Elevated Levels of Hemoglobin-associated Acetaldehyde Related to Alcohol Drinking in the Atypical Genotype of Low $K_m$ Aldehyde Dehydrogenase

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Abstract

Acetaldehyde is suspected to be the ultimate carcinogen in alcohol-related carcinogenesis. The atypical genotypes of low $K_m$ aldehyde dehydrogenase (ALDH2) have higher blood concentrations of free acetaldehyde after drinking alcohol. We measured levels of acetaldehyde reversibly bound to haemoglobin (HbAA) after drinking 0.4 ml/kg ethanol using fluorogenic high performance liquid chromatography method in volunteers with the two major ALDH2 genotypes. In the ALDH2*1/*1 genotype with high ALDH2 activity, the increase of HbAA was small. By contrast, in the ALDH2*1/*2 genotype with low ALDH2 activity, HbAA increased considerably at 1–6 h after the drink, and the elevated levels persisted up to 48 h. We also measured HbAA in 81 male workers. Although HbAA levels were significantly correlated with alcohol consumption levels in both of the ALDH2 genotypes, the slope was significantly steeper in the ALDH2*1/*1 genotype than in the ALDH2*1/*2 genotype. In summary, we demonstrated for the first time a significant difference in the increase of HbAA levels after drinking alcohol, depending on the ALDH2 genotype. The HbAA levels are not only a good biomarker for increased internal exposure levels to acetaldehyde but may also be a predictive biomarker for acetaldehyde-mediated carcinogenesis.

Introduction

Acetaldehyde is suspected to be the ultimate carcinogen in alcohol-related carcinogenesis (1). A recent finding that alcoholics and habitual drinkers with the heterozygous genotype of low $K_m$ ALDH2 showed a significantly higher risk for esophageal cancer suggests an important contribution of acetaldehyde in the carcinogenic process (2). The atypical ALDH2 genotypes occur in about half the population of Orientals (3, 4), and those with these genotypes have moderately elevated levels of acetaldehyde after drinking alcohol, which greatly contributes to alcohol hypersensitivity (3–7). The atypical ALDH2 allele (ALDH2*2) has a bp change in exon 12, causing a single amino acid change (8). Acetaldehyde is very active and exerts a variety of health effects including cytotoxicity (9), DNA damage (10), and carcinogenesis in rodents (11). Peterson and Polizzi measured HbAA using fluorogenic HPLC (12) and found a significant difference in HbAA levels between teetotaters and alcoholics among Caucasians probably with the typical ALDH2 genotype (ALDH2*1/*1; Ref. 12). Because the ability to detoxify acetaldehyde is very low in those with the atypical ALDH2 genotypes (ALDH2*1/*2 and ALDH2*2/*2), HbAA levels among Orientals may differ depending on the ALDH2 genotypes. In the present studies, we measured HbAA levels in volunteers with the two major ALDH2 genotypes (ALDH2*1/*1 and ALDH2*1/*2) 0–7 days after drinking certain amounts of alcohol. We also measured HbAA levels in a Japanese occupational population with the two different ALDH2 genotypes and correlated the levels with the amounts of alcohol consumed recently.

Materials and Methods

Subjects and Questionnaire. For the first part of the study, eight Oriental male volunteers participated with informed consent. The subjects with the ALDH2*1/*1 (n = 4) and ALDH2*1/*2 (n = 4) genotypes were ages 25.3 ± 3.9 and 27.0 ± 8.1 years (mean ± SD), respectively. After cessation of drinking alcohol for at least 1 week, the subjects drank whiskey containing 0.4 ml/kg pure ethanol, followed by cessation of drinking for another week. Heparinized blood was drawn immediately before and at 1, 3, 6, 24, 48, 96, and 168 h after drinking.

For the second part of the studies, 81 Japanese male workers ages 44.3 ± 7.7 years in one plant participated with informed consent. The participants completed a questionnaire asking lifestyle questions including drinking habits and the amount of alcohol consumption during the last 2 days before drawing blood. The amount of alcohol consumption was calculated based on the concentration of ethanol contained in the beverages (beer, sake, whisky, and so on).

Measurement of HbAA. Pretreatment of the blood samples was as described previously (12). The assay for acetaldehyde was based on a modification of the procedure reported previously (12). All solvents and reagents were of HPLC or analytical grade. Four hundred mg of 1,3-cyclohexanedione (Tokyo Kasei, Tokyo, Japan), 10 g of NH4OCOCH3, and 3.2 ml of concentrated HCl were mixed in 30 ml of H2O. Equal volumes of the reagent and the hemolyzed sample were placed in a sealed glass tube and reacted for 40 min at 70°C. The reaction was stopped by placing the sample in an ice bath, and 5 µl of each sample were injected onto an HPLC system (Shimadzu LC-lOA with a 370 nm excitation filter and an integrator (Shimadzu)).

Hemoglobin concentration was adjusted to 30 mg/ml before the reaction as determined by measurement of absorbance at 540 nm. Hemoglobin samples were prepared for injection by centrifuging the reaction mixture at 12,000 × g for 15 min at 4°C. The aqueous supernatant was injected directly. Results were expressed as micromolar acetaldehyde as determined from the standard curve (concentration = 8.56 × 10⁻⁵ × area – 0.187) of acetaldehyde concentrations of 0, 1, 10, and 100 µM. Nanomoles acetaldehyde/g protein was calculated by dividing the acetaldehyde concentration by the hemoglobin concentration.

Determination of the ALDH2 Genotype. The ALDH2 genotype was determined by the method described previously (4). Briefly, PCR products were digested with restriction enzyme Ksp6321 and separated on 4% agarose gels.

Statistical Analysis. We used a paired t test in comparisons between HbAA levels before and after the alcohol challenge. We used either Student’s or Welch’s t test for unpaired samples in comparisons of HbAA levels between the different ALDH2 genotypes and the different drinking levels. A simple linear regression model was utilized to examine correlations of HbAA levels with mean amounts of alcohol consumption. A significance of the difference of the slopes between the two ALDH2 genotypes was tested using general linear methods procedure. P values of 0.05 or less were considered as statis-
A WH2*1fI@2, and 5 were judged as AWH2*2fI@2 using PCR-RFLP associated formaldehyde. The concentration of HbAA, and a peak for formaldehyde indicate that of hemoglobin elevations of HbAA (P < 0.05) were found at 1, 24, and 48 h, but of formaldehyde. The intercept was constant. All statistical analyses were performed using the SAS version 6.07 program with the MSP Fujitsu computer system in the Data Processing Center of Kyoto University.

**Results**

A representative chromatogram of the hemolyzed sample is shown in Fig. 1. A peak of acetaldehyde was sufficiently separated from that of formaldehyde.

The time-dependent changes of the HbAA levels after drinking 0.4 ml/kg ethanol in eight volunteers with the two ALDH2 genotypes are summarized in Fig. 2. In the ALDH2*1/*1 genotype, significant elevations of HbAA (P < 0.05) were found at 1, 24, and 48 h, but means of the increases compared to the levels before the challenge were relatively small (+16.0 ± 4.1, +11.8 ± 2.1, and +13.7 ± 3.5, respectively). In contrast, in the ALDH2*1/*2 genotype, large increases were observed at 1, 3, and 6 h (+52.3 ± 9.1, +58.3 ± 9.6, and +50.0 ± 5.5, respectively). The increases at 24 h (+26.6 ± 8.5; P = 0.053) and at 48 h (+18.3 ± 4.8; P < 0.05) were close to the significant level and significant, respectively.

The concentration of HbAA was measured in 81 male workers. Of 81 workers, 38 were judged as ALDH2*1/*1, 38 were judged as ALDH2*1/*2, and 5 were judged as ALDH2*2/*2 using PCR-RFLP analysis (4). We further classified the subjects into two categories, i.e., 0–0.05 and ≥0.05 ml/kg/day by the average amount of alcohol consumed during the last 2 days divided by body weight. As shown in Table 1, those with the ALDH2*1/*2 genotype that consumed ≥0.05 ml/kg/day had a significantly higher (P < 0.01) HbAA level than those with the ALDH2*1/*1 genotype that consumed ≥0.05 ml/kg/day and those with the ALDH2*1/*2 genotype that consumed 0–0.05 ml/kg/day.

As shown in Fig. 3, the HbAA levels were significantly correlated with the average amount of alcohol consumed during the last 2 days in both the ALDH2*1/*1 and ALDH2*1/*2 genotypes. The slope (3.1) for ALDH2*1/*2 was significantly steeper (P < 0.001) than that (0.5) for ALDH2*1/*1 in the general linear models procedure, assuming that the intercept was constant.

The group of those with the ALDH2*2/*2 genotype that consumed ≥0.05 ml/kg/day consisted of only one person, and the HbAA level was 137.8. Those with the ALDH2*2/*2 genotype that consumed 0–0.05 ml/kg/day included four persons and had a comparable HbAA level (131.4 ± 5.5) compared to the other genotypes with little alcohol consumption.

**Discussion**

The present study revealed for the first time a significant elevation of HbAA levels after drinking certain amounts of ethanol among those with the ALDH2*1/*2 genotype. The HbAA levels increased very rapidly after the challenge, in the same manner as the serial changes of free acetaldehyde (7, 13). However, the free acetaldehyde decreased greatly at 3 h after drinking 0.4 g/kg ethanol (13) and returned to a level close to the baseline level (13) at 4 h. Because HbAA levels at 6 h were still high in the current study, the elevated HbAA levels seem to persist longer than those of the free acetaldehyde. Furthermore, the elevated HbAA levels persisted up to 48 h after drinking, suggesting the possibility of biomonitoring recent alcohol drinking by HbAA level. In the second part of the studies, we actually found a sharp correlation of HbAA levels with the amount of recent alcohol consumption. The relatively longer persistence of the increased HbAA levels suggests a possibility of accumulation of HbAA in those with the ALDH2*1/*2 genotype when they drink habitually. High HbAA levels (>300 nm/g) observed in a few subjects with the ALDH2*1/*2 genotype may be a combined effect of recent drinking and accumulation of HbAA by habitual drinking. Studies are underway to examine the extent of the accumulation of HbAA with repeated alcohol drinking in volunteers. Persistence of HbAA may also be influenced by blood levels of reducing agents such as ascorbate because Schiff base adducts, a most likely formation of HbAA may also be influenced by blood levels of reducing agents such as ascorbate because Schiff base adducts, a most likely effect of recent drinking and accumulation of HbAA by habitual drinking. Studies are underway to examine the extent of the accumulation of HbAA with repeated alcohol drinking in volunteers. Persistence of HbAA may also be influenced by blood levels of reducing agents such as ascorbate because Schiff base adducts, a most likely candidate for HbAA, are easily converted to covalent-binding adducts in the presence of these agents (14).

In the ALDH2*1/*1 genotype, the HbAA levels increased significantly at 1, 24, and 48 h, but the extent of the increase was much smaller than in the ALDH2*1/*2 genotype. The slope (3.1) for ALDH2*1/*2 was significantly steeper (P < 0.001) than that (0.5) for ALDH2*1/*1 in the general linear models procedure, assuming that the intercept was constant.

The group of those with the ALDH2*2/*2 genotype that consumed ≥0.05 ml/kg/day consisted of only one person, and the HbAA level was 137.8. Those with the ALDH2*2/*2 genotype that consumed 0–0.05 ml/kg/day included four persons and had a comparable HbAA level (131.4 ± 5.5) compared to the other genotypes with little alcohol consumption.

**Table 1** Mean HbAA levels in the different ALDH2 genotypes and the different levels of alcohol consumption

<table>
<thead>
<tr>
<th>Average alcohol consumption during the last 2 days</th>
<th>ALDH2*1/*1</th>
<th>ALDH2*1/*2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–0.05 ml/kg/day *</td>
<td>131.8 ± 5.0 (8)</td>
<td>134.9 ± 3.8 (19) NS</td>
</tr>
<tr>
<td>≥0.05 ml/kg/day *</td>
<td>145.6 ± 4.4 (30)</td>
<td>200.1 ± 18.4 (19) P &lt; 0.01</td>
</tr>
</tbody>
</table>

* Calculated as average volume (in milliliters) of pure ethanol consumed per day divided by body weight.

Discussion

The present study revealed for the first time a significant elevation of HbAA levels after drinking certain amounts of ethanol among those with the ALDH2*1/*2 genotype.

The HbAA levels increased very rapidly after the challenge, in the same manner as the serial changes of free acetaldehyde (7, 13). However, the free acetaldehyde decreased greatly at 3 h after drinking 0.4 g/kg ethanol (13) and returned to a level close to the baseline level (13) at 4 h. Because HbAA levels at 6 h were still high in the current study, the elevated HbAA levels seem to persist longer than those of the free acetaldehyde. Furthermore, the elevated HbAA levels persisted up to 48 h after drinking, suggesting the possibility of biomonitoring recent alcohol drinking by HbAA level. In the second part of the studies, we actually found a sharp correlation of HbAA levels with the amount of recent alcohol consumption. The relatively longer persistence of the increased HbAA levels suggests a possibility of accumulation of HbAA in those with the ALDH2*1/*2 genotype when they drink habitually. High HbAA levels (>300 nm/g) observed in a few subjects with the ALDH2*1/*2 genotype may be a combined effect of recent drinking and accumulation of HbAA by habitual drinking. Studies are underway to examine the extent of the accumulation of HbAA with repeated alcohol drinking in volunteers. Persistence of HbAA may also be influenced by blood levels of reducing agents such as ascorbate because Schiff base adducts, a most likely candidate for HbAA, are easily converted to covalent-binding adducts in the presence of these agents (14). Additional studies are required to clarify the effects of such agents on HbAA levels.

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ALDH2*1f@1 genotype. Additional extensive studies are required to confirm whether HbAA levels are correlated with alcohol consumption and the HbAA levels in the occupational population with the ALDH2*1f@1 genotype. Regression lines and equations were generated using the methods of least squares. r, correlation coefficient. HbAA AND THE ALDH2 GENOTYPE

Fig. 3. Correlations of the levels of HbAA with average amounts of alcohol consumption per body weight (ml/kg/day) during the last 2 days in the ALDH2*1f@2 (n = 38) and ALDH2*1f@1 (n = 38) genotypes. Regression lines and equations were generated using the methods of least squares. r, correlation coefficient.

smaller than that in the ALDH2*1f@2 genotype. The small elevation of HbAA is consistent with the previous study (15). Peterson and Polizzi observed significantly elevated HbAA levels among alcoholics but not among social drinkers (12). In the present study, we found a weak but significant correlation between the amount of alcohol consumption and the HbAA levels in the occupational population with the ALDH2*1f@1 genotype. Additional extensive studies are required to confirm whether HbAA levels are correlated with alcohol consumption even in social drinkers with the ALDH2*1f@1 genotype.

Acetaldehyde is volatile and reacts easily with cellular components such as DNA and proteins. Acetaldehyde induces cytotoxicity (9), DNA damage (10), and carcinogenesis in rodents (11). Alcoholics and habitual drinkers with the ALDH2*1f@2 genotype showed a significantly high risk for esophageal cancer, suggesting an important contribution of acetaldehyde in the carcinogenic process (2). Nonetheless, ALDH2 is not expressed in esophageal tissue (16). One possibility is that in this genotype, acetaldehyde not sufficiently inactivated in the liver may overflow from the liver and be efficiently transported to target organs such as the esophagus by reversibly binding to blood proteins, such as in the form of HbAA (17).

We have recently reported that the members of the ALDH2*1f@2 group who drink habitually have a significantly higher frequency of sister chromatid exchange (18) and significantly elevated 8-hydroxydeoxyguanosine levels (19) compared with other groups. It may be that the cell nuclei of habitual drinkers with the ALDH2*1f@2 genotype are continuously attacked by acetaldehyde released from reservoir proteins and are susceptible to DNA damage induced by the acetaldehyde.

Mechanisms for alcohol-related carcinogenesis may be complicated; however, acetaldehyde is likely to be involved in some pathways including DNA damage, depletion of glutathione, and inactivation of O6-methylguanine transferase (20). Utilization of HbAA as a biomarker in epidemiological studies would be of use to clarify the significance of acetaldehyde in alcohol-related carcinogenesis in the human.

In summary, we demonstrated for the first time a significant difference in the increase of HbAA levels after drinking alcohol, depending on the ALDH2 genotype. The elevated HbAA levels were not temporary but lasted up to 48 h after drinking alcohol in the ALDH2*1f@2 genotype. The HbAA levels are not only a good biomarker for increased internal exposure levels to acetaldehyde but may also be a predictive biomarker for acetaldehyde-mediated carcinogenesis.

References

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