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GENDER DIFFERENCES IN THE PHARMACOKINETICS OF ETHANOL IN SALIVA AND BLOOD AFTER ORAL INGESTION

Wojciech Gubała, *Dariusz Zuba*[#]

Institute of Forensic Research, Westerplatte 9, PL 31-033 Kraków, Poland

Gender differences in the pharmacokinetics of ethanol in saliva and blood after oral ingestion. W. GUBAŁA, D. ZUBA. Pol. J. Pharmacol., 2003, 55, 639–644.

The aim of this study was to compare the pharmacokinetics of ethanol in saliva and blood according to gender and to evaluate the determination of ethanol in saliva for evidential sobriety testing. Twenty-four persons, 12 men and 12 women, took part in the experiments. The subjects received ethanol, as neat 40% v/v vodka, in the amount which should lead according to Widmark formula to the blood alcohol concentration equal to 1.0 g/l. Duplicate samples of an unstimulated mixed saliva secretion and venous blood were taken at 15 min intervals timing from the end of consumption, and ethanol concentrations in both specimens were determined by means of gas chromatography. The pharmacokinetic calculations were done using first-order absorption and Michaelis-Menten or zero order elimination models. In most cases ethanol reached higher maximal concentration in saliva than in venous blood, and was faster eliminated from saliva. The significant gender differences in the time-concentration profiles were observed. The maximal ethanol concentrations, both in blood and saliva, were lower in women compared to men. In females, ethanol was faster excreted from the body. Both experimental (C_{max}) and extrapolated to zero time (C_0) maximum ethanol concentrations were lower in females. The apparent volumes of distribution after oral dose for saliva and blood were very close and did not differ statistically. The study shows that the same factor equivalent to volume of distribution should be used in back calculation of alcohol concentration, and saliva alcohol analysis can be treated as independent method to test sobriety.

Key words: *ethanol kinetics, oral administration, concentration-time profile, saliva alcohol, male, female*

[#] *correspondence*; e-mail: dzuba@ies.krakow.pl

INTRODUCTION

At the present time, there is increasing interest in saliva as a biological specimen for analyzing drugs of abuse, therapeutic agents, ethanol, environmental chemicals and many endogenous substances [17]. A great advantage of saliva as a testing material is the fact that it is obtained in a non-invasive way. This is especially important at a time when a real threat exists of inadvertently contracting HIV virus, and many people are opposed to submitting to blood testing. Of course, saliva analysis is not completely free of flaws. Some people, particularly the elderly, can have problems producing an adequate amount of material for analysis. The homogeneous nature of the sample seems to pose a considerable problem, although, as it was pointed out in the literature [7], proper collection and storage procedure ensures satisfactory accuracy and precision.

Ethanol is absorbed and rapidly distributed in every body compartment soon after consumption, therefore, it can be determined by the analysis of alternative materials [3, 16, 23]. The choice of the biological specimen can be made on the basis of practicability or on the aim of the determination.

To date mainly breath testing is used for this purpose. In the eighties, evidential breath alcohol instruments were approved for law enforcement purposes and threshold limits of breath alcohol concentration (BrAC) were introduced alongside the existing statutory blood alcohol concentration (BAC) limits [8, 14]. Nevertheless measurements of breath alcohol may not be accurate in some cases, i.e. in febrile or hypothermic patients, unconscious victims, or patients with bronchopulmonary disease [21].

The ratio of blood flow to tissue mass of the salivary gland is so large that the concentration of alcohol entering saliva accurately reflects the concentration in arterial blood [20]. It is very favorable because acute alcohol effects on the brain function are correlated with the concentrations in this type of blood [10]. The saliva specimens serve as substitutes for blood, and the analytical finding is usually translated into the presumably equivalent BAC [2].

The aim of this study was to compare the pharmacokinetics of ethanol in saliva and blood according to gender and to evaluate the determination of ethanol in saliva for evidential sobriety testing.

MATERIALS and METHODS

Study subjects

Twelve healthy men, aged 21–48 (mean age 31.8 ± 1.7), weighting 64–82 kg (mean weight 72.1 ± 1.1 kg), and 12 healthy women, aged 25–55 (mean age 35.8 ± 2.0), weighting 52–75 kg (mean weight 59.7 ± 1.3 kg), taking no drugs, took part in the study. The volunteers were social drinkers with no history of alcohol abuse. They were informed about the purpose of the study and gave their consent to participate. In each experiment, the subjects consumed alcohol in the form of 40% v/v vodka within 15 min, 2 h after last meal. They received a dose of ethanol of 0.7 g per kg of body weight for men, and 0.6 g per kg of body weight for women. The above dose of alcohol should lead, according to Widmark formula [25], to maximal ethanol concentration in blood equal to 1.0 g/l. Duplicate samples of an unstimulated mixed saliva secretion and venous blood were simultaneously taken at 15 min intervals up to elimination of ethanol from the body (the actual concentrations of ethanol were controlled by measurement in exhaled air using Alcomat V5).

Determination of ethanol in blood and saliva

Blood and saliva ethanol concentrations were determined by means of gas chromatography using Perkin Elmer Autosystem apparatus equipped with headspace autosampler HS 40. Separation was achieved on a 0.2% Carbowax 1500/Graphpack-GC column under isothermal conditions (at 100°C). The temperature of flame ionization detector (FID) was 200°C. A 0.2 ml of saliva or blood was mixed with 1.8 ml of 0.2 g/l solution of 2-methyl-2-propanol (*tert*-butyl alcohol) used as an internal standard (IS). The samples were incubated in the autosampler for 22 min at 60°C. Chromatograms were recorded and basic calculations were done using Turbochrom computer program.

Pharmacokinetics modelling

The pharmacokinetic calculations were done using first-order absorption and Michaelis-Menten or zero order elimination models. The computer program ADAPT II (Biomedical Simulations Resource, University of Southern California) was used. The following equations were applied:

$$\frac{dC_{EtOH}}{dt} \quad \frac{k_a D}{V} \quad e^{-k_a \cdot t} \quad \frac{V_{Max} C_{EtOH}}{(K_M \quad C_{EtOH})}$$

$$\frac{dC_{EtOH}}{dt} \quad \frac{k_a D}{V} \quad e^{-k_a \cdot t} \quad \beta_{60}$$

where: k_a is absorption rate constant, β_{60} – zero order rate of elimination, D – dose of alcohol, V – apparent volume of distribution after oral dose, V_{Max} – maximum velocity of ethanol elimination, K_M – Michaelis' constant, C_{EtOH} – ethanol concentration (the units are given in Table 1).

In the study, the following parameters were estimated: absorption constant (k_a), absorption half time ($t_{1/2a}$), time to peak concentration (t_{max}), peak concentration (C_{max}), parameters of the Michaelis-Menten model (V_{Max}, K_M), elimination rates (β and β_{60}), distribution volume (V), extrapolated maximum concentration (C_0), area under the concentration-time curve (AUC), area under the first moment curve (AUMC) and mean residence time (MRT) [11].

Statistical analysis

Results were expressed as means ± standard error of a mean. The statistical significance of differences between groups of data was determined by F-Snedecor test of variance homogeneity followed by Student's *t*-test. A probability p value of less than 0.05 was taken to indicate statistical significance. The statistical analyses were performed with use of STATISTICA software (Statsoft Inc., Tulsa, OK, USA).

RESULTS

The correlation of ethanol concentrations in blood and saliva

The concentrations of ethanol in blood and saliva were highly correlated. The Pearson's correlation coefficient equaled to 0.944 ($p < 0.0001$) and the regression line fitted to the experimental data by means of least squares method was:

$$SAC = 1.065 \times BAC - 0.023$$

where: SAC – saliva alcohol concentration, BAC – saliva alcohol concentration.

The time vs. concentration profiles

Simultaneous collection of blood and saliva samples allowed for the comparison of the time-

Table 1. The pharmacokinetic parameters determined using first-order absorption and Michaelis-Menten or zero-order elimination models, and the statistical analysis of gender differences

Parameter [unit]	Blood			Saliva		
	Males	Females	Student's *t*-test value	Males	Females	Student's *t*-test value
K_a [h^{-1}]	4.015 ± 0.45	2.338 ± 0.42	2.73*	3.91 ± 0.48	3.96 ± 0.33	0.08
$t_{1/2a}$ [h]	0.206 ± 0.100	0.400 ± 0.210	2.89*	0.214 ± 0.103	0.201 ± 0.108	0.31
t_{max} [h]	0.874 ± 0.275	1.056 ± 0.247	1.71	0.869 ± 0.352	0.736 ± 0.114	1.25
C_{max} [g l^{-1}]	0.785 ± 0.165	0.606 ± 0.118	3.06*	0.801 ± 0.219	0.656 ± 0.112	2.03
V_{max} [g l^{-1} h^{-1}]	0.284 ± 0.182	0.474 ± 0.375	1.57	0.326 ± 0.237	0.373 ± 0.434	0.33
K_M [g l^{-1}]	0.420 ± 0.481	0.366 ± 0.438	0.29	0.374 ± 0.403	0.203 ± 0.367	1.09
β [g l^{-1} h^{-1}]	0.151 ± 0.052	0.175 ± 0.038	1.34	0.172 ± 0.055	0.185 ± 0.034	0.71
B_{60} [g kg^{-1} h^{-1}]	0.112 ± 0.035	0.136 ± 0.023	1.96	0.119 ± 0.034	0.132 ± 0.025	1.03
V_d [l kg^{-1}]	0.777 ± 0.215	0.795 ± 0.145	0.24	0.728 ± 0.208	0.723 ± 0.125	0.07
C_0 [g l^{-1}]	0.951 ± 0.202	0.777 ± 0.139	2.46*	1.025 ± 0.255	0.852 ± 0.145	2.05
AUC [g l^{-1} h]	3.486 ± 1.199	2.109 ± 0.422	3.75*	3.319 ± 1.164	1.961 ± 0.503	3.71*
AUMC [g l^{-1} h^2]	14.015 ± 7.519	5.666 ± 1.717	3.75*	11.149 ± 5.836	4.625 ± 1.871	3.69*
MRT [h]	3.841 ± 0.857	2.737 ± 0.434	3.98*	3.207 ± 0.629	2.289 ± 0.379	4.33*

* statistically significant (p = 0.05)

concentration profiles of ethanol in both specimens. The respective time courses are shown in Figure 1a and 1b, and the blood/saliva ratios are presented in Figure 1c. As it is seen in Figure 1, the concentration-time profiles of ethanol in saliva and blood followed a similar but not identical time course. Ethanol appears to reach a higher peak concentration in saliva than in venous blood, and to be eliminated from both compartments at different rates. In most of the cases, the concentrations of ethanol in saliva were higher than in blood during absorption (see Fig. 1c, up to 60 min), and slightly lower during elimination (see Fig. 1c, above 120 min).

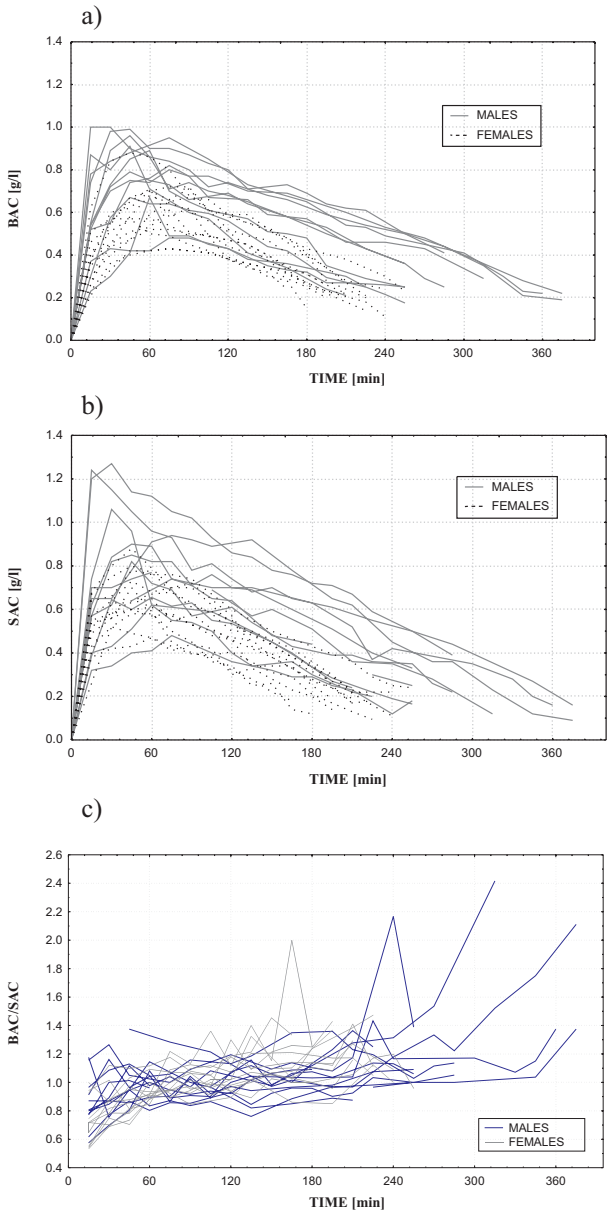

Fig. 1. Time-concentration profiles of ethanol in blood (a) and saliva (b), as well as the differences in ethanol concentration in both specimens during the whole course of its changes in the body (c). BAC – blood alcohol concentration, SAC – saliva alcohol concentration

The significant gender differences in the time concentration profiles could be observed. In contrast to Widmark's model prediction, the maximal ethanol concentrations, both in blood and saliva, were lower in women compared to men. In none of the tested person, the blood ethanol concentration exceeded the value calculated according to the Widmark formula. Moreover, in females ethanol was faster excreted from the body. They became sober after 3–4 h, whereas the excretion of ethanol in males persisted from 3.5 to over 6 h.

The pharmacokinetic parameters

In the study, the pharmacokinetic parameters describing processes of ethanol absorption, distribution and elimination were estimated according to the formulas shown in the Materials and Methods section. The results were summarized in Table 1.

The study showed that ethanol in blood was absorbed slower in females compared with males. It caused differences in shape of blood alcohol curves. Time to peak concentration for females appeared longer in relation to males, however, the difference turned out statistically insignificant ($p > 0.05$). Both experimental (C_{max}) and extrapolated to zero time (C_0) maximum ethanol concentrations were lower in females. The gender difference was statistically significant for ethanol concentrations in blood, but insignificant in saliva ($p = 0.054$ for C_{max} and $p = 0.053$ for C_0). The extrapolated values of C_0 in relation to gender and specimen were shown in Figure 2. The statistically significant differences were also observed for other pharmacokinetic parameters, i.e. AUC, AUMC and MRT. The values of the calculated apparent volumes of distribution after oral dose for the tested persons were shown in Figure 3.

DISCUSSION

The value of Pearson's correlation coefficient, equaled to 0.944 ($p < 0.0001$), shows high correlation between ethanol concentration in blood and saliva. The correlation was comparable with those obtained by other authors [12, 19], and very close

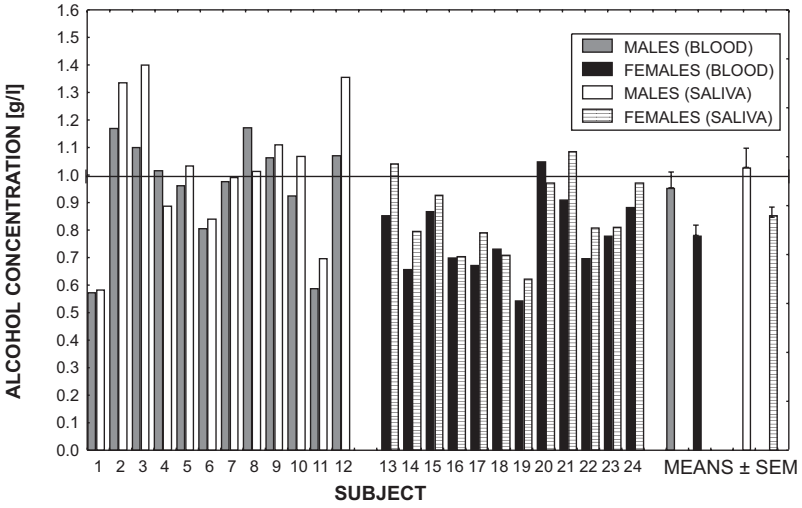

Fig. 2. Comparison of the maximum ethanol concentrations, extrapolated to zero time, depending on gender and specimen (in blood: 0.95 ± 0.20 and 0.78 ± 0.14 ($t = 2.46$, $p < 0.05$) for males and females, respectively; in saliva: 1.03 ± 0.26 and 0.85 ± 0.15 ($t = 2.05$, $p > 0.05$) for males and females, respectively)

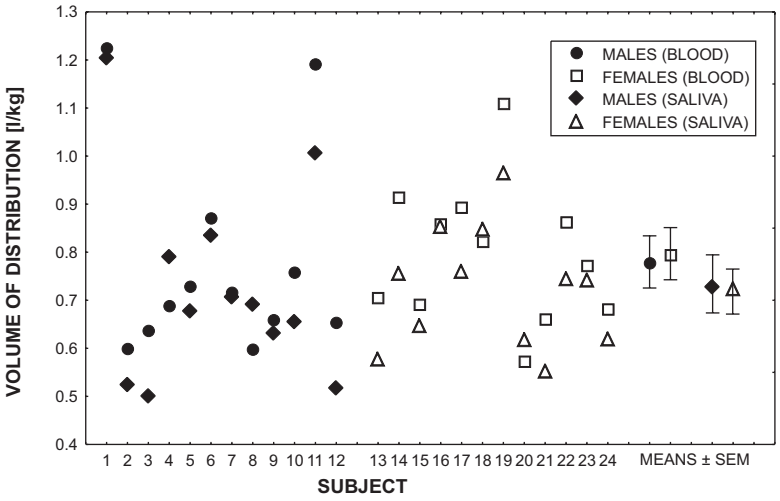

Fig. 3. The calculated apparent volumes of ethanol distribution after oral dose for the tested persons, depending on gender and specimen (in blood: 0.78 ± 0.22 and 0.80 ± 0.15 ($t = 0.24$, $p > 0.05$) for males and females, respectively; in saliva: 0.73 ± 0.21 and 0.73 ± 0.13 ($t = 0.07$, $p > 0.05$) for males and females, respectively)

to the values obtained for the relationship between ethanol concentrations in blood and breath [6, 18].

On the other hand, ethanol appeared to reach a higher peak concentration in saliva than in venous blood, and to be eliminated from both compartments at different rates. This phenomenon of different elimination rates, which leads to cross-over of the both concentration versus time curves, can be explained by differences in the water and lipids content of both body fluids [9]. Because saliva contains more water than blood, and respectively fewer lipids, there should be a higher concentration of alcohol in salivary secretion [13]. But in our study, the ethanol concentration in saliva was higher only during absorption and distribution phase. The faster elimination of ethanol from saliva caused that during elimination concentration of ethanol was higher in venous blood. The concentration of alcohol in saliva runs closer to the concentration in arterial blood compared with the venous blood. This might account for the somewhat different time profiles, namely arteriovenous differences in the pharmacokinetics of ethanol. Concentrations of ethanol in breath are also closer to concentrations in arterial blood [15], therefore, the time profiles for BrAC and SAC appears very similar [7].

The distinctions in many pharmacokinetic parameters can be caused by the fact that, according to Widmark's formula, different amounts of alcohol were given to women and men, whereas the calculated apparent volumes of distribution after oral dose were very similar for both groups. The values of this parameter both for saliva and blood were very close and did not differ statistically ($p > 0.05$). This finding might be explained by change in life style and diet of the women since Widmark has created his formula. The adequacy of Widmark equation's coefficient r (distribution factor – the ratio of body water to blood water), equal to 0.6 for women and 0.7 for men was questioned [5, 22]. Several alternative models, based on total body water volumes (TBW) and the body mass index (BMI), have been proposed in the literature [1, 4, 24]. Nevertheless, the gender differences in volume of distribution were included in those models. According to the study, we suggest using the same factor equivalent to volume of distribution in back calculation of alcohol concentra-

tion. Moreover, both blood and saliva can be used interchangeably for estimation of this parameter for ethanol. In our opinion, saliva alcohol analysis should be treated, similar to breath testing, as an independent method of assessment of the sobriety.

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