DETECTION AND ASSESSMENT
Urinary Ethyl Glucuronide and Ethyl Sulfate Testing for Recent Drinking in Alcohol-Dependent Outpatients Treated with Acamprosate or Placebo

Helen Dahl1, Anders Hammarberg2, Johan Franck2 and Anders Helander1,*

1Alcohol Laboratory, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden and 2Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

*Corresponding author: Anders Helander, C1:74, Clinical Chemistry, Karolinska University Hospital Huddinge, SE-141 86 Stockholm, Sweden. Tel.: +46-8-58581293; E-mail anders.helander@ki.se

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Abstract — Aims: Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are sensitive and specific biomarkers for recent alcohol ingestion. This study compared urinary EtG and EtS measurement with self-reports for detection of prior drinking in alcohol-dependent outpatients treated with the anti-craving medication acamprosate or placebo. Methods: Alcohol-dependent outpatients (26 women, 30 men) were randomized to 21 days of oral acamprosate (2 g/day) or placebo treatment in a double-blind design. They were instructed to refrain from drinking during the study. Return visits to the ward for blood and urine sampling and filling out questionnaires were made on Day 7, 14 (urine sample optional) and 22 (urine sample mandatory). EtG and EtS were determined by liquid chromatography-mass spectrometry. Results: On the first day (Day 0), 72% of all patients (acamprosate 65%, placebo 78%) tested positive for recent drinking according to urinary EtG (reporting limit ≥0.5 mg/l) and EtS (≥0.1 mg/l). On the final day (Day 22), the frequency of positive tests was significantly reduced to 30% in the acamprosate group (P = 0.0374) and 33% for placebo (P = 0.0050). However, there was no difference between the treatment groups. When both groups were combined, the EtG (P = 0.025) and EtS (P = 0.015) concentrations were lower on the final day. Altogether, EtG and EtS were detected in 76 of 156 (49%) urine samples. When drinking in the day before sampling was admitted, 93% of urines tested positive; when drinking was denied, still 28% of the samples were positive. Conclusion: These results confirmed the value of urinary EtG and EtS as reliable indicators of recent drinking during outpatient treatment of persons with alcohol-related problems, and as objective outcome measures when evaluating new alcohol treatment strategies and pharmacotherapies.

INTRODUCTION
Excessive use and abuse of alcohol represent major health, social and economic problems for the individual and for the society. Efforts aiming to reduce alcohol-related harm include early detection of those with riskful drinking habits by clinical interview or use of questionnaires and standardized instruments such as the Alcohol Use Disorders Identification Test (AUDIT) and time-line follow-back methods. However, because many people deny alcohol ingestion or at least underreport the true amount they ingested or at least underreport the true amount they ingested, obtaining reliable information about a person’s drinking behavior and identifying early-stage alcohol-related problems are difficult tasks (Helander et al., 1999).

Various laboratory tests (alcohol biomarkers) are also employed to estimate the quantity and frequency of drinking (Helander, 2003; Hannuksela et al., 2007; Niemelä and Alatalo, 2010). Alcohol biomarkers are generally considered more objective indicators than self-report measures and have demonstrated useful in a number of clinical and forensic situations (Erim et al., 2007; Neumann et al., 2008; Helander et al., 2009b; Palmer, 2009; Hermansson et al., 2010), such as detection of relapse and confirmation of abstinence during outpatient treatment. Sensitive and specific biomarkers targeting riskful long-term drinking are carbohydrate-deficient transferrin (CDT) (Arndt, 2001; Bortolotti et al., 2006) and phosphatidylethanol (Helander and Zheng, 2009).

To confirm abstinence or rule out very recent alcohol use, measurement of ethanol in breath or blood is a standard method. A drawback is that ethanol is rapidly eliminated from the body, limiting the maximum detection time to <12 h even after binge drinking (Helander et al., 1996a). Measurement of conjugated ethanol metabolites, ethyl glucuronide (EtG) (Schmitt et al., 1995) and ethyl sulfate (EtS) (Helander and Beck, 2004) is an alternative way to detect recent drinking, because they exhibit an extended excretion time window compared with ethanol. Although accounting for only <0.1% of the ingested ethanol dose, EtG and EtS are detectable in urine for several hours up to some days longer than the parent compound, the time-lag largely being dose-dependent (Dahl et al., 2002; Hoiseth et al., 2008; Helander et al., 2009a). Given that EtG and EtS are direct ethanol metabolites, a positive finding is considered a reliable indicator of recent intake of alcohol, even when ethanol is no longer detectable. However, false-negative and false-positive EtG (but not EtS) results may occur in case the infected samples are not stored properly prior to analysis (Helander et al., 2007, 2009b).

In people diagnosed with alcohol abuse or dependence, pharmacological medications are employed to prevent (or reduce) drinking (Garbutt, 2009). Disulfiram (Antabuse) is a well-known alcohol-deterrent drug that acts by inhibiting the enzyme aldehyde dehydrogenase. Alcohol ingestion while on disulfiram thus causes acetaldehyde (the first metabolite of ethanol oxidation) to accumulate, leading to unpleasant adverse effects (such as facial flushing and nausea) known as the disulfiram–ethanol reaction. Agents that modulate neurotransmitter systems, thereby presumably reducing the craving for alcohol, are also in routine use. Naltrexone, a non-selective opioid antagonist and acamprosate (calcium acetyl homotaurinate), a modulator of glutamate neurotransmission, have demonstrated some ability to reduce drinking and increase the time spent abstinent (Kiefer et al., 2005; Verheul et al., 2005; Morley et al., 2006).

This outpatient study compared urinary EtG and EtS testing with self-reports as ways to detect drinking in
alcohol-dependent subjects participating in a randomized double-blind evaluation to determine the effect of 21-days acamprosate medication on alcohol-cue reactivity and alcohol priming (Hammarberg et al., 2009a,b).

MATERIALS AND METHODS

Patients
The study involved 56 treatment-seeking individuals (26 women and 30 men; mean age 50 years) recruited via advertisements in a local newspaper (Hammarberg et al., 2009a). Inclusion criteria were a male or a non-pregnant/non-nursing female aged 18–65 years fulfilling the criteria for alcohol dependence according to the Diagnostic and Statistical Manual of Mental Disorders, fourth version (DSM-IV), with a goal of controlled drinking, willingness to give informed consent and comply with the study procedures, and having consumed alcohol on at least 15 of the last 90 days according to self-report. Exclusion criteria were seeking complete abstinence, a psychiatric disorder diagnosis according to DSM-IV (including all forms of substance dependence other than nicotine and alcohol), current use of psychoactive medications to manage schizophrenia, a bipolar disorder or major depression, inpatient alcohol detoxification within the last 4 days, acamprosate medication during the last 12 months and use of illegal drugs during the course of the study.

The study was conducted at the Stockholm Addiction Centre at the Karolinska University Hospital (Stockholm, Sweden). It was approved by the regional ethical review board and the Swedish Medical Products Agency and was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki (Dnr KI 2007/995-31). All patients gave written informed consent to participate.

Study design
Patients were randomized to 21 days of either acamprosate [2 g/day; n = 28, 14 female/14 male; mean age 50.2 ± 7.6 (SD) years] or placebo (n = 28, 12 female/16 male, mean age 49.8 ± 7.3 years) treatment in a double-blind design, as detailed elsewhere (Hammarberg et al., 2009a). The length of the treatment period was set based on observations of plasma steady state acamprosate levels after ~1 week of treatment (Mason et al., 2002). Return visits to the ward to meet a nurse for blood and urine sampling and the study coordinator for relapse prevention and filling out questionnaires were made on Day 7, 14 (leaving a urine sample was optional on these days) and 22 (leaving urine was mandatory). The patients were also questioned about any alcohol consumption, expressed as the number of standard drinks (12 g ethanol) per day over the past 3 days, using a time-line follow-back method. While they were instructed to refrain from alcohol during the treatment period, evidence of drinking was not grounds for study discontinuation unless warranted for clinical safety. However, they were required to give a negative breath alcohol test on Day 22. Compliance to acamprosate medication was confirmed by analysis of urine and plasma (Hammarberg et al., 2009a).

RESULTS
On the initial day of the study (Day 0), a urine sample was obtained from 46 (82%) patients. Because leaving urine was optional on Day 7 and 14, much fewer urine samples were collected on those days (52 and 61%, respectively), and these results were therefore excluded from the calculations. On the final study day (Day 22), 47 patients (87%) delivered the mandatory urine sample. From 19 patients on acamprosate medication and 21 on placebo, a urine sample was obtained on both Day 0 and 22.

On the first study day, 33 of the 46 patients (72%; 65% for acamprosate and 78% for placebo) who delivered a urine sample tested positive for recent drinking according to EtG and EtS (i.e. concentrations above the reporting limit). On the final day, the frequency of positive urine tests was significantly reduced to 30% among those randomized to acamprosate medication (P = 0.0374) and to 33% for the placebo group (P = 0.0050) (Fig. 1). However, there were no significant differences between the treatment groups. When the results for both groups were combined, the urinary EtG (n = 40, P = 0.0251) and EtS (P = 0.0146) concentrations on Day 22 were significantly lower compared with those on Day 0.

EtG and EtS analysis in urine samples
The urine samples were stored at −20°C until taken for EtG and EtS analysis using a liquid chromatography-mass spectrometry method with selected ion monitoring in negative mode (Helander and Beck, 2005). The ions monitored were m/z 221 for EtG, m/z 226 for EtG-d5 (penta-deuterated internal standard), m/z 125 for EtS and m/z 130 for EtS-d5 (internal standard). The EtG and EtS concentrations were calculated from the peak-area ratio to the corresponding internal standard, with reference to calibration curves. The detection limits (LOD) were <0.05 mg/l for both substances and the routine reporting limits were 0.5 mg/l for EtG and 0.1 mg/l for EtS (Helander et al., 2009a, 2010).

![Fig. 1. Frequency of urine samples testing positive for EtG and EtS in alcohol-dependent patients randomly allocated to 21 days of acamprosate medication (2 g/day) or placebo. Samples were collected on the initial (Day 0) and final (Day 22) day of the study.](https://academic.oup.com/alcalc/article-abstract/46/5/553/128804)
Altogether, 63 urine samples from 41 patients (21 on acamprosate and 20 on placebo) of the totally 156 (40.4%) specimens collected within this study tested positive for EtG (range: 0.50–300 mg/l, mean 44.6, median 15.2) and EtS (range: 0.11–61.0 mg/l, mean 10.2, median 3.78). When separated by treatment group, there were 30 of 80 (36.5%) EtG and EtS positive samples in the patients on acamprosate, and 33 of 76 (43.4%) positives in the placebo group. The proportion of positive urine samples ($P=0.4739$), or the corresponding EtG ($P=0.5357$) and EtS ($P=0.9397$) concentrations, did not differ significantly between acamprosate medication and placebo.

In addition to the 63 EtG and EtS positive urine samples, another 13 (8.3%) contained measurable amounts (i.e. >LOD) of both metabolites but at levels below the routine reporting limit. Accordingly, based on EtG and EtS testing, close to half (48.7%) of all the urine specimens indicated recent drinking. In 26 of the 63 EtG and EtS positive cases (41.3%), the patient admitted alcohol consumption (range: 1–8 standard drinks; mean: 4.3 ±50 g) on the previous day; in 19 of these, drinking was also admitted 2 and/or 3 days back (Fig. 3). In another 33 cases (52.4%), drinking was not admitted on the previous day but 2 and/or 3 days back. Only two patients denied drinking over the past 3 days. Self-report data were missing for two subjects. Among 28 cases where the patients reported alcohol consumption (range 1–8 drinks, mean 4.1 ±50 g ethanol) on the day before urine sampling, 26 (92.9%) specimens tested positive for EtG and EtS. In the remaining cases where no drinking on the previous day was admitted, 35 (27.6%) samples were positive for EtG and EtS.

In the 93 urine samples that tested negative for EtG and EtS (i.e. not detectable or below the reporting limit), there were no positive self-reports of drinking on the day prior to sampling, while in 15 cases, alcohol consumption was admitted 2 and/or 3 days back.

Altogether there were 77 cases of self-reported drinking over the 3-days period prior to each sampling, ranging from 1 to 25 standard drinks (i.e. 12–300 g ethanol, mean 7.3 drinks ±90 g ethanol). In 25 of 26 patients allocated to acamprosate medication and in 23 of 25 on placebo (data missing for one patient), drinking was admitted on a total of 130 of 465 (28%) individual days (each interview covered the preceding 3 days). Overall the self-reported quantity of drinking over the past 3 days prior to urine sampling showed a good correlation with the EtG ($r=0.662$, $P<0.001$) and EtS ($r=0.716$, $P<0.001$) concentrations.


discussion

This study compared urinary EtG and EtS measurement with self-report as methods to obtain information about recent alcohol use during outpatient treatment of alcohol-dependent subjects. The study population comprised treatment-seeking patients participating in a 3-week randomized double-blind study to determine the effect of acamprosate medication on alcohol-cue reactivity and alcohol priming (Hammarberg et al., 2009a,b). The patients were instructed to refrain from alcohol during the treatment period, and the goal of abstinence was aided by relapse prevention in connection with weekly return visits to the ward. However, evidence of drinking was not a ground for study discontinuation, except for having a positive breath test on the day of the cue- and alcohol-priming sessions.

Given that self-reports of drinking may be unreliable (Helander et al., 1996b, 1999), particularly in situations when admitting alcohol use is likely to have negative consequences for the individual, this study employed urinary EtG and EtS measurement as a sensitive and specific objective indicator of recent alcohol consumption. The present results indicated a dose- and time-dependent sensitivity of urinary EtG and EtS as biomarkers for detection of recent drinking. This agrees with previous studies, demonstrating that both metabolites can pick up ingestion of even small amounts of...
alcohol for roughly 1 day, and large amounts for 2–3 days afterwards (Stephanson et al., 2002; Halter et al., 2008; Hoiseth et al., 2008, 2010; Helander et al., 2009a).

Despite the efforts taken to reach abstinence during the treatment period, there were numerous indications of recent alcohol use, both according to the results of urinary EtG and EtS testing and self-report using a time-line follow-back method. In many cases, the positive EtG and EtS tests were supported by self-reports of drinking but the biomarkers also revealed a large number of occasions when prior drinking had been denied. Overall, the results indicated that both the frequency and amount of drinking were significantly reduced at the end of the 3-week treatment period compared with the starting values. However, in this respect, acamprosate offered no advantage over placebo. The present observations are in line with those of a 12-week clinical trial (Kiefer et al., 2003), reporting a significant effect of acamprosate medication over placebo regarding time to first drink and time to relapse based on self-report data, but with no difference in alcohol biomarker levels (e.g. CDT) between study groups.

In the original study (Hammarberg et al., 2009a), acamprosate was found to attenuate the subjective craving induced by alcohol priming, and patients also reported reduced craving during the 3-week treatment period prior to the priming session. Despite this, no treatment effect on the quantity and frequency of drinking was demonstrated in this evaluation. It should be noted that the patients were instructed not to use alcohol during the study, which might explain the discrepancy between craving and drinking measures. Furthermore, the correlation between the alcohol craving and drinking measures is not straightforward; alcohol-dependent patients may experience craving without consuming alcohol (Drummond, 2001).

In conclusion, the results of the present study highlighted the value of urinary EtG and EtS as reliable indicators of recent drinking during outpatient treatment of patients with alcohol-related problems, and as objective outcome measures when evaluating new treatment strategies and pharmacotherapies.

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REFERENCES


