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The Relationship Between Ingested Dose of Ethanol and Amount of Ethyl Glucuronide Formed in Blood

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Abstract

A positive non-linear relation between the dose of ethanol ingested and the area under the curve (AUC) for ethyl glucuronide (EtG) in urine is previously observed. The relation between both doses and AUC of ethanol and the AUC for EtG in blood is not previously published, and this study aimed to investigate this relationship. After an overnight fast, 10 healthy volunteers ingested 0.5-g ethanol per kilo body weight (low dose) in one occasion and 1.0-g ethanol per kilo body weight (high dose) in the next occasion. Results showed that there was a significant higher median ratio between blood AUC for EtG and dose of ethanol in the high-dose (8.99; range 7.37–10.94) group compared to the low-dose (5.02; range 4.25–6.15) group ($P = 0.005$). The median ratio between the AUC for EtG and AUC for ethanol was actually significantly higher in the low-dose (1.77; range 1.51–2.24) group compared to the high-dose (1.67; range 1.30–2.02) group ($P = 0.005$), although values are quite similar. This study therefore showed that the ratio between the AUC for EtG in blood and dose of ethanol is higher after intake of 1.0 g/kg than 0.5 g/kg. This pattern is however not seen when AUC for EtG is compared to AUC for ethanol. Results therefore support that the percentage of ethanol converted to EtG is not increasing when the doses increase. An explanation for the positive non-linear relation previously observed between the dose of ethanol ingested and amount of EtG formed may be a relative higher first-pass metabolism of ethanol at lower doses.

Introduction

Ethanol is mainly metabolized by the hepatic oxidative system, and the main responsible enzyme is the alcohol dehydrogenase (ADH) (1). Ethyl glucuronide (EtG) is a minor non-oxidative ethanol metabolite formed by the enzyme 5′-diphospho-glucuronosyltransferase. EtG is widely used as a biomarker for alcohol intake (2).

Previous studies indicate that a very small amount (<0.1%) of the ingested alcohol is converted to EtG (3,4), but the relation between the dose of ethanol ingested and the percent of ethanol glucuronidated is not extensively studied. Only one previous article addresses this question properly, and Perez-Mana et al. conclude in

a comprehensive study that a positive non-linear relation between dose of ethanol ingested and area under the curve (AUC) for EtG in urine is observed. When the dose of ethanol was doubled, a more than doubling of the AUC for EtG in urine was seen. However, the relation between AUC of ethanol and AUC of EtG in urine is linear (5). This linear relation is expected, as it is difficult to imagine, from a pharmacokinetic point of view, that a higher percentage of metabolite is formed when more parent drug is present in blood. In fact, the opposite, a situation of enzyme saturation, where less proportion of the metabolite would be formed at higher concentrations of parent drug, is more likely expected. Experimental studies using very high

doses of ethanol are problematic because of ethical concerns. It is therefore important to know the relation between increasing doses of ethanol and which concentrations of EtG that can be expected. If the relation is linear, extrapolation can then be performed to very high ethanol doses. Although the blood kinetics of EtG is relatively well described, the relation between both doses and AUC of ethanol and the AUC for EtG in blood is not previously published, and this study aimed to investigate this relationship.

Materials and Methods

Study design and protocol

The study protocol and maximum concentrations (C_{max}), time to maximum concentrations and detection times as well as the concentration–time profiles for EtG are previously published (6). Briefly, 11 healthy volunteers ingested 0.5-g ethanol per kilo body weight in one occasion (over 15 min) and 1.0-g ethanol per kilo body weight in the next occasion (over 1 h) after an overnight fast (data missing for one subject after high-dose ingestion). Blood samples were collected before ingestion of ethanol and then at 1.5, 3.5, 5.5, 8.5, 11.5 and 24 h after intake and analyzed for ethanol and EtG using fully validated analytical methods. Ethanol was analyzed using a previously published method (7). EtG was analyzed using a previously published ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS-MS) method (8). The vodka ingested in the study was confirmed by the same method not to contain EtG.

Statistics

AUC values for EtG and ethanol in blood were not normally distributed. For all results, median and range values are reported for continuous variables. Results from one subject after ingestion of high dose were missing, and this subject was also excluded from the low-dose results due to use of paired sample statistics.

Differences between the high and low ethanol dose group were studied using nonparametric related sample Wilcoxon signed-rank test. EtG and ethanol concentrations are measured in milligrams per liter and grams per kilogram, respectively, and for AUC calculations, the concentrations are multiplied with hours.

The Kinetica software version 5.1 (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used for computing AUC for ethanol and EtG. The AUC from zero to the last sampling point (AUCall), calculated by using all the measured concentrations of ethanol and EtG, is reported. The software's standard parameters were used.

Results

Results are reported from 10 subjects after ingestion of both low and high doses of ethanol. The median C_{max} for EtG in blood was 0.35 mg/L (range 0.28–0.41) after ingestion of low dose of ethanol (0.5 g/kg body weight) and 1.06 mg/L (range 0.8–1.22) after ingestion of high dose of ethanol (1.0 g/kg body weight). The median C_{max} for ethanol was 0.55 g/kg (range 0.51–0.68) after ingestion of low dose of ethanol and 1.25 g/kg (range 1.11–1.52) after ingestion of high dose of ethanol.

The median and range AUC values for ethanol and EtG are presented in Table I. Also, Table I shows the relation between AUC for both ethanol and EtG and the dose of ethanol. This shows that there is a significant higher ratio between AUC for EtG and dose

of ethanol in the high-dose group compared to the low-dose group ($P = 0.005$). The ratio between the AUC for EtG and AUC for ethanol is actually significantly higher in the low-dose group compared to the high-dose group ($P = 0.005$), although values are quite similar. There is also significantly higher ratio between AUC for ethanol and the dose of ethanol in the high-dose group compared to the low-dose group ($P = 0.005$).

Discussion

This study showed a significantly higher AUC for EtG compared to dose of ethanol in a high-dose ingestion group compared to a low-dose ingestion group, while this pattern was not seen between the two dosing groups for the ratio between AUC for EtG and AUC for ethanol. This study therefore demonstrated, by the use of EtG kinetic data in blood, what was previously shown using only EtG concentrations in urine (5). When the dose of ethanol is doubled, it seems that EtG formation is more than doubled, but this is not the case when studying the relation between AUC for ethanol and AUC for EtG.

The difference when using the dose of ethanol ingested compared to the AUC for ethanol in the calculations could be explained by the fact that when increasing from low to higher doses of ethanol, the proportion of ethanol undergoing first-pass metabolism decreases (9), resulting in a larger fraction of ingested ethanol reaching the systemic bloodstream. Actually, our data support this theory by showing that the AUC for ethanol shows more than four times increase when the dose of ethanol is doubled. It is probable from a theoretical point of view that this difference in first-pass metabolism would be smaller when increasing from a high to a very high dose of ethanol (9). A very low dose of ethanol would be expected to show even higher first-pass metabolism compared to a 0.5g per kilo body weight dose, and the formation of EtG after such ingestion would be interesting to study with respect to the relation between the dose ingested, the AUC for ethanol and the AUC for EtG. It should also be noted that the present results cannot conclude on the reason for the difference when studying doses of ethanol compared to AUC for ethanol. First-pass metabolism is one possible explanation, but other factors could also be present.

From a theoretical point of view, it is difficult to imagine how the proportion of EtG formed would increase when more ethanol is available for metabolism. The opposite situation, where the enzymes become saturated, is more probable and would lead to an inverse non-linear relation. There are no clear published data indicating this for EtG or other glucuronidation reactions (10), but the main metabolizing system for ethanol, ADH, becomes saturated at very low concentrations of ethanol (11,12). It should be noted that the present results showed significantly higher ratio between AUC for EtG and AUC for ethanol in the low-dose group compared to the high-dose group, possibly indicating saturation of the glucuronidation process, but the absolute difference was very small.

The present findings are relevant when extrapolating from experimental data to real-life high ethanol concentration cases. The present results indicate that a doubling of ethanol concentration will not lead to more than a doubling of the EtG concentration. This is relevant when using EtG measurements in blood in, for instance, evaluation of hip-flask defense cases (13). If, for instance, an apprehended drunk driver has a measured blood ethanol concentration of 2.4 g/kg 1 h after driving, and claims that he only ingested alcohol after he left the car, no experimental data are available that tells us which maximum

Table I. Area Under the Curve (AUC) for Ethanol and EtG and the Ratio Between AUC for Ethanol or EtG and the Dose Ingested and the Ratio Between AUC for EtG and the AUC for Ethanol (Median and Range Values)

Dose of ethanol (g/kg body weight)	AUC ethanol (g/kg∗h)	AUC ethanol/dose of ethanol (g/kg∗h)/(g/kg)	AUC EtG (mg/L∗h)	AUC EtG/dose of ethanol (mg/L∗h)/(g/kg)	AUC EtG/AUC ethanol (mg/L∗h)/(g/kg∗h)
0.5 (n = 10)	1.33 (1.06–2.00)	2.67 (2.11–3.99)	2.51 (2.12–3.07)	5.02 (4.25–6.15)	1.77 (1.51–2.24)
1.0 (n = 10)	5.64 (4.21–6.25)	5.64 (4.21–6.25)[a]	8.99 (7.37–10.94)	8.99 (7.37–10.94)[a]	1.67 (1.30–2.02)[b]

[a]Significantly higher ratio in the high-dose group compared to the low-dose group ($P = 0.005$). [b]Significantly higher ratio in the low-dose group compared to the high-dose group ($P = 0.005$).

concentrations of EtG could be seen after such a high intake of ethanol. The present results support that experimental data from subjects showing a blood ethanol concentration of 1.2 g/kg after a single dose (6) can be used and that expected concentrations of EtG will be approximately doubled.

The present study has some weaknesses which need to be addressed. Firstly, the blood sampling was not frequent enough to include the exact C_{max} values for ethanol, and the differences in ethanol concentrations after low- and high-dose ingestion are less evident from the C_{max} values compared to the AUC values. Also, the infrequent sampling made calculation of distribution volume (V_d) for ethanol impossible, information that would be interesting when first-pass metabolism is considered. Finally, the data did not include ethyl sulfate concentrations, which could be interesting to compare to EtG.

In conclusion, this study supported that the percentage of ethanol converted to EtG is not increasing when the doses increase. An explanation for the non-linear relation previously observed between the dose of ethanol ingested and amount of EtG formed may be a relative higher first-pass metabolism of ethanol at lower doses, but other explanations are also possible.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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