1, bottom). One immunoreactive band for CYP1A1 was also observed with the microsomes of control mice and those administered ethanol solution. This was highly specific in the microsomal protein of the mice in the commercial alcoholic solution-treated groups, since the immunoreactive band of CYP1A2 was not detected using MAb 1-31-2 (not shown). MAb 2-66-3 (anti-CYP2B1) did not react with any proteins in the
Fig. 1. Western blot analysis of CYP2E1, CYP1A1 and CYP1A2. Hepatic microsomal proteins (20 μg) were analyzed by immunoblot analysis using monoclonal antibodies against EtOH 1-98-1 (top) and MC1-7-1 (bottom), respectively. Microsomes were prepared from C3H/He male mice 2 h after the oral administration of ethanol at 5 g/kg body weight after chronic administration of water (control) (lane 1), ethanol solution (lane 2), sake (lane 3), red wine (lane 4) and mirin (lane 5) each containing 5% (v/v) ethanol. Lane 6 contains a molecular weight standard.

Specific activity of AlDH in the hepatic mitochondria

Table 5 shows the specific activities of AlDH in the hepatic mitochondrial fraction at 2 h after the oral administration of ethanol to mice which had received...
chronic administration of ethanol solution, sake, red wine or mirin. The low-Km AlDH activity did not change following chronic administration of any of the alcoholic solutions, but the high-Km AlDH activity in the mice given mirin increased significantly. The total AlDH activity did not change in the four groups.

**Specific activities of AlDH in the hepatic microsome**

Table 6 shows the specific activities of AlDH in the hepatic microsomal fraction at 2 h after the oral administration of ethanol following chronic administration of the four alcoholic solutions. The low-Km, high-Km and total AlDH activities increased slightly in all four groups except the total AlDH activity in the mice given sake, which was almost equal to that in the control group.

| Treatment group | Low-Km (NADH nmol/min/mg prot.) | High-Km (NADH nmol/min/mg prot.) | Total (NADH nmol/min/mg prot.) |
|-----------------|---------------------------------|---------------------------------|-------------------------------|
| Control         | 3.50 ± 0.35                     | 4.19 ± 0.22b                   | 8.99 ± 0.35                   |
| Ethanol         | 3.27 ± 0.32                     | 4.13 ± 0.34b                   | 8.78 ± 0.46                   |
| Sake            | 3.45 ± 0.24                     | 4.09 ± 0.28b                   | 8.06 ± 0.56                   |
| Red wine        | 3.15 ± 0.38                     | 3.66 ± 0.27b                   | 8.00 ± 0.46                   |
| Mirin           | 2.93 ± 0.55                     | 5.35 ± 0.29a                   | 9.10 ± 0.50                   |

Water (control), ethanol solution, sake, red wine or mirin containing 5% (v/v) ethanol were given ad libitum for 45 d. Values (means ± SE for 8 mice) in the same column not bearing the same superscript letter are significantly different by Duncan’s new multiple range test at $p < 0.05$.

Table 5. Specific activities in liver mitochondrial aldehyde dehydrogenase of mice 2 h after the oral administration of ethanol after chronic administration of ethanol solution, sake, red wine or mirin.

| Treatment group | Low-Km (NADH nmol/min/mg prot.) | High-Km (NADH nmol/min/mg prot.) | Total (NADH nmol/min/mg prot.) |
|-----------------|---------------------------------|---------------------------------|-------------------------------|
| Control         | 1.17 ± 0.22                     | 1.19 ± 0.27                    | 2.69 ± 0.62                   |
| Ethanol         | 1.71 ± 0.29                     | 2.08 ± 0.66                    | 4.79 ± 1.23                   |
| Sake            | 1.61 ± 0.31                     | 1.42 ± 0.17                    | 2.64 ± 0.45                   |
| Red wine        | 1.49 ± 0.37                     | 1.64 ± 0.40                    | 3.68 ± 0.38                   |
| Mirin           | 1.72 ± 0.19                     | 1.54 ± 0.31                    | 4.07 ± 0.72                   |

Water (control), ethanol solution, sake, red wine or mirin containing 5% (v/v) ethanol were given ad libitum for 45 d. Values are means ± SE for 4 mice.
Table 7. Specific activities of liver cytosolic aldehyde dehydrogenase of mice 2h after the oral administration of ethanol after chronic administration of ethanol solution, sake, red wine or mirin.

| Treatment group | Low-Km (NADH nmol/min/mg prot.) | High-Km (NADH nmol/min/mg prot.) | Total |
|-----------------|---------------------------------|---------------------------------|-------|
| Control         | 1.51 ± 0.22                     | 5.94 ± 0.44a                   | 7.86 ± 0.34a |
| Ethanol         | 1.65 ± 0.13                     | 6.38 ± 0.45a                   | 8.56 ± 0.44a |
| Sake            | 1.36 ± 0.16                     | 4.55 ± 0.29b                   | 6.51 ± 0.42b |
| Red wine        | 1.65 ± 0.26                     | 5.64 ± 0.57ab                  | 7.92 ± 0.67a |
| Mirin           | 1.19 ± 0.16                     | 4.07 ± 0.29b                   | 5.82 ± 0.33b |

Water (control), ethanol solution, sake, red wine or mirin containing 5% (v/v) ethanol were given ad libitum for 45d. Values (means±SE for 8 mice) in the same column not bearing the same superscript letter are significantly different by Duncan’s new multiple range test at p<0.05.

Specific activities of AlDH in the hepatic cytosol

Table 7 shows the specific activities of AlDH in the hepatic cytosolic fraction at 2h after the oral administration of ethanol. Following the chronic administration of sake or mirin, the total and high-Km AlDH activities decreased significantly and the low-Km AlDH activity decreased slightly as compared to activities in the control mice.

The serum levels of ethanol, acetaldehyde and acetate did not change significantly at 2h after the oral administration of ethanol to mice having received a chronic administration of ethanol solution, sake, red wine or mirin (data are not shown).

DISCUSSION

C3H/He male mice showed a preference for mirin and avoided drinking red wine. The reason is believed to be because the mirin was sweet and red wine was bitter. Although the food intake in the four groups given alcoholic solutions decreased as compared to that in the control group, the total energy intake of the groups given ethanol solution, sake or red wine was equal to that of the control group. The body weights of the mice given mirin reflected the increased amounts of total energy and ethanol intake. The body weights of mice given red wine, which showed small amounts of total energy and ethanol intake, decreased slightly. The relative liver weights of mice given sake, red wine or mirin were almost equal to that of the control mice. In our previous study (14), we noted that the liver weights of C3H/He male mice increased following the chronic administration of 10% (v/v) ethanol solution for 80d. It was suggested that liver weight is affected by the ethanol content and period of ethanol administration.

The cytosolic ADH carries on the most important role in the ethanol oxidation...
of liver. Cytosolic ADH activity showed no change in the four groups given alcoholic solutions. In the hepatic microsomal fractions of these four groups, the ANH activity increased significantly although the MEOS activity and cytochrome P-450 content were not altered as compared with those of the control mice. These results suggest an increase of CYP2E1 in the hepatic microsome due to alcoholic solution consumption, as ANH activity is used as a measure of CYP2E1 in animal tissues (10). Perhaps because the mice of the mirin group consumed a large amount of ethanol, the highest activity of ANH in the hepatic microsome was seen in this group.

To investigate whether the enhanced ANH activity under these conditions was accompanied by an increase in cytochrome P-450 protein in the hepatic microsome, we performed an immunoblot analysis. Forkert et al (25) reported that MAb 1-98-1 against rat CYP2E1 interacted with a major band of Mr 51,000 on Western immunoblotting of mouse liver microsomes. The molecular weight of CYP2E1 estimated by Western blotting was the same as reported by them. The CYP2E1 increased in the hepatic microsomes of mice administered ethanol solution, red wine or mirin, and the ANH activity in these groups showed high values. The intensity of this band in sake-administered mice was almost equal to that of the control mice, despite the high value of ANH activity in this group. The cause could not be explained in this experiment. CYP2E1 was considered a major component of the MEOS (9). The CYP2E1 enzyme implies high activity for the p-hydroxylation of aniline, oxidation of alcohols and N-demethylation of N-nitrosodimethylamine (NDMA), which is a potent hepatocarcinogen (26, 27) and is regulated by testosterone in the renal mouse microsome (28). These results suggest that, in the hepatic microsome of mice given ethanol solution, red wine or mirin, the MEOS activity did not indicate a significant difference and the oxidation of ethanol to acetaldehyde was increased. Crankshaw and Hines (11) reported that at least one unidentified component in beer blocked the CYP2E1 in the hepatic microsome of rats. However, in our study, the CYP2E1 in the hepatic microsome of mice given red wine or mirin increased as compared to that in the control mice. There may be differences in the constituents between beer, red wine and mirin. In the immunodetection using MAb 1-7-1 that cross-links to the two immunoreactive proteins CYP1A1 and CYP1A2, a single band of CYP1A1 was detected in the hepatic microsomes from all groups of mice. The bands in the control and ethanol solution groups showed lower intensities than those in the other three groups. These results suggest that the increased intensity of CYP1A1 in the mice given sake, red wine or mirin might be due to constituents other than ethanol in these beverages. However, the activity of aryl hydrocarbon hydroxylase as a marker of CYP1A1 was not well determined in this experiment because of the insufficient amount of hepatic microsome. CYP1A1 is well known as an enzyme that is inducible by carcinogens such as 3-methylcholanthrene and benzo[a]pyrene. Grønbæk et al (29) reported that wine drinkers experience a significantly lower cause of mortality than subjects who drink no wine. Thus, it is not distinct whether wine drinking is
related to the risk factor of cancer. Hamilton et al (5) reported that experimental colonic tumorigenesis by chronic beer and ethanol consumption in the rat is due to alcohol itself rather than other constituents of the beverage. Thus, we speculated that CYP1A1 intensity in the hepatic microsome is increased by an unidentified component(s) of sake, red wine or mirin.

It is well known that the low-Km AlDH in the mitochondrial fraction is the most important enzyme in acetaldehyde oxidation in the liver. In the hepatic mitochondrial fraction, the low-Km and total AlDH activities did not change following the administration of sake, red wine or mirin, while high-Km AlDH activity increased in the mirin-administered mice which consumed the largest amount of ethanol.

In the cytosolic fraction, the high-Km and total AlDH activities decreased in the mice given sake or mirin. These groups consumed amounts of ethanol similar to or larger than the mice given the ethanol solution. The activities of high-Km and total AlDH in the mice given ethanol solution or red wine did not change, and in our previous study (14), the activities of low-Km and high-Km AlDH in the hepatic cytosolic fraction did not change following the chronic administration of 10% (v/v) ethanol solution. The decrease in total AlDH activity may be related to the decrease in high-Km AlDH activity. The high-Km AlDH activity in the cytosolic fraction may be inhibited by constituents other than ethanol in sake and mirin. These results suggest that the high-Km AlDH activity in the hepatic mitochondrial and cytosolic fractions is affected by ethanol or other constituents. In this regard, further experiments should be carried out in the future. Recently, a genetic study was conducted with respect to AlDH enzymes in the mouse. Thirteen types of AlDH were identified in mouse tissues, of which 11 were found in the liver (30). It is unknown whether AlDH is the most important in catalyzing the oxidation of acetaldehyde. It has been reported that cytosolic AlDH catalyzes the oxidation of retinaldehyde to retinoic acid (31). It is likely that the oxidation of retinoic acid is decreased in the livers of mice chronically given sake or mirin. In the hepatic microsomal fractions of the mice given ethanol solution, red wine or mirin, the high-Km and total AlDH activities increased slightly. Such an increase in AlDH activity in this fraction might have been due to the rise in high-Km AlDH activity. It has been reported that CYP2E1 in rat liver microsome is an aldehyde oxidase and thus metabolizes both ethanol and its primary oxidation product (32) and horse liver ADH catalyze aldehyde oxidation as well (33).

Our results of studying C3H/He mice following the chronic administration of sake, red wine or mirin indicate that the activities of hepatic cytosolic, mitochondrial and microsomal enzymes relating to the oxidation of both ethanol and acetaldehyde are affected by the mutual action of ethanol or other constituents in sake, red wine and mirin.
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