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Original Article

# A Detailed Analysis of Alcohol Pharmacokinetics in Healthy Korean Men

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To determine blood alcohol concentration (BAC) by extrapolation, an understanding of basal pharmacokinetics is indispensable. Breath alcohol concentration (BrAC) has been used for the determination of body alcohol concentration replaced by BAC in Korea. Therefore, the determination of BAC/BrAC ratio is a key problem in alcohol pharmacokinetics. Among several factors, the ingested dose of alcohol and the allelic variation of mitochondrial aldehyde dehydrogenase 2 (ALDH2) are the most significant factors influencing the pharmacokinetic parameters, particularly in the absorption and elimination phases. This study shows a detailed pharmacokinetic analysis of BAC and BrAC associated with genetic polymorphism including ALDH2 in 42 healthy Korean men. The change in the alcohol dose ingested influenced the maximum concentration ($C_{max}$), the time to reach $C_{max}$ ($T_{max}$), the absorption rate constant ($K_{01}$), the area under the concentration-time curve ($AUC_{last}$), and the hourly elimination rate. The conversion of wild-type 487Glu (*ALDH2*1*) to 487Lys (*ALDH2*2*) in human ALDH2 resulted in changes in $C_{max}$ (*ALDH2*1/*1*, 0.03±0.01 g/dL [±standard deviation] vs. *ALDH2*1/*2*, 0.05±0.004 g/dL [P<0.01]), $AUC_{last}$ (*ALDH2*1/*1*, 4.48±2.19 g·min/dL vs. *ALDH2*1/*2*, 7.52±1.26 g·min/dL [P<0.05]), and the BAC elimination rate (*ALDH2*1/*1*, 0.05±0.02 g/L/hr vs. *ALDH2*1/*2*, 0.09±0.01 g/L/hr [P<0.05]). Moreover, the comparison of BAC and BrAC by Bland-Altman plot showed good agreement, suggesting that the measurement of BrAC can be a good alternative for the determination of BAC, particularly in the post-absorption phase. These results provide fundamental information about the pharmacokinetics of alcohol and the determination of BAC in forensics.

**Key Words:** Alcohols; Pharmacokinetics; Korean; Blood; Breath; Alcohols; Concentration; Forensic sciences

## Introduction

Alcohol (ethanol) has been reported to be the most harmful legal substance to individuals and societies [1], and one of the frequently abused agents worldwide [2]. Furthermore, alcohol drinking is frequently associated with many violations and crimes, such as drunken driving, violent fighting, sexual assaults, arson, and suicides or homicides [3]. Therefore, physiological research on alcohol metabolism in the body becomes important in the fields of forensic science and legal medicine. The measurement of alcohol in biological materials is the most commonly performed analysis in a forensic toxico-

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logical laboratory [2].

The pharmacokinetics of alcohol in the body is influenced by multiple factors. The absorption of alcohol is largely affected by the concentration of alcohol and the consumption of food while drinking [2]. The distribution of alcohol is positively associated with the water content of organs, with tissues having a higher water content showing a higher alcohol concentration [2,3]. After the absorption phase, the alcohol concentration of whole blood is approximately the same, and therefore, the venous concentration of alcohol can be considered as the blood alcohol concentration (BAC) [2]. Determining the BAC at the time of an accident by back-calculation is often required because blood samples are sometimes taken a few hours later. In forensic casework, breath alcohol concentration (BrAC) has been used as an alternative due to its non-invasive application. Therefore, knowledge of factors affecting the BAC and BrAC including the ratio of BAC/BrAC in alcohol pharmacokinetics is important in forensic applications.

Approximately 90% to 98% of alcohol is eliminated in the liver via oxidative metabolism [3]. Oxidative metabolism of alcohol occurs in two main steps: the first step is the oxidation of alcohol to acetaldehyde by the alcohol dehydrogenase (ADH) enzymes, mainly by class I ADH (ADH1A, ADH1B, and ADH1C) enzymes, and the second step is the oxidation of acetaldehyde to acetate by aldehyde dehydrogenase (ALDH) enzymes, primarily by mitochondrial ALDH2, which is a homertetrameric enzyme with a very low $K_M$ value for acetaldehyde among the many ALDH isozymes, as recently reviewed [4]. Single nucleotide polymorphisms (SNPs) of the gene loci of the alcohol-metabolizing enzymes affect alcohol metabolism and have been reported to significantly affect alcohol consumption and risk of alcoholism in East Asian populations [4-6]. ADH1B, ADH1C, and ALDH2 have alloenzymes with different SNPs: *ADH1B*1, ADH1B*2* (Arg47His, rs1229984), and *ADH1B*3* (Arg369Cys, rs2066702) for ADH1B; *ADH1C*1* and *ADH1C*2* (Arg271Gln, rs1693482 and Ile349Val, rs698) for ADH1C; and *ALDH2*1* and *ALDH2*2* (Glu487Lys, rs671) for ALDH2 [6,7]. The *ALDH2*2* allele encodes a nearly inactive subunit with dominant negative inactivation, which leads to accumulation of high levels of acetaldehyde in the blood and other tissues, resulting in alcohol-associated adverse reactions, such as flushing, palpitation, and general discomfort [6,8,9]. The *ALDH2*2* allele is best known for protection against alcoholism and is essentially absent in populations across the world, except in East Asian populations [6,10,11].

Although, many studies of alcohol pharmacokinetics have been conducted in an international context, no investigation for the relationship between alcohol pharmacokinetics and genetic polymorphism has been conducted in a Korean context. The aim of this study was to study the alcohol pharmacokinetic parameters in the blood and breath in healthy human Korean subjects to determine whether the measurement of BrAC can be used as a good alternative for the determination of BAC. We observed that the dose of alcohol and the *ALDH2* polymorphism are important factors in controlling many pharmacokinetic parameters. At the same time, our time-dependent analysis revealed a robust correlation between BAC and BrAC. Therefore, these results establish some critical points to be considered during extrapolation of BAC.

## Materials and Methods

### 1. Experimental procedure

Data were obtained from 42 healthy Korean male volunteers. Before the experiment, we collected a 10-mL venous blood sample to assess complete blood count and liver function. We asked the participants to abstain from alcohol and other drugs for 24 hours before testing. Approval for the drinking experiments was granted by the Scientific Investigation Department of the Ministry of National Defense. The participants were given a detailed explanation of the study schedule, procedures to be performed, and possible adverse events, and all participants submitted voluntarily written informed consent before screen-

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ing. The participants received prorated compensation for their participation after trial completion.

On the day of the experiment, the participants did not consume any food after PM 13:00 and ingested no fluids after PM 15:00. They started drinking exactly 2 hours after finishing the last meal. Before drinking commenced, all participants were asked to provide a breath sample to confirm the absence of alcohol. The participants were classified into two groups based on their alcohol-drinking history (frequency and quantity in the past year). The classification criteria were based on the method reported in a previous study [12]: group A (0–72 g ethanol/wk) and group B (80–244 g ethanol/wk). Group A consumed 0.5 g ethanol per kilogram of body weight, whereas group B consumed 0.8 g ethanol per kilogram of body weight. All volunteers drank soju, popular Korean liquor that contains 21% (v/v) ethanol, within 20 minutes. In order to avoid complexity in data interpretation, no mixing of different types of alcohol was allowed. During the experiment, alcohol beverages were served without food.

An indwelling venous catheter was inserted into the antecubital vein, and a baseline blood sample was drawn. Two samples of venous blood were drawn every 30 or 60 minutes from 30 to 420 minutes (30, 60, 90, 120, 150, 180, 240, 300, 360, and 420 minutes) after the end of drinking. BAC was determined by headspace gas chromatography. Each sample was examined in duplicate and put into a head-space vial with 200 $\mu$L of a 0.02% solution of 2-methyl-2-propanol (tert-butyl alcohol, Aldrich Chemical, Milwaukee, WI, USA), which was used as an internal standard. The vial was tightly sealed using a rubber septum and an aluminum cap. The temperature of the oven, sample valve, and transfer line was 900°C. The sample was heated at 900°C for 30 minutes, and 1 mL of the head space (7694 static sampler, Hewlett Packard, Palo Alto, CA, USA) was injected onto a gas chromatography system (6890N, Agilent Technologies, Santa Clara, CA, USA) equipped with a glass column (HP-B ALC) and a flamed ionization detector. The temperature of the column and injection port was maintained at 2,500°C. Nitrogen, controlled at 20 psi, was used as the carrier gas at a flow rate of 20 mL/min. At a mean ethanol concentration of 0.05%, the precision of this headspace gas chromatography method, expressed as a coefficient of variation, was 1.6%. The limit of detection for BAC was 0.001%.

BrAC was determined by breath alcohol testing using Alcomat (Siemens, Munich, Germany), which has been approved by military police authorities for alcohol testing. This device detects alcohol concentration by measuring the absorption of infrared radiation. Two Alcomat instruments were used and calibrated by the technical laboratory of the military police. In order to prevent inaccuracy originating from residual alcohol, room-air blanks were analyzed during each test. The participants were asked to exhale deeply into the heated inlet tube of the instrument for at least 6 seconds. The participants had to exhale twice, separately, at an interval of 1–2 minutes. Duplicate determinations were made 1–2 minutes apart and as close as possible to when blood was sampled. The mean of the duplicate breath alcohol determinations was used for assessment.

### 2. Pharmacokinetics

The BAC and BrAC versus time data for each group were analyzed separately. Noncompartmental pharmacokinetic analysis of alcohol was performed using Phoenix ver. 6.2 (Pharsight Co., Sunnyvale, CA, USA). Individual maximum concentrations ($C_{max}$) and times to reach $C_{max}$ ($T_{max}$) were obtained directly from the observed data. The area under the concentration-time curve ($AUC_{last}$) was estimated by linear interpolation using the trapezoidal rule. A one-compartment model with Michaelis-Menten kinetics was used to determine the pharmacokinetic parameters (absorption rate constant, $K_{01}$; Michaelis-Menten constant, $K_M$; and maximum elimination rate, $V_M$). The alcohol elimination rate was determined by linear regression.

### 3. Genotyping of ADH and ALDH

Genomic DNA from each participant was isolated

from venous blood samples for genotyping. The *ADH1B* His47Arg (rs1229984), *ADH1C* Ile349Val (rs698 SNP), and *ALDH2* Glu487Lys (rs671) SNPs were analyzed using predesigned TaqMan SNP genotyping assays (Life Technologies, Carlsbad, CA, USA).

### 4. Statistical analysis

Comparison of two different measurements (BAC and BrAC) was carried out by Bland and Altman's method as previously described [13]. The statistical significance of differences between the pharmacokinetic parameters of the various *ADH1B*, *ADH1C*, and *ALDH2* genotypes was evaluated using the Mann-Whitney U test. A P-value of less than 0.05 was accepted to be statistically significant.

## Results

### 1. Baseline characteristics of participants

A total of 42 Korean healthy men aged 22 to 61 years (mean, 40.4 years; standard deviation [SD], 10.1) participated in the study. The average body height and weight of the participants were 172.7 cm (SD, 6.0) and 72.1 kg (SD, 7.9), respectively. The average body height and weight of group A (n=20) were 172.3 cm (SD, 6.4) and 69.9 kg (SD, 7.9), respectively. The average body height and weight of group B were 173.2 cm (SD, 5.7) and 74.1 kg (SD, 7.5), respectively. All 42 participants completed the study.

### 2. Effect of dose on alcohol pharmacokinetics

The ingested dose is an important factor influencing the pharmacokinetics of alcohol, particularly in the absorption phase. To investigate the effect of alcohol dose, we compared the pharmacokinetic parameters of alcohol between the two groups: group A (low dose, 0.5 g/kg) and group B (moderate dose, 0.8 g/kg). Fig. 1 illustrates the mean concentration-time profiles of alcohol in venous blood and the end-expired breath of each group. Notably, the BAC and BrAC curves displayed similar patterns in the absorption, distribution, and elimination phases of alcohol metabolism. To assess the difference between BAC and BrAC in each group, we performed a Bland-Altman plot analysis, which is usually used to estimate the agreement between two different assays [13]. The Bland-Altman plot in Fig. 2 shows the difference in BAC and BrAC plotted against the mean concentration of alcohol for BAC and BrAC ([BAC+BrAC]/2). The observed bias was −0.00075 g/dL and the SD of the differences was 0.0576 g/dL. The 95% lower and upper limits of agreement by the two methods were −0.11363 and 0.11210, respectively. In addition, the estimated pharmacokinetic parameters of each group were not statistically different between BrAC and BAC (Table 1). These results suggest that the BAC and BrAC were in good agreement, regardless of the alcohol dose.

Comparison of the pharmacokinetic data between groups A and B showed that several parameters varied depending on the alcohol dose (Table 1). In the absorption phase, $C_{max}$, $T_{max}$, and $K_{01}$ were approximately 2-fold higher in group B. In addition, $AUC_{last}$, which represents a measure of alcohol exposure, showed more than a 2-fold increase in group B. In the elimination phase, the elimination rate increased approximately 2-fold in group B. However, intergroup differences in $K_M$

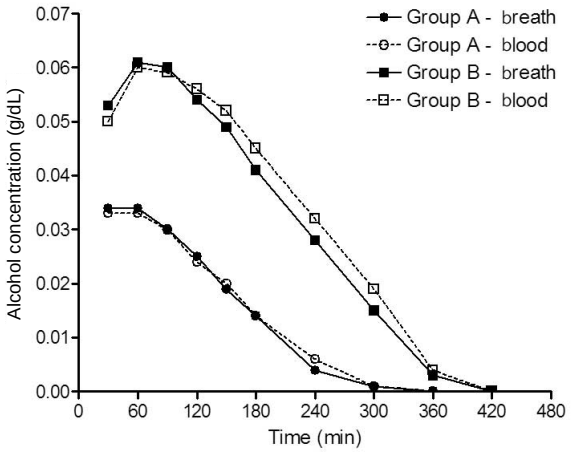


Fig. 1. Mean breath alcohol concentration (BrAC) and blood alcohol concentration (BAC) versus time profiles in group A (0.5 g/kg) and group B (0.8 g/kg) participants. After drinking alcohol within a period of 20 minutes, BrAC and BAC were determined every 30 or 60 minutes (30, 60, 90, 120, 150, 180, 240, 300, 360, and 420 minutes) as described in the Materials and Methods section.


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and $V_M$ were not statistically significant. Thus, the dose of alcohol influenced several pharmacokinetic parameters, particularly in the absorption and elimination phases.

Given that forensic casework often requires back-calculation of alcohol concentration, determination of the alcohol elimination rate of an individual is important. Table 2 demonstrates the elimination rate of each

Table 1. Pharmacokinetic parameters in group A (0.5 g/kg, n=20) and group B (0.8 g/kg, n=22) participants

| Parameter | Group A (n=20) | Group B (n=22) |
|---|---|---|
| Breath | | |
| $T_{max}$ (min) | 51.00±21.98 | 77.73±28.77 |
| $C_{max}$ (g/dL) | 0.04±0.01 | 0.06±0.01 |
| $AUC_{last}$ (g·min/dL) | 5.01±2.25 | 12.50±3.73 |
| $K_{01}$ (1/min) | 0.26±0.06 | 0.42±0.21 |
| $K_M$ (g/dL) | 3.76±0.26 | 3.66±0.31 |
| $V_M$ (1/min) | 1.16±0.11 | 1.22±0.07 |
| Elimination rate (g/L/hr) | 0.06±0.02 | 0.12±0.03 |
| Blood | | |
| $T_{max}$ (min) | 37.50±13.33 | 80.45±36.32 |
| $C_{max}$ (g/dL) | 0.03±0.01 | 0.06±0.01 |
| $AUC_{last}$ (g·min/dL) | 5.09±2.36 | 13.39±3.79 |
| $K_{01}$ (1/min) | 0.24±0.11 | 0.45±0.22 |
| $K_M$ (g/dL) | 3.71±0.50 | 3.69±0.42 |
| $V_M$ (1/min) | 1.29±0.31 | 1.21±0.07 |
| Elimination rate (g/L/hr) | 0.06±0.02 | 0.12±0.03 |

Values are presented as mean±standard deviation.
$T_{max}$, times to reach $C_{max}$; $C_{max}$, maximum concentrations; $AUC_{last}$, area under the concentration-time curve; $K_{01}$, absorption rate constant; $K_M$, Michaelis-Menten constant; $V_M$, maximum elimination rate.

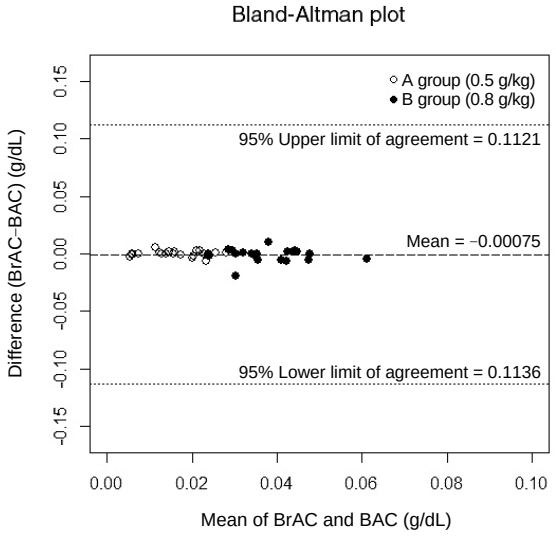


Fig. 2. Bland-Altman plot of each participant's difference (blood alcohol concentration [BAC]–breath alcohol concentration [BrAC]) against the mean of the two measurements ([BAC+BrAC]/2). Empty circles and filled circles represent data from group A (0.5 g/kg) and group B (0.8 g/kg), respectively. The horizontal lines show a mean bias of −0.00075, and 95% lower and upper limits of agreement of −0.1136 and 0.1121.

Table 2. Individual elimination rate (g/L/hr) of breath alcohol concentration and blood alcohol concentration calculated by linear regression

| Subject | BrAER (g/L/hr) | BAER (g/L/hr) |
|---|---|---|
| Group A | | |
| 1 | 0.05621 | 0.06346 |
| 3 | 0.09002 | 0.08939 |
| 4 | 0.02035 | 0.0189 |
| 5 | 0.07603 | 0.08267 |
| 6 | 0.07588 | 0.08411 |
| 11 | 0.05574 | 0.05126 |
| 12 | 0.0943 | 0.08655 |
| 14 | 0.09101 | 0.07913 |
| 15 | 0.03101 | 0.02767 |
| 16 | 0.06819 | 0.0535 |
| 17 | 0.05895 | 0.05457 |
| 19 | 0.02597 | 0.02991 |
| 26 | 0.0913 | 0.07507 |
| 27 | 0.05648 | 0.04249 |
| 28 | 0.05648 | 0.05588 |
| 29 | 0.02087 | 0.02018 |
| 32 | 0.10093 | 0.0987 |
| 33 | 0.07055 | 0.08359 |
| 34 | 0.05467 | 0.04774 |
| 37 | 0.05562 | 0.0571 |
| Group B | | |
| 2 | 0.11733 | 0.05994 |
| 7 | 0.08885 | 0.08245 |
| 8 | 0.15333 | 0.16091 |
| 9 | 0.08907 | 0.09739 |
| 10 | 0.09105 | 0.07435 |
| 13 | 0.09485 | 0.114 |
| 18 | 0.11505 | 0.11612 |
| 20 | 0.14395 | 0.13333 |
| 21 | 0.15114 | 0.11176 |
| 22 | 0.12256 | 0.16537 |
| 23 | 0.11484 | 0.11114 |
| 24 | 0.10726 | 0.0969 |
| 25 | 0.12091 | 0.14696 |
| 30 | 0.15995 | 0.15785 |
| 31 | 0.11956 | 0.12306 |
| 35 | 0.08055 | 0.11785 |
| 36 | 0.08333 | 0.08058 |
| 38 | 0.14584 | 0.1371 |
| 39 | 0.12058 | 0.1153 |
| 40 | 0.15114 | 0.14416 |
| 41 | 0.15052 | 0.16384 |
| 42 | 0.12422 | 0.11703 |

BrAER, alcohol elimination rate in breath test; BAER, alcohol elimination rate in blood test.

participant calculated by linear regression analysis. In group A, the minimum and maximum values of blood-alcohol elimination rate were 0.0189 (participant 4) and 0.0987 g/L/hr (participant 3), respectively, with an approximate 5-fold difference. In group B, an approximate 3-fold difference was observed between the minimum (0.05994 g/L/hr [participant 2]) and maximum (0.16537 g/L/hr [participant 30]) values of blood-alcohol elimination rate. Therefore, individual variation is a critical factor to be considered when determining BAC by back-calculation.

### 3. Effect of genotype on alcohol pharmacokinetics

Next, we investigated the effect of the genotype of alcohol-metabolizing enzymes such as ADH1B, ADH1C, and ALDH2 on the pharmacokinetics of alcohol. Among the several SNPs identified in alcohol-metabolizing enzymes, we selected the following three for analysis: the *ADH1B* His47Arg (A to G) polymorphism denoted as *ADH1B*1* and *ADH1B*2*, the *ADH1C* Ile349Val (A to G) polymorphism denoted as *ADH1C*1* and *ADH1C*2*, and the *ALDH2* Glu487Lys (G to A) polymorphism denoted as *ALDH2*1* and *ALDH2*2*. Genotype distributions and allele frequencies of the three SNPs in the 42 study participants are listed in Table 3. No participants were homozygous for *ALDH2*2/*2* (AA) in either group, and only 1 participant with an inactive *ALDH2*1/*2* (GA) genotype was identified in group B. Given the low frequency of some genotypes in group B, statistical analysis was performed only in group A.

Table 4 summarizes the pharmacokinetic parameters of each genotype group for *ADH1B*, *ADH1C*, and *ALDH2*. There was no significant difference in parameters between groups of different *ADH1B* and *ADH1C* genotypes. In the case of the *ALDH2* genotype, $C_{max}$, $AUC_{last}$, and the elimination rate increased in subjects with the *ALDH2*1/*2* (GA) genotype compared with the *ALDH2*1/*1* (GG) genotype in both the BAC and BrAC analysis. $K_{01}$ changed significantly only in the BrAC analysis. Therefore, these findings suggest that compared to *ADH1*, the *ALDH2* polymorphism is a more important determining factor in the pharmacokinetics of alcohol.

Table 3. Allelic and genotypic frequencies of *ADH1B*, *ADH1C*, and *ALDH2* polymorphisms

| Genotypic frequency | Group A (n=20) | Group B (n=22), |
|---|---|---|
| *ADH1B* His47Arg (rs1229984 SNP, A-to-G polymorphism) | | |
| AA | 14 (70.0) | 8 (36.4) |
| AG | 6 (30.0) | 11 (50.0) |
| GG | 0 (0) | 3 (13.6) |
| A | 34 (85.0) | 27 (61.4) |
| G | 6 (15.0) | 17 (38.6) |
| *ADH1C* Ile349Val (rs698 SNP, A-to-G polymorphism) | | |
| AA | 17 (85.0) | 15 (68.2) |
| AG | 3 (15.0) | 7 (31.8) |
| GG | 0 (0) | 0 (0) |
| A | 37 (92.5) | 37 (84.1) |
| G | 3 (7.5) | 7 (15.9) |
| *ALDH2* Glu487Lys (rs671, G-to-A polymorphism) | | |
| GG | 16 (80.0) | 21 (95.5) |
| GA | 4 (20.0) | 1 (4.5) |
| AA | 0 (0) | 0 (0) |
| G | 36 (90.0) | 43 (97.7) |
| A | 4 (10.0) | 1 (2.3) |

Values are presented as number (%).

## Discussion

The determination of alcohol concentration is frequently required in alcohol-related crimes and is one of the major issues in forensic science. For precise estimation, an understanding of the basal pharmacokinetics of alcohol and the detailed measurement of key parameters are necessary. In this study, we performed a detailed pharmacokinetic analysis of alcohol in healthy Korean men. We found that alcohol dose and *ALDH2* polymorphism, but not *ADH1B* or *ADH1C* polymorphism, were important influencing factors for BAC. To our knowledge, this is the first report to analyze the effect of polymorphisms in alcohol-metabolizing enzymes on BAC by calculating detailed pharmacokinetic parameters from absorption to elimination.

The absorption, distribution, metabolism, and excretion of alcohol are influenced by numerous factors. Alcohol absorption occurs primarily in the small intes-


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Table 4. Comparison of pharmacokinetic parameters according to the *ADH1B*, *ADH1C*, and *ALDH2* genotypes in group A participants

| Parameter | *ADH1B* (His47Arg, rs1229984, A to G) | | | *ADH1C* (Ile349Val, rs698, A to G) | | | *ALDH2* (Glu487Lys, rs671, G to A) | | |
|---|---|---|---|---|---|---|---|---|---|
| | AA (n=14) | AG (n=6) | P-value[a] | AA (n=17) | AG (n=3) | P-value[a] | GG (n=16) | AG (n=4) | P-value[a] |
| Breath | | | | | | | | | |
| $T_{max}$ (min) | 51.42±21.79 | 50.00±24.49 | 0.8575 | 49.41±21.06 | 60.00±30.00 | 0.4893 | 52.50±23.24 | 45.00±17.32 | 0.6070 |
| $C_{max}$ (g/dL) | 0.04±0.01 | 0.04±0.01 | 0.7724 | 0.04±0.01 | 0.04±0.01 | 0.6714 | 0.03±0.01 | 0.05±0.003 | 0.0060[b] |
| $AUC_{last}$ (g · min/dL) | 4.91±2.36 | 5.26±2.17 | 0.5634 | 4.93±2.37 | 5.52±1.61 | 0.5964 | 4.35±1.92 | 7.67±1.41 | 0.0061[b] |
| $K_{01}$ (1/min) | 0.26±0.07 | 0.24±0.02 | 0.6801 | 0.26±0.07 | 0.24±0.02 | 0.9578 | 0.26±0.07 | 0.23±0.01 | 0.0182[c] |
| $K_M$ (g/dL) | 3.74±0.31 | 3.79±0.05 | 0.6801 | 3.75±0.28 | 3.78±0.02 | 0.711 | 3.75±0.29 | 3.80±0.04 | 0.5708 |
| $V_M$ (1/min) | 1.15±0.13 | 1.17±0.06 | 0.4579 | 1.15±0.12 | 1.16±0.09 | 0.9578 | 1.17±0.12 | 1.10±0.03 | 0.2568 |
| Elimination rate (g/L/hr) | 0.06±0.03 | 0.07±0.02 | 0.4831 | 0.06±0.03 | 0.07±0.02 | 0.4585 | 0.06±0.02 | 0.09±0.01 | 0.0061[b] |
| Blood | | | | | | | | | |
| $T_{max}$ (min) | 38.57±14.06 | 35.00±12.25 | 0.5829 | 37.06±13.12 | 40.00±17.32 | 0.7245 | 39.38±14.36 | 30.00±0 | 0.2083 |
| $C_{max}$ (g/dL) | 0.03±0.01 | 0.04±0.01 | 0.5087 | 0.03±0.01 | 0.03±0.01 | 0.791 | 0.03±0.01 | 0.05±0.004 | 0.0093[b] |
| $AUC_{last}$ (g · min/dL) | 4.98±2.27 | 5.35±2.78 | 0.7415 | 5.16±2.48 | 4.69±1.95 | 0.7913 | 4.48±2.19 | 7.52±1.26 | 0.0182[c] |
| $K_{01}$ (1/min) | 0.24±0.13 | 0.23±0.03 | 0.4095 | 0.24±0.12 | 0.25±0.02 | 0.9578 | 0.26±0.11 | 0.18±0.12 | 0.1859 |
| $K_M$ (g/dL) | 3.67±0.60 | 3.80±0.07 | 0.8046 | 3.69±0.54 | 3.82±0.10 | 0.711 | 3.68±0.56 | 3.84±0.05 | 0.3447 |
| $V_M$ (1/min) | 1.31±0.36 | 1.23±0.17 | 0.8046 | 1.31±0.33 | 1.15±0.11 | 0.3683 | 1.24±0.18 | 1.45±0.63 | 0.7768 |
| Elimination rate (g/L/hr) | 0.06±0.03 | 0.06±0.02 | 0.7415 | 0.06±0.03 | 0.06±0.02 | 0.6338 | 0.05±0.02 | 0.09±0.01 | 0.0182[c] |

$T_{max}$, times to reach $C_{max}$; $C_{max}$, maximum concentrations; $AUC_{last}$, area under the concentration-time curve; $K_{01}$, absorption rate constant; $K_M$, Michaelis-Menten constant; $V_M$, maximum elimination rate.
[a] Mann-Whitney U test; [b] P<0.01; [c] P<0.05.

tine, with the concentration of alcohol, the consumption of food while drinking, and gastrointestinal motility being major determining factors in this process [2]. Alcohol is distributed via blood vessels into tissues and organs according to water content, and the time to equilibrium is mainly dependent on the sex, weight, and height of the individual [2]. In this study, we found that the alcohol dose affected several pharmacokinetic parameters, such as $C_{max}$, $T_{max}$, and $K_{01}$, particularly in the absorption phase.

Alcohol is eliminated mainly (90%–98%) by the oxidizing enzymes in the liver. The remaining 2%–10% is excreted directly into breath, urine, and sweat, and small portions (less than 0.1%) are metabolized via a nonoxidative pathway, resulting in ethyl sulfate and ethyl glucuronide [3]. The oxidation of alcohol in the liver occurs in two steps. The first step, the conversion of alcohol to aldehyde, is carried out mainly by class I ADH (ADH1A, ADH1B, and ADH1C) enzymes, which exist in the cytosol of hepatocytes, and have a low $K_M$ value for alcohol, resulting in saturation at low BACs [14,15]. Cytochrome p450 2E1 (CYP2E1) is another enzyme that mediates this first step. CYP2E1 has a higher $K_M$ value, and plays an important role in the elimination of alcohol in heavy drinkers and alcoholics [16,17]. The second step, the conversion of aldehyde to acetate, is catalyzed primarily by ALDH2, which has a low $K_M$ value and is found in the mitochondria of hepatocytes [3,6]. The acetate produced is further metabolized into acetyl-CoA, which is used in the tricarboxylic acid cycle and ATP generation [15].

Polymorphisms in alcohol-oxidizing enzymes are a determining factor that affects the elimination phase of alcohol, because these enzymes are responsible for the majority of alcohol metabolism. Among class I ADHs, ADH1B and ADH1C have alloenzymes with different SNPs: *ADH1B*1*, *ADH1B*2* (Arg47His, rs1229984), and *ADH1B*3* (Arg369Cys, rs2066702) for ADH1B; *ADH1C*1* and *ADH1C*2* (Arg271Gln, rs1693482, and Ile349Val, rs698) for ADH1C [6,7]. Except for the change at position 349 in ADH1C, the other three amino acid substitutions result in enzyme kinetic

changes because they are located in coenzyme binding sites [18-20]. Previous studies have shown that *ADH1B*1* and *ADH1C*2* have lower $K_M$ values than *ADH1B*2* and *ADH1C*1*, respectively [6,21]. However, in this study, these polymorphisms resulted in no significant changes in the pharmacokinetic parameters of alcohol (Table 4). ALDH2 also has 2 allelic variants: *ALDH2*1* and *ALDH2*2* (Glu487Lys, rs671) [6,7]. *ALDH2*2* is considered to be nearly inactive because it has an extremely high $K_M$ for NAD+ and a low $V_{max}$, while the activity of the *ALDH2*1/*2* heterodimer has been predicted to be 25% that of the normal *ALDH2*1/*1* homodimer [9,22]. In this study, we found that $C_{max}$ and $AUC_{last}$ are influenced by the *ALDH2* polymorphism (Table 4). This result and the estimated $C_{max}$ and $AUC_{last}$ values are compatible with those of previous studies (*ALDH2*1/*1* genotype: $C_{max}$, $0.044\pm0.006$ g/dL; $AUC_{last}$, $4.168\pm0.442$ g·min/dL; *ALDH2*1/*2* genotype: $C_{max}$, $0.054\pm0.004$ g/dL; $AUC_{last}$, $5.106\pm0.304$ g·min/dL) [6,23,24]. However, the fold changes in $C_{max}$ and $AUC_{last}$ between the two genotypes are much greater in our study (1.67 and 1.37 for $C_{max}$; 1.68 and 1.23 for $AUC_{last}$). Unexpectedly, the elimination rate observed with the *ALDH2*1/*2* genotype was higher than that observed with the *ALDH2*1/*1* genotype, which is probably due to the secondary effect of the increased $C_{max}$ and $AUC_{last}$.

The frequencies of allelic variance in alcohol-oxidizing enzymes differ according to ethnicity. Eastern Asians, including Han Chinese, Japanese, and Koreans, show unique allelic distributions compared to those shown by Caucasians, American Indians, and Africans. The *ADH1B*2* and *ADH1C*1* alleles are prevalent in eastern Asians compared to other populations [6]. The *ALDH2*2* allele is found only in eastern Asian areas, and very rarely in other regions [6]. Notably, the *ALDH2*2* allele was found to influence several pharmacokinetic parameters in this study, suggesting that the *ALDH2* genotype must be considered when calculating the BAC in cases involving these ethnic groups.

Breath alcohol testing is an easy method of measuring alcohol concentration in the body, and is widely used for screening tests and as evidence [13]. Bland-Altman analysis of the results from this study showed good agreement between BAC and BrAC regardless of alcohol dose (Fig. 2), which is consistent with previous reports showing a high correlation ($r=0.95-0.98$) between these two values [13]. However, there is a limitation in our Bland-Altman analysis because only the mean difference of each participant throughout the experiment was analyzed, ignoring the time effect. Previous studies suggest that the BAC/BrAC ratio tends to vary according to the pharmacokinetic phase [13]. This temporal variation is probably due to the fact that BrAC reflects the alcohol concentration of arterial blood rather than that of venous blood [25]. Therefore, BrAC can be used for the determination of body alcohol concentration by back-calculation in the post-absorption phase, when the alcohol concentration of arterial and venous blood is approximately at equilibrium [2,13].

For the determination of BAC by back-calculation, knowledge about the elimination rate of alcohol from blood, which is denoted as $\beta$-slope [3], is indispensable. A previous study reported a mean $\beta$ value of $0.133\pm0.029$ g/L/hr for men [3], which is higher than our estimation. A confounding factor may be the duration of fasting because the participants in this study participated in the experiment after 2 hours of fasting, while previous studies recommended overnight (10 hours) fasting [3]. In addition, our results demonstrate that there are large variations among participants in the elimination rate of alcohol (Table 2). Therefore, individual variations in elimination rate need to be considered when determining BAC, and further studies are required to clarify the factors affecting variation in elimination rate.

In this study, a detailed pharmacokinetic analysis of alcohol was carried out in 42 healthy Korean males. Among several factors, the ingested dose of alcohol and *ALDH2* allelic variation were identified as decisive factors in the pharmacokinetic parameters, particularly in the absorption and elimination phases.

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Moreover, the comparison of BAC and BrAC showed that they were in good agreement, suggesting that the measurement of BrAC is an appropriate alternative for the determination of BAC, particularly in the post-absorption phase. These results provide fundamental information about the pharmacokinetics of alcohol and the determination of body alcohol concentrations in the field of forensics.

### Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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