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Genetic and Environmental Influences on Alcohol Metabolism in Humans

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This manuscript represents the proceedings of a symposium at the 2000 RSA Meeting in Denver, Colorado. The organizer/chair was Ting-Kai Li. The presentations were: (1) Introduction to the Symposium, by Ting-Kai Li; (2) ALDH2 polymorphism and alcohol metabolism, by Shih-Jiun Yin; (3) ALDH2 promoter polymorphism and alcohol metabolism, by David W. Crabb; (4) Use of BrAC clamping to estimate alcohol elimination rates: Application to studies of the influence of genetic and environmental determinants, by Sean O'Connor; and (5) Effect of food and food composition on alcohol elimination rates as determined by clamping, by Vijay A. Ramchandani.

Key Words: ADH/ALDH Polymorphisms, Alcohol Clamping, Food Effects on Elimination.

INTRODUCTION

Ting-Kai Li

The effects of ethanol on bodily functions, e.g., those of the brain, heart, and liver, are dependent upon the systemic concentrations of ethanol over time. Therefore, the pharmacokinetics of ethanol play a pivotal role in the pharmacodynamic actions of ethanol and of its metabolic product acetaldehyde (Eckardt et al., 1998).

After oral ingestion, ethanol pharmacokinetics must take into account: (1) Absorption from the gastrointestinal tract. Because ethanol is absorbed most efficiently from the small intestine, the rate of gastric emptying becomes an important factor that governs the rate of rise of blood alcohol concentration (BAC), i.e., the slope of the ascending limb of the BAC-time curve, and the extent of first pass metabolism of ethanol by the liver and stomach. (2) The distribution of ethanol in the body. Ethanol distributes equally in total body water, which is related to the lean body mass of the person. (3) The elimination of ethanol from the body which occurs primarily by metabolism in the liver, first to acetaldehyde and then to acetate (Bosron et al., 1993).

All three processes are influenced both by genetic and environmental factors. For example, gastric emptying and ethanol absorption vary with the concentration of ethanol in the beverage, the rate of ingestion of ethanol, and the presence of food in the stomach or its concomitant ingestion. The peak BAC and the time to reach peak BAC have been shown to be influenced by genetic factors. Total body water is related to height and body weight, both of which are influenced by genetic as well as environmental factors. Alcohol elimination rate varies as much as 3-fold from person to person. Studies in monozygotic and dizygotic twins have shown that the heritability of alcohol elimination rate (i.e., genetic component of variance) is about 40–50% (Kopun and Propping, 1977). Ethanol metabolic rate is influenced by the genetic variations in the principal alcohol metabolizing enzymes, cytosolic alcohol dehydrogenase (ADH), and mitochondrial aldehyde dehydrogenase (ALDH2). Both ADH and ALDH use NAD^+ as a cofactor in the oxidation of ethanol and acetaldehyde. The rate of metabolism as well as the NAD^+/NADH ratio in liver varies with the fed or fasted state (Bosron et al., 1993).

Genetic Polymorphisms of ADH and ALDH2

In humans, there are multiple molecular forms of both ADH and ALDH, each encoded by a family of genes whose members are homologous in sequence to different degrees. As many as 7 ADH genes and as many as 12 ALDH genes have been identified in humans (Edenberg and Bosron, 1997; Yoshida et al., 1998). However, the ADH isoforms that are important for ethanol metabolism are the Class I, Class II, and Class IV isozymes, and the ALDH isozymes important for acetaldehyde metabolism are the mitochon-

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drial ALDH2 and one of the cytosolic aldehyde dehydrogenases, ALDH1.

Enzymatically active ADH is composed of two protein subunits. All Class I isozymes are found in liver, and consist of homo- and heterodimeric forms of the three α, β, and γ subunits (i.e., $\alpha\alpha$, $\alpha\beta$, $\beta\beta$, $\beta\gamma$, etc.). The corresponding genes are *ADH1*, *ADH2*, and *ADH3*. The Class II ADH is a single homodimeric enzyme $\pi\pi$ found in liver, and the class IV enzyme is a single homodimeric enzyme $\sigma\sigma$, found primarily in stomach. There are functionally different forms of the β and γ subunits encoded by variant alleles of the *ADH2* gene and the *ADH3* gene. Thus the *ADH2*1*, *ADH2*2*, and *ADH2*3* alleles give rise to β_1, β_2, and β_3 subunits, respectively, and the *ADH3*1* and *ADH3*2* alleles give rise to the γ_1 and γ_2 subunits, respectively. The relative order of catalytic efficiency (k_{cat}/K_m) for ethanol oxidation at ethanol concentrations of about 100 mg% and saturating coenzyme NAD^+ concentration (0.5 mM) is: $\beta_2 > \beta_1 > \gamma_1 > \gamma_2 \simeq \sigma \gg \beta_3 > \alpha \gg \pi$. However, the relative order of k_{cat} at saturating concentrations of both ethanol and NAD^+ is $\sigma > \beta_3 \simeq \beta_2 > \gamma_1 > \gamma_2 \approx \pi > \beta_1$. Thus, as ethanol concentrations in blood/liver change, so do the relative contributions of each of the ADH isozymes to ethanol oxidation change (Bosron et al., 1993).

There are differences in frequency of the *ADH2* and *ADH3* alleles in different ethnic/racial populations. Whereas *ADH2*1* predominates in white and black populations, *ADH2*2* predominates in East Asian populations (e.g., Chinese and Japanese). The *ADH2*2* allele is also found in about 25% of white subjects with Jewish ancestry, and *ADH2*3* is found in about 25% of black subjects. With respect to the *ADH3* polymorphism, *ADH3*1* and *ADH3*2* appear with about equal frequency in white populations, but *ADH3*1* predominates in black and East Asian populations (Li, 2000).

ALDH is a tetrameric enzyme. As with ADH, there are multiple molecular forms of ALDH in human liver. The mitochondrial ALDH2, which has the lowest K_m for acetaldehyde (3 μM), and one of the cytosolic ALDH1 forms with intermediate affinity for acetaldehyde ($K_m \simeq 30$ μM), are the major forms responsible for acetaldehyde oxidation. There is one known, functionally significant genetic polymorphism of the *ALDH2* gene. The allelic variants are *ALDH2*1* and *ALDH2*2*, encoding for the high activity and low activity forms of the subunits. The low activity form arises from a single amino acid exchange at the coenzyme binding site of the enzyme subunit causing a 100-fold increase in the K_m for NAD^+ (Steinmetz et al., 1997). The variant allele has been seen only in certain East Asian populations (e.g., Han Chinese, Japanese). It has not been observed in populations of White origin.

Control of Alcohol Metabolic Rate

The rate of alcohol metabolism is determined not only by the amount of ADH and ALDH2 enzyme in tissue and by their functional characteristics (vide supra), but also by the concentrations of the cofactors, NAD^+ and NADH, and of ethanol and acetaldehyde in the cellular compartments (i.e., cytosol and mitochondria of hepatocytes). The equilibrium of the ADH reaction is poised toward the formation of ethanol and NAD^+ from acetaldehyde and NADH, whereas that of the ALDH2 reaction is very strongly directed toward the oxidation of acetaldehyde to acetate. Accordingly, acetaldehyde exerts strong product inhibition on the ADH reaction and the elimination of acetaldehyde is the most critical factor for ethanol to be oxidized at an efficient rate. In agreement with this scheme of regulation of ethanol metabolism, the usual concentrations of ethanol and acetate in the circulation during ethanol oxidation are millimolar, whereas that of acetaldehyde is less than 10 micromolar. Furthermore, the total activity of ALDH2 (amount of enzyme and its functional properties) becomes a key determinant of the rate of ethanol metabolism (Bosron et al., 1993). Environmental influences on elimination rate can occur through changes in the redox ratio of NAD^+/NADH and through changes in hepatic blood flow.

Alcohol Metabolism, Alcohol Drinking Behavior, and Alcohol Dependence

To date, ADH and ALDH are the only genes which have been firmly established to influence vulnerability to alcohol dependence or alcoholism (Li, 2000). There is consensus in the literature that the allele frequencies of *ADH2*2*, *ADH3*1*, and *ALDH2*2* are significantly decreased in subjects diagnosed with alcohol dependence (as defined in DSM III R) compared with the general population of East Asians, including the Japanese, Han Chinese, and Koreans. The *ALDH2*2* allele and the *ADH2*2* allele also significantly influence drinking behavior in nonalcoholic individuals. Association between reduced alcohol consumption or reduced risk of alcohol dependence and the *ADH2*2* variant allele has recently been found in other ethnic groups that did not carry the *ALDH2*2* allele, including Europeans (Borràs et al., 2000; Whitfield et al., 1998), Jews in Israel (Neumark et al., 1998), Mongolians in China (Shen et al., 1997), and the Atayal natives of Taiwan (Thomasson et al., 1994). This is consistent with the finding that *ADH2* affects vulnerability to alcohol dependence independent of *ALDH2* (vide infra).

This symposium presented advances in certain aspects of our knowledge and understanding of the genetic and environmental influences on alcohol metabolism in humans. New findings on the relationship of the genetic polymorphisms of *ALDH2* (Shih-Jiun Yin and David Crabb) and on the use of the alcohol clamp to study alcohol elimination rates (Sean O'Connor and Vijay Ramchandani) were discussed.

ALDH2 POLYMORPHISM AND ALCOHOL METABOLISM

Shih-Jiun Yin

The genetic epidemiologic evidence summarized above seemed to support the hypothesis proposed by Thomasson et al. (1991) that *ADH2*2* and *ADH3*1*, encoding the high-activity β_2 and γ_1 subunits of ADH, respectively, as well as *ALDH2*2*, encoding the low activity subunit form of ALDH2, protect individuals from alcohol dependence. This could occur through either the faster production or the slower removal of acetaldehyde, which has been shown to trigger aversion reactions. This hypothesis also implied that these three variant alcohol-metabolism gene alleles may act synergistically to produce higher systemic concentrations of acetaldehyde and, hence, produce more protection in Asians. However, two recent studies have refined and modified this hypothesis.

First, the allelic variation at *ADH3* appears to be in linkage disequilibrium with that at *ADH2*, and the polymorphic *ADH3* gene itself does not significantly affect susceptibility to alcohol dependence (Chen et al., 1999a; Osier et al., 1999). Second, the protection against alcohol dependence afforded by the *ADH2*2* allele appears to be independent of that afforded by *ALDH2*2* in the Asians. Multiple logistic regression analysis has demonstrated that there is no significant interaction between the functional polymorphisms of *ADH2* and *ALDH2* in risk for alcohol dependence (Chen et al., 1999a). Independent effects of the polymorphic *ADH2* and *ALDH2* genes on risk for alcohol dependence would imply that the molecular protection mechanisms of *ADH2* may not be mediated through blood acetaldehyde accumulation after alcohol ingestion, as has been firmly established with *ALDH2*. Indeed, in ethanol challenge experiments with a dose of 0.4 g/kg ethanol, allelic variations at *ADH2* did not cause significant elevation of blood acetaldehyde levels, which were actually near zero, in homozygous *ALDH2*1/*1* Japanese subjects (Mizoi et al., 1994). This has been confirmed in our laboratory in Han Chinese at a dose of 0.5 g/kg ethanol (Wang and Yin, unpublished data, 2000). The rates of elimination from blood at saturating ethanol concentrations for Class I ADHs also did not show a significant difference among the three *ADH2* genotypes (Mizoi et al., 1994). Further studies are required to clarify whether the alcohol flush reaction occurs with *ADH2* polymorphism in individuals who are homozygous for the *ALDH2*1* allele under a setting similar to social drinking, e.g., administration of multiple doses during a long session of alcohol consumption that is thought to be associated with the slow flush reaction in East Asians. Similar studies should also be performed in whites who have the *ADH2*2* variant allele and who drink less than those who do not have this allele. Such individuals do not appear to have the flush reaction, yet they report having dysphoria with drinking. The mechanism underlying this *ADH2*2*-related reaction is a topic of ongoing interest.

ALCOHOL METABOLISM, CARDIOVASCULAR, AND SUBJECTIVE RESPONSES IN SUBJECTS WITH DIFFERENT *ALDH2* GENOTYPES

Shih-Jiun Yin

Higuchi et al. (1994) have reported that, in a survey of 1300 Japanese alcoholics, none were found to be homozygous for *ALDH2*2*. The strong protection afforded by the *ALDH2*2* allele is attributed to acetaldehyde accumulation, and the physiologic reactions it invokes, including the alcohol-flush reaction. Recently, Peng et al. (1999) correlated blood ethanol and acetaldehyde concentrations, cardiovascular hemodynamic responses, and subjective reactions after challenge with a low dose of ethanol (0.2 g/kg) in healthy men with *ALDH2*2/*2*, *ALDH2*1/*2*, and *ALDH2*1/*1* genotypes. All subjects were homozygous for *ADH2*2* and *ADH3*1*, and matched for age, body weight, and body mass index. The peak ethanol concentration and the area under the concentration-time curve (AUC) during 130 min postingestion were 2–3-fold higher in the *ALDH2*2* homozygotes than in the *ALDH2*1* homozygotes, which indicates a slower rate of ethanol elimination. The AUC for acetaldehyde in the *ALDH2*2* homozygotes was 220- and 5-fold greater than that of the *ALDH2*1* homozygotes and the heterozygotes, respectively. Interestingly, at 130 min after ingestion of such a low dose of ethanol, blood acetaldehyde concentration (17 μM) in the *ALDH2*2* homozygotes was still similar to the peak concentration (24 μM) in the heterozygotes. These findings indicate that, whereas the mitochondrial ALDH2 activity in *ALDH2*1* homozygotes is sufficient to oxidize virtually all acetaldehyde derived from ethanol via cytosolic ADH in liver, there is residual mitochondrial ALDH2 enzyme activity in the heterotetrameric heterozygote individuals as was predicted by Crabb et al. (1989). Cytosolic ALDH1 must be the enzyme responsible for acetaldehyde removal in the *ALDH2*2* homozygotes, because there is virtually undetectable activity of ALDH2 enzyme in these subjects (Farrés et al., 1994).

The homozygous *ALDH2*2* subjects showed the greatest cardiovascular hemodynamic effects due to the high blood acetaldehyde levels (Peng et al., 1999). Increases in blood flow rate of the facial artery (4-fold) and of the common and internal carotid arteries (2-fold) were found at peak times of blood acetaldehyde in the *ALDH2*2* homozygotes. Moreover, they persistently showed a decrease (20–50%) in systemic vascular resistance for at least 2 hr postingestion. The mechanisms underlying the acetaldehyde-induced cardiovascular alterations remain obscure.

Elevation of blood acetaldehyde also caused dysphoric symptoms. Reports of "feeling terrible" together with palpitations and facial warming were intensely perceived by the *ALDH2*2* homozygotes even 2 hr after drinking. Therefore, the results of direct correlation of physiologic and subjective responses after alcohol challenge in the subjects with different *ALDH2* allelotypes support the no-

tion that elevated blood acetaldehyde levels, after even a small amount of alcohol, cause the homozygous *ALDH2*2* individuals persistent discomfort. Nearly full protection against alcohol dependence in these subjects may derive from either abstinence or deliberate moderation in alcohol consumption due to prior experience with an unpleasant reaction. The neurochemical and physiologic mechanisms by which elevated acetaldehyde discourages drinking remains unclear.

The proportion of alcoholics heterozygous for *ALDH2*2* has significantly increased during the past 10–20 years in Japan, from 2.5% in 1979 to 13.0% in 1992 (Higuchi et al., 1994). An increasing trend has also been observed in Han Chinese alcoholics living in Taiwan where the proportion has gone from 12% in 1989–1990 (Thomasson et al., 1991) to 26% in 1998–1999 (Chen et al., 1999b). These results would imply that although *ALDH2*2* homozygosity may protect against the development of alcohol dependence in a nearly complete fashion, the protection by heterozygosity of the gene is incomplete. This incompleteness of protection by a single copy of the *ALDH2*2* allele suggests that sociocultural factors contribute substantially to development of the disease in such individuals, offering a unique opportunity to study gene-environment interactions.

Physiologic Features and Drinking Pattern of an Alcoholic Patient Homozygous for ALDH2*2

A recent study reported identifying an alcoholic patient who was *ALDH2*2/*2*, *ADH2*2/*2*, *ADH3*1/*2* (Chen et al., 1999b). He exhibited five positive DSM III-R criteria for alcohol dependence and had comorbid diagnoses of anxiety and major depression. He did not have a family history of alcohol dependence. This unexpected finding has revised the long-standing thought that individuals homozygous for *ALDH2*2* would be completely protected from becoming alcoholic. The study estimated that individuals carrying the combinatorial genotype of *ADH2*2/*2-ALDH2*2/*2* are at 100-fold lower risk for alcohol dependence than the *ADH2*1/*1-ALDH2*1/*1* individuals, the latter being the predominant combinatorial genotype in white populations. A protective effect of the *ADH2*2* allele was seen also in subjects who were homozygous for *ALDH2*1*, and heterozygous for *ALDH2*1* or *ALDH2*2*.

How did this alcoholic patient overcome the protection provided by inheriting two copies of the *ALDH2*2* variant allele? Correlation of alcohol pharmacokinetics and cardiovascular hemodynamics of the patient after challenge with a moderate dose of 0.5 g/kg ethanol (Chen et al., 1999b) suggest that developed tolerance or innate insensitivity to the pharmacodynamic effects of acetaldehyde rather than altered alcohol metabolism per se may have been the reason. This may be applicable also to the development of alcohol dependence in persons who are heterozygous for *ALDH2*2* (Chen and Yin, unpublished data, 2000).

Notably, to accommodate the inborn impairment of acetaldehyde metabolism, this patient had adopted a drinking pattern characterized by slow and prolonged consumption of alcohol, but low in total overall quantity (Chen et al., 1999b). The unique drinking pattern of this patient consisted of (a) beer as a favorite beverage type; (b) sipping the beverage almost continuously throughout the day rather than fast, binge drinking; and (c) consuming a relatively low amount of alcohol with 3–5 bottles (i.e., 350 ml of 4.5% by volume of ethanol or 12.4 g ethanol per bottle) of beer per day. Even though this patient satisfied DSM-III-R criteria for alcohol dependence, he never became heavily intoxicated. It is worth noting that significant "great feeling" and a less intense "terrible feeling" subjective responses have been observed in Asian-American social drinkers who are heterozygous for *ALDH2*1/*2* compared with the homozygous *ALDH2*1/*1* individuals 30 to 120 min after ingestion of 0.59 g/kg of ethanol (Wall et al., 1992).

Summary

The functional polymorphisms at the alcohol-metabolism gene loci, *ADH2*, *ADH3*, and *ALDH2*, exhibit a complex pattern of influences on susceptibility to alcoholism in East Asians. The *ADH3* polymorphism, which is in linkage disequilibrium with *ADH2*, contributes negligibly to the vulnerability to alcohol dependence. The *ALDH2*2* homozygosity can tremendously, but not completely, as thought previously, protect against alcohol dependence. Alcohol elimination is significantly slower in the *ALDH2*2* homozygotes due to product inhibition of the ADH activity by acetaldehyde. The current evidence suggests that physiologic tolerance or innate hyposensitivity to the accumulation of blood acetaldehyde after alcohol ingestion may be crucial for the development of alcohol dependence in individuals carrying the *ALDH2*2* variant allele. The protection against alcohol dependence afforded by the *ADH2*2* allele seems to be independent of that afforded by *ALDH2*2*, a finding that requires further studies to define the underlying molecular mechanisms.

MITOCHONDRIAL ALDEHYDE DEHYDROGENASE GENE PROMOTER POLYMORPHISM AND ALCOHOL METABOLISM

David W. Crabb

The gene for mitochondrial aldehyde dehydrogenase (*ALDH2*) is expressed in many tissues as shown by Northern blot analysis, but by far the highest level of expression is in the liver (Stewart et al., 1996a). Studies to date have revealed that the *ALDH2* promoter contains a binding site for the ubiquitous transcription factor NF-Y. This factor binds to a CCAAT box at about 370 bp from the translation start site, and probably serves to drive basal activity of the promoter (Stewart et al., 1996b). Promoter constructs containing this site retain transcriptional activity. This site may play a role in the expression of the gene at relatively low levels in most tissues.

Upstream from this site is a complex transcription factor binding site best characterized as a nuclear receptor response element (NRRE). The nuclear receptor family is a very large group of transcription factors that are localized to the cytosol or nucleus (Giguere, 1999). They share common motifs in the DNA binding domains (including zinc finger motifs). Many are the receptors for lipid soluble hormones, such as the classical steroid hormones, thyroxine, vitamin D, and vitamin A (retinoic acid). Other members of the family have regions homologous to the ligand binding domains of the former group, but have no ligand yet identified. It is not clear if a ligand is required in many cases, because other pathways, such as phosphorylation-dephosphorylation of the receptor, may mediate the transcriptional activating capability of the receptor.

We have demonstrated that the NRRE in the *ALDH2* promoter can be divided into a 5′ and a 3′ segment. The 3′ segment is bound and activated by hepatocyte nuclear factor 4 (HNF-4) and retinoid X receptor (RXR). It is similar to other elements that can bind multiple members of the steroid receptor family (Nakshatri and Bhat-Nakshatri, 1998). HNF-4 may be the dominant factor that drives high levels of expression of ALDH2 in liver and kidney (Stewart et al., 1998). The role of RXR is not clear. In the presence of cotransfected RXR, activation of the promoter (as studied by transient transfection assays using the NRRE linked to a heterologous promoter) requires the presence of 9-cis retinoic acid. Dietary vitamin A deficiency results in a modest reduction in hepatic ALDH2 protein levels, which suggests a subsidiary role for RXR in the liver expression of ALDH2, but there may be a greater role in tissues not expressing HNF-4 (Pinaire et al., 2000). The activation of the *ALDH2* promoter constructs by HNF-4 and RXR is repressed by members of the chicken ovalbumin upstream promoter-transcription factor (COUP-TF) family (Tsai and Tsai, 1997), namely, COUP-TFI, ARP-1, and Ear2 (Pinaire et al., 2000). The 5′ segment is also bound by members of the COUP-TF family, but we have been unable to show that cotransfection of the COUP-TF factors suppresses transcription from a reporter containing this site (Chou et al., 1999). The majority of reports of COUP-TF function have indicated that it is a repressor of transcription; however, there are other reports to suggest an activating role that may be modulated by phosphorylation of the factor. Additional work is needed to define the roles of these factors in influencing ALDH2 expression.

Immediately upstream from this 5′ segment is a polymorphism (A or G) that was detected by polymerase chain reaction (PCR) sequencing of samples of DNA from several ethnic groups (Chou et al., 1999). Unlike the well-known polymorphisms in alcohol dehydrogenase (ADH) or that causing ALDH2 deficiency in Asians, this polymorphism is common in Asians, African-Americans, and whites. Subsequent studies have established the allele frequencies in a number of other geographically separated populations (Peterson et al., 1999). The frequency of the A allele was 34% in African-Americans, 17% in Asians, and 44% in whites. The activity of this polymorphism was tested by generating reporter plasmids containing a copy of the 5′ NRRE with either an A or a G allele at the polymorphic site upstream of a heterologous promoter. The A allele was less active when transfected into hepatoma cells than the G allele. There was no difference in the transcriptional activity in a nonhepatoma cell line. At present, it is not known how the polymorphism affects *ALDH2* promoter activity. It does not affect the affinity of COUP-TFI or ARP-1 binding to oligonucleotides containing the polymorphism, and there is no obvious difference in binding of liver nuclear factors to the oligonucleotides in electrophoretic mobility shift assays.

This polymorphism was simultaneously reported by Harada et al. (1999). His group noted that homozygotes for the A allele were less common (1.6%) among alcoholics in Japan than among controls (6.8%) when males homozygous for the *ALDH2*1* allele were studied. If the A allele is transcriptionally less active in the liver, as is suggested by the transfection data, individuals with one or two A alleles might have lower ALDH2 mRNA levels, protein levels and, therefore, lower ALDH activity than homozygotes with two G alleles. If this is the case, they may experience aversive reactions to alcohol similar to, but probably less intense than, those with the Asian flush reaction due to the *ALDH2*2* allele. Thus, this polymorphism may modulate the risk of alcohol dependence and possibly the frequency of flushing reactions in both Asian and non-Asian subjects.

USE OF BrAC CLAMPING TO ESTIMATE ALCOHOL ELIMINATION RATES: APPLICATION TO STUDIES OF THE INFLUENCE OF GENETIC AND ENVIRONMENTAL DETERMINANTS

Sean O'Connor

Human alcohol challenge studies usually seek to measure kinetic (absorption, distribution, elimination, metabolic) or dynamic (sensitivity, acute tolerance of dependent measures of brain function) properties of alcohol. In either case, variable gastric emptying after oral ethanol challenge yields large interindividual variation in the time course of breath alcohol concentration (BrAC). BrAC is close to the arterial concentration of ethanol and can differ substantially from the venous concentration (BAC) on the ascending limb. In our laboratory, we have also observed that the slope of the ascending limb and peak BrAC are as variable within individuals as among subjects given equal doses of alcohol by mouth per kg of body weight (adjusted for gender) on different days (Christian et al., 1996).

Intravenous administration of ethanol provides the most promising route to overcome the variability in alcohol kinetics in humans, because of easy sterile access, reliable indwelling catheters and pumps, and complete avoidance of absorption kinetics. With use of 6% alcohol in Ringer's lactate solution, our laboratory has been able to produce

nearly identical time courses of BrAC, both within and among subjects (O'Connor et al., 1998; Ramchandani et al., 1999a). If initial infusion rates can exceed 1000 ml/hr, we have demonstrated a reliable linear ascension of BrAC from 0 to 60 mg% at 10 ± 1 min, followed by maintenance of the BrAC within ± 5 mg% of the target concentration for 2.8 hr (O'Connor et al., 2000b).

The infusion rate profiles required to achieve consistent exposure of the brain to alcohol vary substantially across subjects because the distribution and elimination of alcohol depends on highly variable physiology, e.g., hemodynamics, vascular and peripheral water volumes, and the V_{max} of alcohol metabolism. Some a priori method of computing the infusion rate profile for an individual is required, and we have used a 3-compartment physiologically based pharmacokinetic (PBPK) mass-flow model of alcohol distribution and elimination.[1] The rapidly-perfused "vasculature," volume Vb, sums the mg/min of ethanol flowing from three sources: out of the liver, into an antecubital vein from the infusion pump, and from the slowly perfused "periphery," volume Vp. The vascular compartment mixes these sources using first order kinetics, and the sum is divided between the liver and the periphery. The liver compartment subtracts the alcohol elimination rate determined by the Michaelis-Menten equation from the ethanol flowing through it. The periphery compartment is modeled as a vascular pipe running through a pool of water. A concentration gradient, between arterial and tissue concentration of ethanol, that is greater than zero drives ethanol into the tissues, and a positive gradient between the tissue and venous blood alcohol concentrations drives ethanol in the reverse direction. An individual's kinetics can be simulated by specifying his/her cardiac output and the parameter set {Vp, Vb, and V_{max}} each approximated from an individual's height, age, weight, and gender (Ramchandani et al., 1999a).

Calculation of the infusion rate profile required to achieve the desired time course of BrAC for an individual is performed by forcing the difference between the modeled concentration and the desired time course to be negligibly small as the solution is computed using Simulink® (MATLAB®, MathWorks, Inc., Natick, MA). When the computed infusion rate profile is used in the laboratory, only minor adjustments are needed to achieve the desired result in the individual that was modeled. Real-time measurements of BrAC, with consequent adjustments of the infusion profile by the technician, can accommodate practical deviations from the desired time course (e.g., from modeling errors, inability to know V_{max} ahead of time, or from the need to interrupt the infusion so the subject can void his/her bladder from time to time). Compared with oral dosing methods, substantial reductions in the variability of BrAC within and among subjects are achieved with the BrAC clamp (O'Connor et al., 1998).

We have published results derived from the last 45–60 min of the BrAC clamp, when both the BrAC and the infusion rate were steady: ethanol mass flow is in equilibrium throughout the body, and the rate of alcohol being infused is the rate of alcohol being eliminated. By multiplying the infusion pump rate during this steady-state interval by the measured concentration of ethanol in the infusate, a direct assessment of the subject's alcohol elimination rate (AER, g/hr) can be obtained under the conditions of the experiment. The technique has a 6.7% coefficient of variation and does not depend on any knowledge of the subject's actual total body water (O'Connnor et al., 1998). With measurements of the AER derived from clamping experiments, one can begin to ask questions of kinetic and genetic interest. For example, how does the AER vary with gender, race, age, and *ADH* or *ALDH2* allelic status? Does eating, physical activity, or recent drinking history change the AER?

Preliminary data from our recent clamping experiments have been presented as examples of the application of BrAC clamping to studies of differences in the alcohol elimination rate by gender (Kwo et al., 1998; O'Connor et al., 2000a; Ramchandani et al., 2000). In an on-going study, a sample of 118 men and women, all social drinkers aged 21–38, including equal numbers of subjects with and without a family history of alcohol dependence, were clamped at 60 mg% so that dependent measures of brain function could be examined for the acute response to alcohol. A subset of 112 of these reached a steady-state sufficient for the calculation of the AER. After the infusion of alcohol was terminated, the BrAC was measured at least six times on the descending limb, to obtain an estimate of the descending limb slope (DLS: mg%/hr). These data confirmed several reports (Jones and Andersson, 1996; Li et al., 2000; Taylor et al., 1996; Thomasson, 1995), based on observations after oral challenges, that American women have a slightly greater DLS than men (mean ± SEM mg%/hr: females 18.6 ± 0.4; men 16.0 ± 0.4; $p < 0.001$). Using the BrAC clamp, however, the study also demonstrated that women have a significantly lower AER (7.1 ± 0.2 g/hr) than men (9.4 ± 0.3 g/hr) at 60 mg% ($p < 10^{-5}$). Men are larger and proportionally more muscular than women, and hence total body water would be larger.

Moreover, the solubility of alcohol is much greater in water than in lipids. If an estimate of the total body water (TBW, liters) is assumed to be the volume of distribution of alcohol, then multiplying the DLS by TBW should yield a close approximation of the AER. In our study, this procedure did not perform well: the product, DLS*TBW, underestimated the AER by an average of 1.2 g/hr in women and 1.8 g/hr in men, and the correlation coefficient between it

[1] *The published version of the PBPK model is available at no cost by e-mail to* oconnor1@iupui.edu. *The model is offered as a .mdl file for the Simulink® toolbox of MATLAB®, a ubiquitous graphical language that will run on virtually any modern computer platform. More advanced versions of the model, and training in clamping techniques, are available through collaboration.*

and the AER at 60 mg% for the same individual was modest: $r = 0.26$ for women; $r = 0.50$ for men. This variation might be accounted for by inaccuracies in estimates of TBW based on height, age, weight, and gender and/or in measurements of DLS and/or in measurements of the AER. However, the regression coefficients of the six or more points used to estimate DLS were 0.90 or better, and the test-retest reliability of the direct measurement of the AER measurement was even better (O'Connor et al., 1998). It is more likely, therefore, that the assumption about the volume of distribution of alcohol may have been incorrect under the conditions of the experiment.

EFFECT OF FOOD AND FOOD COMPOSITION ON ALCOHOL ELIMINATION RATES AS DETERMINED BY CLAMPING

Vijay A. Ramchandani

Several studies have investigated the effect of food on alcohol pharmacokinetics after oral ingestion and found a decrease in absorption rate and an increase in first-pass metabolism, resulting in lower and delayed peak blood alcohol levels (Gentry, 2000; Jones et al., 1997; Pikaar et al., 1988; Rogers et al., 1987; Sedman et al., 1976; Watkins and Adler, 1993). Studies have also indicated an increase in the disappearance rate of alcohol after a meal (e.g., Gentry, 2000; Jones et al., 1997; Kalant, 2000; Rogers et al., 1987). However, most of these studies have employed oral alcohol administration, and thus cannot reconcile the confounding effects of food on alcohol absorption from the effects of food on alcohol elimination. Breath alcohol clamping uses intravenous infusions of alcohol to achieve and maintain breath alcohol concentrations (BrAC) at a target level for prolonged periods of time (Kwo et al., 1998; O'Connor et al., 1998; Ramchandani et al., 1999a). During the steady-state, the alcohol infusion rate is a direct measure of the alcohol elimination rate. Studies using the BrAC clamp thus allow the evaluation of the influence of food on alcohol elimination rates (AER, g/hr) without the confounding effect on alcohol absorption.

We have conducted two studies to evaluate the effect of food and food composition on AER in healthy men and women. The objective of the first study was to evaluate the effect of food intake on AER in healthy men and women. This was a two-session study in 20 subjects (10 men and 10 women), all 21–30 years old and healthy social drinkers. In one session, subjects underwent an alcohol clamp at 50 mg% after a 12-hr overnight fast; in the other session, subjects underwent the same alcohol clamp procedure 1 hr after the consumption of a standard 530-calorie breakfast. Sessions were conducted in counter-balanced order across subjects. The AER estimates were compared between sessions and by gender using two-way repeated measures ANOVA. Results showed an average increase in AER of 25% after the intake of a 530-calorie meal compared with that after an overnight fast. The percent change in AER ranged from 9–49% across subjects. There was a significant effect of gender, with men showing significantly higher AERs compared with women; however, the effect of food was similar in both men and women.

The objective of the second study was to evaluate the effect of food composition on AER in healthy men and women. This was a four-session, randomized, alcohol clamping study (at 50 mg%) in 8 subjects (4 men and 4 women) under four different conditions: (1) after a 12-hr overnight fast; (2) after a high-fat breakfast; (3) after a high-protein breakfast; and (4) after a high-carbohydrate breakfast. All breakfasts were 550 calories and consumed within 20 min. The alcohol clamp was started exactly 1-hr after the start of meal consumption. AER estimates were compared across sessions using repeated measures ANOVA. Results showed an average increase of 45% in AER after consumption of the 550-calorie meal, regardless of meal composition, compared with that after an overnight fast. The percent change in AER ranged from 16–74% across subjects. The percent change in AER was similar for high-fat, high-carbohydrate, and high-protein containing meals.

The findings of these two studies indicate that food intake results in an increase in alcohol elimination rates (AER), as measured by clamping at 50 mg%. The increase in AER after food intake was similar for men and women. The increase in AER was also similar for meals of different compositions compared with the fasted session, which indicated that the food effect was not due to any specific interaction with meal constituents. Probable mechanisms for the increased alcohol elimination include food-induced increases in liver blood flow, in the activity of alcohol metabolizing enzymes, or in the rate of NADH reoxidation.

CONCLUSIONS

Ting-Kai Li

This symposium focused on the large degree of within-subject and between-subject variation in alcohol absorption, distribution, and elimination in humans and how genetic and environmental factors influenced this variation. In particular, the effects of allelic variations of the *ADH2* and *ALDH2* genes on alcohol elimination rate and on drinking behavior and susceptibility to alcohol dependence were described. The key role that acetaldehyde plays in alcohol metabolism and in the pharmacodynamic effects of alcohol ingestion was emphasized. The existence of allelic variations in the promoter region of the *ALDH2* gene and how this variation can influence the amount of the ALDH2 enzyme synthesized in the liver were summarized.

Although the aversive systemic response to acetaldehyde can be reproduced by ALDH inhibitors such as disulfiram, it is much less certain what acetaldehyde's effects are in brain. As has been reviewed recently (Hunt, 1996; Zimatkin et al., 1998), acetaldehyde may have central nervous system stimulant as well as sedative/hypnotic effects. How-

ever, because there is so little acetaldehyde in the systemic circulation, it is questionable whether acetaldehyde from the periphery would reach the brain. On the other hand, the brain can oxidize ethanol to acetaldehyde, and the enzyme systems that affect this transformation remain unsettled (Person et al., 2000). This is a promising area of future research. In this regard, it is significant that an abstract report at this meeting indicates that acetaldehyde in μM quantities is initially reinforcing and then becomes aversive as the concentration increases, as demonstrated by intracranial self-administration studies (Rodd-Henricks et al., 2000).

The symposium also presented the methodology to attain a constant blood/breath ethanol concentration over time by means of intravenous ethanol infusion, thereby bypassing the variations introduced by absorption and distribution kinetics. The rate of ethanol elimination is obtained in the process of maintaining the "clamp" on the blood/breath ethanol concentration. Gender and ethnic differences in AER can be easily discerned in this manner, as well as the effects of environmental factors such as food on AER. The BrAC clamps provides a useful platform to study pharmacodynamic effects of ethanol and their adaptation over time as has been demonstrated in two studies (O'Connor et al., 1998; Ramchandani et al., 1999b) and several abstracts at this meeting (Blekher et al., 2000; Morzorati et al., 2000; O'Connor et al., 2000a).

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