Comparison of Urinary Excretion Characteristics of Ethanol and Ethyl Glucuronide

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Abstract

This study compared the urinary excretion characteristics of ethyl glucuronide (EtG) with that of ethanol, with focus on the effect of water-induced diuresis. Six healthy volunteers ingested an ethanol dose of 0.5 g/kg (range 25.0–41.5 g) as 5% (v/v) beer in 30 min and the same volume of water after 3 h. Urine collections were made before starting the experiment and at timed intervals over 31.5 h. The concentration of EtG was determined by an LC–MS method (LOQ = 0.1 mg/L). The urine samples collected immediately before starting drinking were all negative for ethanol and EtG, thus confirming that the participants had not recently ingested alcohol. Intake of beer resulted in a marked increase in excreted urine volume and a concomitant drop in creatinine concentration. The concentration of ethanol peaked at a mean value of 17 mmol/L in the 1.5-h urine collection. Except for one subject, EtG was first detectable (range 0.9–5.5 mg/L) at 1 h. Intake of water at 3 h produced another increase in urine volume and a drop in creatinine. The ethanol concentration curve was not influenced by the water diuresis, whereas this caused a distinct drop in the EtG concentration. When EtG was expressed relative to the creatinine value, this ratio was seemingly not affected by the intake of water. The ethanol concentration returned to zero at 6.5 h, whereas EtG was still detectable for up to 22.5–31.5 h, albeit at low levels in the end (< 1 mg/L). Only about 0.02% of the administered dose of ethanol (on a molar basis) was recovered in the urine as EtG. The results demonstrated that EtG remains detectable in the urine for many hours after the ethanol itself has been eliminated. Moreover, it was possible to lower the concentration of EtG by drinking large amounts of water prior to voiding, whereas this strategy did not influence the EtG/creatinine ratio or the concentration of ethanol.

Introduction

Following ingestion of alcoholic beverages, between 92 and 95% of the ethanol dose is metabolized mainly in the liver in a two-stage oxidation process, first to acetaldehyde and then to acetic acid (acetate). The remaining part is mainly excreted unchanged in urine, breath, and through the skin with sweat (1). However, another small fraction of the ethanol becomes conjugated by reaction with uridine 5'-diphosphoglucuronic acid (UDPGA), a reaction catalyzed by the endoplasmic reticulum UDP-glucuronosyltransferase (UGT) enzymes (2,3), and is excreted in the urine as ethyl glucuronide (ethyl β-glucuronide, EtG) (4–6). Normally, conjugation pathways (phase II reactions) are important detoxification processes whereby a wide variety of lipophilic endogenous compounds and xenobiotics are made more water-soluble to facilitate their elimination from the body in urine or bile. The liver is the principal site for the formation of glucuronide conjugates.

In recent years, there has been an interest in EtG as a biochemical marker for acute alcohol ingestion that is primarily due to the observation that the washout rate for EtG is much slower than for the parent compound (7,8). Accordingly, a positive finding of EtG in blood or urine provides a strong indication that the person was recently drinking alcohol, even if the ethanol itself is no longer detectable. The observation of a poor correlation between urinary concentrations of ethanol and EtG (7) is therefore not unexpected but may be explained by the time-lag between their excretion profiles. Another important factor, which could possibly also explain the considerable interindividual variation in EtG concentrations (8), is the impact of fluid intake prior to sampling, but this has not yet been investigated. It is thus still unknown whether the concentration of EtG in urine is dependent on dilution of the sample, for example after drinking large volumes of water, whereas it is well known that diuresis does not change the concentration of ethanol even though the creatinine content becomes markedly reduced (9).

This study was carried out to examine further the urinary excretion characteristics of EtG in comparison with that of ethanol, with special emphasis on the possible interference by water-induced diuresis.

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Materials and Methods

Subjects and experimental design

Six healthy volunteers (three women and three men), all social drinkers, with a mean age of 41 ± 11 years (SD, range 25–54) and a mean body weight of 69 ± 12 kg (range 50–83), participated in a controlled experiment. According to self-report, all participants had abstained from alcoholic beverages for at least 48 h before the experiment began. The abstention from alcohol prior to taking part in the experiment was confirmed by urinary 5-hydroxytryptophol to 5-hydroxyindole-3-acetic acid ratios (5HTOL/5HIAA) in the range of 3–8 nmol/μmol, which is well below the reference value of < 15 nmol/μmol for this marker of acute alcohol consumption (10).

A light morning meal was consumed approximately 1.5 h before starting the experiment. A first urine collection was made immediately before drinking export beer (5.3% ethanol, v/v) at a fixed dose of 0.5 g per kg body weight (corresponding to 594–986 mL beer) in 30 min (at ~08:30–09:00 a.m.). At 3.0 h after starting drinking beer, the subjects drank exactly the same volume of tap water in 10 min. Urine collections were made at timed intervals (see figures) until bedtime and in the next day for a total of 31.5 h. The volume of the urine specimens was measured and 10-mL aliquots were stored in plastic tubes at –20°C until analysis. The subjects gave informed consent, and the study protocol was approved by the Ethics Committee at the Karolinska Hospital.

Analysis

The concentration of EtG in urine was determined by a negative ion electrospray liquid chromatographic–mass spectrometric (LC–MS) method as described in detail elsewhere (11). In short, urine was mixed 1:10 (v/v) with an internal standard (deuterium-labeled EtG in distilled water; EtG-d_5) and a 10-μL portion was injected directly into the LC–MS system (PerkinElmer 200 LC system and Sciex API 2000 MS) equipped with a 5-μm Hypercarb analytical column (100 × 2.1-mm i.d.). The mobile phase consisted of 25 mmol/L formic acid with 5% acetonitrile pumped isocratically at 0.2 mL/min at ambient temperature. The ions monitored were *m/z* 221 for EtG and *m/z* 226 for EtG-d_5. A calibration curve covering 0.1–200 mg/L (0.45–900 μmol/L) EtG was run with every batch of samples (the calibration curve was linear up to 1.500 mg/L), and controls in the same range or blanks constituted ~7% of the total number of samples analyzed. The intra-assay CVs of the method were 8.9 and 1.9% at EtG concentrations of 0.9 and 306 mg/L, respectively (n = 10); the interassay CVs were 10.6 and 9.1% at concentrations of 0.8 and 375 mg/L, respectively (n = 12). The EtG concentration of unknown samples was determined from the peak-area ratio between EtG and EtG-d_5 by reference to the calibration curve. The limit of quantitation (LOQ) of the LC–MS method was 0.1 mg/L (signal-to-noise ratio > 5).

The ethanol concentration in urine was determined enzymatically using yeast alcohol dehydrogenase (LOQ = 1 mmol/L) (12). Urinary creatinine was determined by the routine Jaffe reaction on a Hitachi 917 analyzer (LOQ = 0.5 mmol/L).

EtG and EtG-d_5 were purchased from Medichem Diagnostica or prepared enzymatically from ethanol and ethanol-d_5, respectively (11). All other chemicals were of analytical grade. Data are given as means, SDs, and ranges, unless otherwise stated.

Results

The urine samples collected immediately before starting drinking beer (at zero time) were all negative for ethanol (< 1 mmol/L) and EtG (< 0.1 mg/L). Intake of ethanol at 0.5 g/kg resulted in a marked increase in urine volume and a concomitant drop in the creatinine concentration (Figure 1A). The concentration of ethanol increased rapidly and reached a mean peak value of 17.0 ± 2.5 (SD) mmol/L in the 1.5-h urine collection (Figure 1D). The EtG concentration also started to

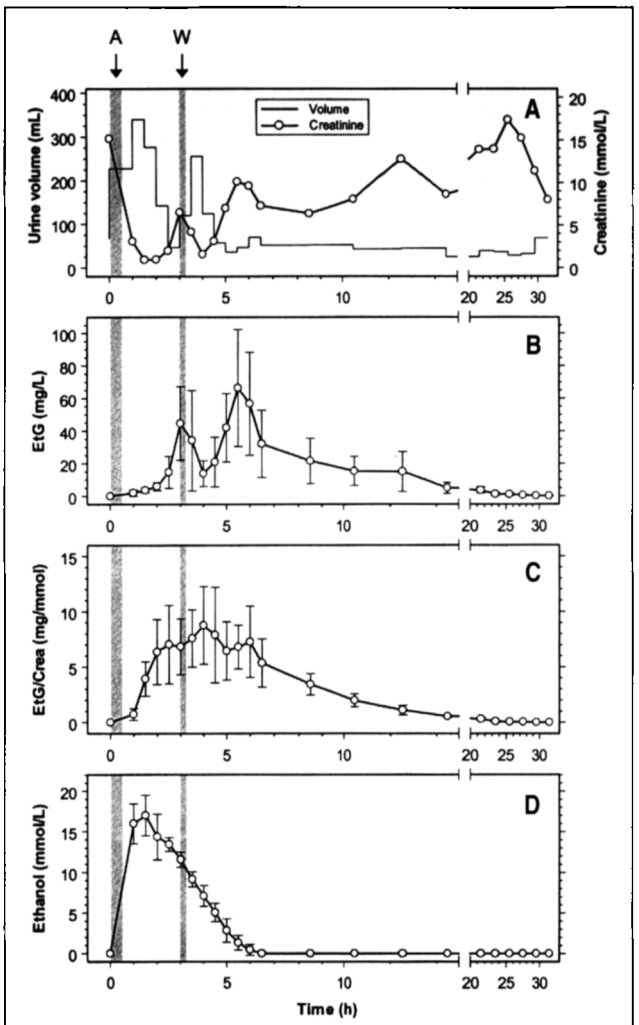

Figure 1. Time-course of diuresis and urinary creatinine concentration (A), the urinary concentration of EtG (B), the urinary EtG/creatinine ratio (C), and the urinary concentration of ethanol (D) after six healthy volunteers drank 594–986 mL export beer (range 25.0–41.5 g ethanol, corresponding to 0.5 g/kg) in 30 min (A, first shaded area) and 3 h later the same volume of tap water in 10 min (W, second shaded area). Data represent mean values ± SD.

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increase and, except in one subject, was detectable (range 0.9–5.5 mg/L EtG) already in the first urine collection after the alcohol intake at 1.0 h (Figure 1B).

At 3 h after starting drinking the beer, when the ethanol-induced diuresis was declining and urinary creatinine increasing, intake of the same volume of water produced another increase in urine volume and a drop in the creatinine concentration (Figure 1A). The water-induced diuresis was much less pronounced (mean AUC ~40%) compared with that observed after drinking beer. The ethanol excretion curve was seemingly not influenced by the water intake (Figure 1D), whereas this caused a distinct drop in the rising urinary EtG concentration curve from a mean value of 44.6 ± 22.6 (SD) mg/L at 3 h to 13.8 ± 7.9 mg/L 1 h later (Figure 1B). Thereafter, EtG increased again and peaked at 66.4 ± 35.9 mg/L (range 20.6–124) at 5.5 h. After the 6-h urine collection, when ethanol returned to zero, the EtG concentration started to fall with a half-life of ~2.5 h.

When EtG was instead expressed relative to the urinary creatinine concentration, this ratio was not markedly affected by the water intake (Figure 1C). The EtG/creatinine ratio increased to a plateau of 6.4–8.8 mg/mmol (mean values; range for individual values 2.7–15.2) at between 2 and 6 h, with the peak value of 8.8 ± 3.5 mg/mmol observed in the 4-h urine collection. Thereafter, the EtG/creatinine values decreased with a half-life of ~2.5 h. As demonstrated in Figure 2, there was a considerable variation in the EtG/creatinine excretion profiles between different individuals.

The urinary ethanol concentration returned to below the limit of quantitation at 6.5 h, whereas EtG was detectable at low levels (< 1 mg/L) for up to 22.5–31.5 h after starting the experiment. Of the orally administered dose of ethanol (mean 34.6 g, range 25.0–41.5 g), a total of 31.3 ± 9.3 mg EtG was recovered in the urine, and this corresponds to about 0.02% (range 0.013–0.025%) on a molar basis.

Discussion

EtG is of interest as a potential marker of acute alcohol intake because it is likely to be very specific for alcohol consumption and, furthermore, can be detected in samples of blood and urine for several hours after the parent compound is no longer present (7,8). Determination of EtG can thus be used to detect recent intake of alcohol, even when the ingested ethanol dose has already been eliminated from the body.

In the present study, healthy volunteers drank beer at a fixed dose of 0.5 g ethanol per kilogram body weight. When ethanol was no longer measurable in urine, EtG was eliminated with a half-life of ~2.5 h which is in good agreement with previous observations (7). Compared with ethanol, EtG was detectable in the urine for up to ~15–25 h extra, albeit at low concentrations (< 1 mg/L) in the end. A few occasional observations on alcohol-dependent patients undergoing detoxification suggested that EtG could be detected in the urine for > 80 h (13), thereby indicating that EtG may accumulate in the body after repeated heavy drinking. However, these preliminary findings have not yet been confirmed or examined in detail.

The present study pointed at one important limitation of this new alcohol marker. It was demonstrated that ingestion of a water load prior to urine sampling lead to a dramatic reduction in the EtG concentration, whereas this, in accordance with previous observations (9), did not influence the concentration of ethanol. This is explained by ethanol being excreted by the kidney according to a passive diffusion process. Accordingly, it is possible to deliberately lower the urinary concentration of EtG, and most likely of other conjugated metabolites as well, simply by drinking moderate to large amounts of water or other fluid prior to voiding. This is a well-known strategy among abusers to avoid detection in testing for illicit drugs (14). Considering the dramatic effect of water diuresis observed when the EtG concentration in urine was rising and close to its maximum, this effect is likely to be even more pronounced on the descending part of the concentration-time curve. It should be pointed out that it is during this time period that EtG could play an important role as a test for recent alcohol consumption, but water-induced diuresis may then cause the already low EtG concentration to fall below the limit of detection, or quantitation, of the analytical method used.

The present study also indicated that the interference by water-induced diuresis on the EtG concentration could be overcome by calculating the EtG/creatinine ratio. The normalization of values to creatinine is common practice to compensate for unusually dilute or concentrated urine samples and has, for example, been used to improve interpretation of the pharmacokinetics of conjugated metabolites of illicit drugs (15). Even when the EtG values were expressed as a ratio to creatinine, there were considerable interindividual variations in the excretion profiles. However, in addition to the diuresis effect due to fluid intake and the water content of the alcoholic beverage consumed (e.g., beer vs. hard liquor), there are several other factors that may possibly influence the urinary concentration of EtG. Additional studies are therefore demanded to consider the potential interactions of enzyme activity, induction, and polymorphism of

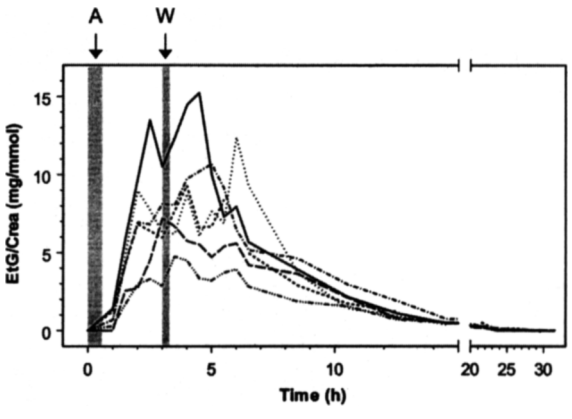

Figure 2. Individual time-course curves for the urinary EtG/creatinine ratio in six healthy subjects after ingestion of 0.5 g/kg ethanol as beer in 30 min (A, first shaded area) and the same volume of tap water 3 h later in 10 min (W, second shaded area).

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the UGT enzymes (2), as well as of individual differences in ethanol distribution and elimination, on the excretion characteristics of EtG. Use of the EtG/creatinine ratio instead of the EtG concentration apparently did not reduce the window of detection (i.e., sensitivity) of the marker to indicate recent alcohol consumption. It should be noted that even this strategy will not be useful once the EtG concentration has become diluted to below the limit of quantitation, and urine dilution will thus remain a way to shorten the detection time.

In studies on rabbits (4), the elimination pathway for ethanol via reaction with UDPGA to form EtG was briefly reported to account for 0.5–1.6% of the administered ethanol dose (~0.8–3.6 g/kg), the amount increasing with increasing ethanol dose. These values have thus far also been employed for humans. However, the results of the present study indicate that this metabolic pathway is considerably less important in humans, as only about 0.02% of the ingested 0.5 g/kg dose of ethanol (on molar basis) was recovered in the form of EtG in the urine. Whether the relative formation of EtG in humans is dependent on the dose of ethanol remains to be elucidated.

In conclusion, the results of the present study confirmed that EtG remains detectable in the urine for many hours after ethanol itself has been eliminated. Thus, testing urine for the presence of EtG provides a means to determine if a person has recently consumed alcohol. However, the results also showed that it is possible to lower the urinary concentration of EtG simply by drinking large amounts of water prior to voiding, whereas this strategy did not influence the concentration of ethanol or the EtG/creatinine ratio. Expressing urinary EtG as a ratio to creatinine therefore should be recommended in routine clinical use to compensate for urine dilution. Finally, it was demonstrated that conjugation with glucuronic acid to form EtG represents a minor pathway for ethanol elimination in humans, and as such even less important than previously believed.

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