ALCOHOL SENSITIVITY IN TAIWANESE MEN WITH DIFFERENT ALCOHOL AND ALDEHYDE DEHYDROGENASE GENOTYPES

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Background and Purpose: Previous studies demonstrated that ADH 2*2, encoding for high-maximal velocity \( V_{\text{max}} \) \( \beta \), alcohol dehydrogenase (ADH) activity, and mutant ALDH 2*2, encoding for null aldehyde dehydrogenase (ALDH) 2 activity, independently influence susceptibility to alcoholism. A single copy of the ALDH 2*2 allele may protect less strongly than a single copy of ADH 2*2 in individuals carrying only one copy of either ALDH 2*2 or ADH 2*2. Individuals with various ADH 2 and ALDH 2 gene status may exhibit different alcohol metabolism and alcohol sensitivity, which affects drinking behavior. To explore the underlying pharmacogenetic mechanism, alcohol metabolism and alcohol sensitivity were tested using ethanol challenge in Taiwanese men with different ADH 2 and ALDH 2 genotypes.

Methods: Twenty-four adults, matched by age, body mass index, nutritional state, and homozygosity at ADH 3, were recruited from a population base of 304 men. Six individuals were chosen with each of the different ADH 2 and ALDH 2 genotypes: ADH 2*1/*1, ALDH 2*1/*1; ADH 2*2/*2, ALDH 2*1/*1; ADH 2*1/*1, ALDH 2*1/*2; and ADH 2*2/*2, ALDH 2*1/*2. After a low-to-moderate challenge with ethanol (0.3 g/kg), blood ethanol and acetaldehyde concentrations, heart rate, and facial capillary blood flow (FCBF) were measured for 130 minutes.

Results: All heterozygous ALDH 2*2 individuals were found to be strongly responsive to low-to-moderate ethanol, as evidenced by pronounced increases in heart rate and FCBF. Conversely, there were no significant differences in alcohol metabolism and alcohol sensitivity between the ADH 2*2 and ADH 2*1 homozygotes with identical ALDH 2 genotype.

Conclusion: Individuals heterozygous for ALDH 2*2 exhibit strong alcohol hypersensitivity caused by persistent accumulation of large amounts of acetaldehyde, but homozygosity for ADH 2*2 is not dependent upon this pathway against alcoholism.

Epidemiologic studies have indicated that heavy drinking is a risk factor for hypertension [1], coronary artery disease [2], and stroke [3]. Alcohol metabolism is a biologic determinant that can significantly affect drinking behavior and alcohol-related organ damage [4–6]. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are the major liver enzymes responsible for ethanol elimination in humans [6–8]. Both enzymes exhibit genetic polymorphism and ethnic variation [9].

Four genes of the ADH family, coding for class I (ADH 1-3; \( K_m < 5 \) mM) and class II (ADH 4; \( K_m = 34 \) mM) enzymes, are significantly involved in the liver metabolism of ethanol [10]. Three allelic variants occur at the ADH 2 locus: ADH 2*1, ADH 2*2 and ADH 2*3. Two variants occur at the ADH 3 locus: ADH 3*1 and ADH 3*2. ADH 2*1 is the predominant allele (~90%) among most world populations, but ADH 2*2 is the predominant allele (~70%) in East Asian populations. ADH 3*1 is the predominant allele among East Asians and...
Africans (~90%), whereas in whites, it is approximately equally distributed with ADH 3*2. Enzyme ADH 2 2-2 exhibits 20-fold greater maximal velocity (V_max) of ethanol oxidation than ADH 2 1-1, and the V_max of ADH 3 1-1 is about twice that of ADH 3 2-2 [6]. These functional polymorphisms are all attributed to single-nucleotide substitutions.

Human ALDH also constitutes a complex family. The major forms responsible for oxidation of acetaldehyde in liver are low-K_m, mitochondrial ALDH2 (0.20 µM) and cytosolic ALDH1 (33 µM) [11]. A functional single-nucleotide polymorphism (SNP) occurs within exon 12 of ALDH2, resulting in a 260-fold increase in K_m for oxidized nicotinamide adenine dinucleotide (NAD+) and an 11-fold reduction in V_max compared with the normal enzymes [12]. About half of several East Asian populations carry this variant ALDH 2*2 allele, which is rarely seen in other ethnic groups. Functional polymorphism for the cytosolic ALDH1 has not been reported in humans.

Studies have shown that the allele frequencies of ADH 2*2 and ALDH 2*2 are significantly decreased in alcohol-dependent individuals as compared to the general population of East Asians, including ethnic Han Chinese [10, 13, 14], Koreans [15] and Japanese [16–23]. Our previous studies on alcoholism and alcohol-metabolizing genes have demonstrated that protection against alcoholism afforded by ADH 2*2 is independent of that afforded by ALDH 2*2 [10].

Alcohol sensitivity, including facial and upper limb/trunk flushing, palpitation, nausea, vomiting, dizziness and other uncomfortable symptoms [24, 25], play protective roles against alcohol-related problems and alcoholism among ethnic Han Chinese [9]. This has been ascribed to the deficiency of mitochondrial ALDH2 activity [26] and high acetaldehyde concentrations in blood [27–29]. In a survey of 1,300 Japanese alcoholics, none were found to be homozygous for ALDH 2*2 [17]. The strong protection afforded by homozygosity of ALDH 2*2 has been attributed to increased alcohol sensitivity ascribable to the prolonged accumulation of large amounts of acetaldehyde in blood [9].

The mechanisms underlying protection against alcoholism afforded by heterozygous ALDH 2*2 and homozygous ADH 2*2 are still unclear. To evaluate the possible protective roles of homozygous ADH 2*2 and heterozygous ALDH 2*2 gene status against heavy drinking, the relationships between alcohol sensitivity and blood ethanol and acetaldehyde concentrations were examined after consumption of a low-to-moderate dose of alcohol (0.3 g/kg) by individuals with different ADH 2 and ALDH 2 genotypes.

### Materials and Methods

#### Subjects

Medical, dental, and pharmacy students (n = 304) underwent genotyping at the ADH 2, ADH 3 and ALDH 2 gene loci. Briefly, DNA was extracted from leukocytes, and alleleotypes of the polymorphic ADH 2, ADH 3 and ALDH 2 loci were determined using polymerase chain reaction (PCR)-directed mutagenesis and restriction-fragment-length polymorphism (RFLP), as described previously [9]. ALDH 2 allele frequencies were confirmed by a recently improved PCR-RFLP method [30].

Twenty-four adult male subjects were recruited for the study from these 304 students. Six subjects with the ADH 2*1/*1, ADH 2*1/*2 genotype (Group 1), six with ADH 2*2/*2, ALDH 2*1/*1 genotype (Group 2), six with ADH 2*2/*2, ALDH 2*2/*2 genotype (Group 3), and six with the ADH 2*2/*2, ALDH 2*1/*2 genotype (Group 4) volunteered for the experiments. All 24 subjects were homozygous for ADH 3*1.

Subjects were requested not to consume alcohol for at least 1 week prior to testing and, on the day of testing, were asked to eat a light lunch before 11:30 AM and to arrive at the testing site at 2 PM, where the room temperature was maintained around 26°C. After measuring body weight and height, fixing the probe holder to the left ear lobe, and setting a retension venous catheter in the right antecubital vein, the subject relaxed on a couch for 30 minutes. Baseline facial capillary blood flow (FCBF) and heart rate were measured and blood was drawn for ethanol and acetaldehyde content. Ethanol solution (10%, v/v) was freshly prepared in natural orange juice, and was orally administered within 10 minutes, with five aliquots at 2-minute intervals (0.3 g/kg). Repeat measurements were conducted at 40, 70 and 130 minutes after the start of ethanol ingestion. Blood was collected from the indwelling catheter into syringe tubes. The experimental procedures were approved by the Institutional Review Board for Human Studies, and informed consent was obtained from each subject after the nature and possible consequences of participation in the study had been explained.

#### Determination of blood ethanol and acetaldehyde

Ethanol concentration was determined using headspace gas chromatography as described elsewhere [9]. Acetaldehyde in blood was determined as a fluorescent adduct, formed by reaction with 1,3-cyclohexanedione and ammonium ion, using high-performance liquid chromatography [9], in a procedure modified from Ung-Chhun and Collins [31].
The area under the curve (AUC) of the blood ethanol and acetaldehyde concentrations was calculated using the trapezoidal method from the start of ethanol administration to 130 minutes thereafter using Excel software (Microsoft Corp., Redmond, WA, USA). In order to calculate the AUC, the curve was divided into one triangle and two trapezoids according to the four time points: 0, 40, 70 and 130 minutes. The AUCs of the concentration-time curve were then summed to give the total AUC.

**Measurement of alcohol response**

Heart rate was recorded by monitoring pulsation of the radial artery for 1 minute. Blood flow in facial capillaries was monitored using noninvasive laser Doppler flowmetry (PeriFlux 4001 Master/4002 Satellite, Perimed AB, Järfälla, Sweden). The basic unit of measurement was the perfusion unit (PU); 1 PU was an arbitrary value equal to an analogue output of 10 mV. To reduce the influence of inter-individual variation, alcohol responses were expressed as percent changes; i.e., difference between the value obtained after alcohol ingestion and that at preingestion divided by the latter value and multiplied by 100.

**Statistical analysis**

Data for the six subjects in each of the four genotypic groups at each time point for blood ethanol and acetaldehyde concentrations (excluding zero value points), for peak concentration, and for AUC, as well as for percent change in heart rate and FCBF, were evaluated using SPSS one-way ANOVA with Scheffe’s test. The homogeneity of variance for the data was checked using SPSS Levene’s test (SPSS Inc., Chicago, IL, USA). The correlation between peak blood ethanol and acetaldehyde concentrations and highest percent change in heart rate and FCBF was evaluated using partial correlation with the SPSS software.

**Results**

All 24 participants had normal findings on cardiovascular examination. There were no significant differences with regard to age, body weight, and body mass index among the four genotype groups (age: 22.0 ± 1.3 vs. 20.3 ± 0.5 vs. 21.7 ± 1.4 vs. 20.7 ± 1.0 yr; body weight: 66.9 ± 9.9 vs. 66.3 ± 5.8 vs. 71.7 ± 9.9 vs. 62.8 ± 5.7 kg; body mass index: 22.7 ± 3.2 vs. 22.6 ± 1.5 vs. 24.9 ± 3.3 vs. 20.9 ± 1.2 kg/m²; all values mean ± standard deviation [SD]). None of the subjects had a family history of alcoholism or drank alcoholic beverages more than occasionally. None of the subjects smoked or used drugs.

**Discussion**

The pharmacologic and toxicologic effects of alcohol are dependent upon the duration of exposure and the concentrations of alcohol and its metabolites attained in body fluids and tissue within that period. The AUC for the blood concentration-time curve can best describe an individual’s exposure to ethanol after a single dose. All heterozygous ALDH 2*2 subjects exhibited a greater increase in AUC for ethanol concentration compared with homozygous ALDH 2*1 subjects (Table; Figure, A), indicating slower alcohol elimination and longer and heavier exposure in heterozygous ALDH 2*2.
Figure. Concentrations of A) blood ethanol and B) acetaldehyde, and percent change in C) heart rate and D) facial capillary blood flow after administration of a low-to-moderate dose of ethanol (0.3 g/kg) to men with different ADH2 and ALDH2 alleles during a 2-hour period. All subjects were homozygous for ADH3*1. ○ = ADH2*1/*1, ALDH2*1/*1 (n = 6); ▲ = ADH2*2/*2, ALDH2*1/*1 (n = 6); ● = ADH2*1/*1, ALDH2*2/*2 (n = 6); △ = ADH2*2/*2, ALDH2*1/*2 (n = 6). Vertical bars (only the upper or lower portion shown) represent standard errors of the means. *p < 0.05 vs. ADH2*1/*1, ALDH2*1/*1; **p < 0.01 vs. ADH2*1/*1, ALDH2*1/*1; ***p < 0.001 vs. ADH2*1/*1, ALDH2*2/*2, ALDH2*1/*1; ****p < 0.001 vs. ADH2*2/*2, ALDH2*1/*1 by ANOVA.

subjects. This is probably caused by a reduction in ADH activity due to product inhibition by acetaldehyde in the liver (Figure, B) [32].

It is well established that blood acetaldehyde rises rapidly in ALDH2-deficient Asians following ingestion of alcoholic beverages [27–29, 33]. This study showed that elevation of acetaldehyde was much greater and much more prolonged in heterozygous ALDH2*2 subjects than in homozygous ALDH2*1 subjects (Table; Figure, B). The AUC of blood acetaldehyde concentration for all homozygous ALDH2*1 subjects was 226-fold less than that in heterozygous ALDH2*2 subjects (Table). This finding indicates that mitochondrial ALDH2 activity in normal homozygous individuals (Figure, B). Heterotetrameric ALDH2 enzymes in heterozygous individuals have residual activity, but they cannot efficiently remove the blood acetaldehyde via the liver because the ALDH2*2 allele encoding the mutant ALDH2 is dominant and the heterotetrameric enzymes have extremely low activity [11, 34]. Cytosolic ALDH1 appears to be responsible for acetaldehyde removal in ALDH2*2 heterozygotes. These explanations are primarily based on the enzymatic properties of human ALDH1 and ALDH2, which differ widely in $K_m$ (33 vs. 0.20 $\mu$M) as well as in catalytic efficiency ($V_{max}/K_{cat}$, 1.05 vs. 129 $\mu$M $^{-1}$min$^{-1}$) for oxidation of acetaldehyde [11].

Cardiovascular responses and facial flush are the most conspicuous physical symptoms presenting in Asians showing alcohol sensitivity [24, 25, 35]. In this study, with
Table. Pharmacokinetic parameters of blood ethanol and acetaldehyde, and peak percent change in heart rate and facial capillary blood flow in men with different ADH2 and ALDH2 allelotypes after alcohol intake (0.3 g/kg)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Peak ethanol (mM)</th>
<th>AU C for ethanol (mM x hr)</th>
<th>Peak acetaldehyde (µM)</th>
<th>AU C for acetaldehyde (µM x hr)</th>
<th>Heart rate (% change)</th>
<th>Facial capillary blood flow (% change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADH 2*1/<em>1, ALDH 2</em>1/*1</td>
<td>7.08 ± 0.82</td>
<td>8.71 ± 0.81</td>
<td>0.33 ± 0.13</td>
<td>0.28 ± 0.44</td>
<td>11.5 ± 1.7</td>
<td>60.5 ± 39.1</td>
</tr>
<tr>
<td>ADH 2*1/<em>2, ALDH 2</em>1/*1</td>
<td>7.12 ± 0.61</td>
<td>8.83 ± 0.64</td>
<td>0.17 ± 0.17</td>
<td>0.25 ± 0.25</td>
<td>7.7 ± 2.3</td>
<td>68.6 ± 34.3</td>
</tr>
<tr>
<td>ADH 2*1/<em>1, ALDH 2</em>1/*2</td>
<td>8.82 ± 0.81</td>
<td>11.42 ± 1.03</td>
<td>59.84 ± 10.76</td>
<td>57.36 ± 12.70</td>
<td>53.9 ± 7.3</td>
<td>809.7 ± 218.3a</td>
</tr>
<tr>
<td>ADH 2*2/<em>2, ALDH 2</em>1/*1</td>
<td>8.22 ± 0.51</td>
<td>10.12 ± 0.66</td>
<td>59.72 ± 14.31</td>
<td>59.53 ± 14.11</td>
<td>66.1 ± 4.6</td>
<td>495.2 ± 175.7</td>
</tr>
</tbody>
</table>

All subjects were ADH 3*1/*1. Mean ± standard error; n = 6 for each genotypic group. *p < 0.05 vs. ADH 2*1/*1, ALDH 2*1/*1; **p < 0.01 vs. ADH 2*1/*1, ALDH 2*1/*1; †p < 0.001 vs. ADH 2*1/*1, ALDH 2*1/*1; ‡p < 0.05 vs. ADH 2*2/*2, ALDH 2*1/*1; *p < 0.01 vs. ADH 2*2/*2, ALDH 2*1/*1; †p < 0.001 vs. ADH 2*2/*2, ALDH 2*1/*1 by one-way ANOVA.

Low-to-moderate dose ethanol challenge (0.3 g/kg), homozygous ADH 2*1 subjects showed no noticeable effect on heart rate and FCBF (Fig. C and D). Heterozygous ADH 2*2 subjects exhibited a significant increase in heart rate and FCBF, corresponding to the time course of blood acetaldehyde concentrations (Fig. B). At least 400% increases in the flow rate in facial capillaries were found during peak blood acetaldehyde concentration in ALDH 2*2 heterozygotes (Fig. D). Interestingly, only Group 3 subjects showed significantly higher FCBF than ALDH 2*1 homozygotes. On the other hand, all ALDH 2*2 heterozygotes exhibited significantly higher heart rate than ALDH 2*1 homozygotes, even up to 130 minutes after ingestion. Heart rate increase may be a better indicator of alcohol reaction than FCBF. The response of FCBF to alcohol is stronger than that of heart rate, but its sensitivity is still limited due to: the larger basal individual variation (9.4 to 143.0 PU at baseline); unequal distribution of capillary vessels; various numbers of capillary vessels monitored; different capacity for full dilatation in capillaries, and sensitivity too high to respond to external stimulation. In this study, all heterozygous ADH 2*2 subjects exhibited persistently high blood acetaldehyde concentrations and increased heart rate, similar to the responses of homozygous ALDH 2*2 subjects after ingestion of a small amount of ethanol (0.2 g/kg) in our previous study [9]. Peak blood acetaldehyde concentration and increase in heart rate in Group 4 subjects and ALDH 2*2 homozygotes (our previous study) showed comparable results (59.7 ± 14.3 vs. 75.4 ± 10.6 μM and 66.1 ± 4.6 vs. 54.2 ± 9.9% (mean ± SE), respectively (Figure, B and C) [9].

Human tissue ADH activities show large variations with respect to isozyme patterns and genetic polymorphism. ADH activities of subjects with the homozygous ADH 2-2 phenotype are about 2-fold and 20-fold higher than those with the heterozygous ADH 2-1 and homozygous ADH 2 1-1 phenotype, respectively [6]. This previous finding suggests that ADH 2*2 homozygotes with high enzyme activity may oxidize ethanol to acetaldehyde more efficiently than ADH 2*1 homozygotes. However, the results of this study showed no significant difference in alcohol metabolism and alcohol sensitivity between ADH 2*2 and ADH 2*1 homozygotes with identical ALDH 2 genotype.

In conclusion, heterozygous ADH 2*2 individuals present strong alcohol sensitivity after low-to-moderate alcohol ingestion that can be ascribed to prolonged accumulation of large amounts of acetaldehyde in the blood. This explains the partial protection against alcoholism afforded by the molecular mechanisms underlying the protection against alcoholism afforded by the ADH 2*2 variant allele.

ACKNOWLEDGMENTS: This work was supported by grants from the National Defense Medical Center (DOD-90-59, DOD-91-77) and the Armed Forces Tao-Yuan General Hospital (No. 049008).

References