ETHYL GLUCURONIDE — A MARKER OF ALCOHOL CONSUMPTION AND A RELAPSE MARKER WITH CLINICAL AND FORENSIC IMPLICATIONS

FRIEDRICH MARTIN WURST*, CHRISTOPH KEMPTER1, STEPHAN SEIDL2 and ANDREAS ALT2

Department of Psychiatry II, University of Ulm, 1 Department of Hydrochemistry and Hydrobiology, University of Stuttgart and 2 Department of Forensic Medicine, University of Ulm, Germany

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Abstract — Ethyl glucuronide (EtG) is a non-volatile, water-soluble, direct metabolite of ethanol that can be detected in body fluids and hair. We investigated urine and serum samples from three patient groups: (1) 33 in-patients in acute alcohol withdrawal; (2) 30 detoxified in-patients (treated for at least 4 weeks) from a ‘motivation station’; and (3) 43 neuro-rehabilitation patients (non-alcoholics; most of them suffering from stroke, traumatic brain injury, Parkinson’s disease etc.) using gas chromatography/mass spectrometry (GC/MS) with deuterium-labelled EtG as the internal standard and additionally in the second group of patients using liquid chromatography (LC/MS-MS). We found no correlation between the concentration of EtG in urine at hospitalization and the blood-ethanol concentration ($r = 0.17$), the time frame of detection ($r = 0.5$) or the total amount of clomethiazole required for the treatment of withdrawal symptoms ($r = 0.28$). In four out of 30 in-patients from the ‘motivation station’ — where neither clinical impression nor routine laboratory findings gave indications of relapse — concentrations of EtG in urine ranged between 4.2 and 196.6 mg/l. EtG concentrations in urine of between 2.89 and 23.49 mg/l were found in seven out of 43 neuro-rehabilitation patients using GC/MS. The GC/MS and the LC/MS-MS results showed a correlation of 0.98 with Pearson’s correlation test and 1.0 with Spearman’s correlation test. We suggest that EtG is a marker of alcohol consumption that can be detected for an extended time period after the complete elimination of alcohol from the body. When used as a relapse marker with a specific time frame of detection intermediate between short- and long-term markers, EtG fills a clinically as well as forensically important gap. Its specificity and sensitivity exceed those of all other known ethanol markers.

INTRODUCTION

Detection of ethanol in the body is possible for only a relatively short time. Of the other markers of alcohol intake, γ-glutamyltranspeptidase (GGT) is also influenced by a variety of substances and diseases. Carbohydrate-deficient transferrin (CDT) which is more specific, and mean corpuscular volume (MCV) provide an indication only of the cumulative amounts of alcohol consumed.

About 90 to 95% of alcohol is eliminated by oxidation, mainly in the liver via alcohol and aldehyde dehydrogenase, catalase, and the microsomal ethanol-oxidizing system. The rest is excreted by the kidneys (0.5–2%), the lungs (1.6–6%) and the skin (max. 0.5%) (Mallach, 1987). The inducible aerobic gastrointestinal flora contribute to ethanol oxidation in the gastrointestinal tract to some extent (Jokelainen et al., 1997).

Until recently, the biotransformation of ethanol to ethyl glucuronide (EtG) has received only limited interest in comparison to hepatic and extra-hepatic oxidative metabolism. The detoxifying pathway of alcohol elimination via conjugation with activated glucuronic acid (uridine-5′-diphospho-β-glucuronic acid) is reported to represent about 0.5% of total ethanol elimination. EtG is a non-volatile, water-soluble, direct metabolite of ethanol, which can be detected in body fluids and hair (Neubauer, 1901; Kamil et al., 1952; Jaakonmaki et al., 1967; Kozu, 1973; Besserer and Schmidt, 1983; Wurst et al., 1995; Schmitt et al., 1995; Skopp et al., 1995; Wurst et al., 1996a,b; Schmitt et al., 1997a,b; Sachs, 1997; Alt et al., 1997; Sticht et al., 1997; Wurst et al., 1997; Seidl et al., 1998; Wurst et al., 1998).
In 1885, Thierfelder and Mering demonstrated the glucuronidation of tertiary, but not primary and secondary, alcohols. The glucuronidation of these alcohols was detected by Neubauer (1901). EtG was isolated by Kamil et al. (1952) as the triacetyl methyl ester from the urine of rabbits. The conjugate was determined in human urine by Jaakonmaki et al. (1967) and by Kozu (1973). These findings have been confirmed by Besserer and Schmidt (1983). We have subsequently reported on EtG values between 1 and 100 mg/l and a detection time of up to 75 h with t-butyl glucuronide as the internal standard using gas chromatography mass spectrometry (GC/MS) in 33 alcoholics with a mean blood-ethanol concentration (BEC) of 183 mg/dl upon hospitalization (Wurst et al., 1995, 1996a,b). Schmitt et al. (1995) reported the synthesis, analytical data, and determination of EtG in serum and urine. Since January 1997, deuterium-labelled EtG (d5-EtG) — the ultimate gold standard — has been available. When we remeasured the urine samples (stored at −18°C) of the above-mentioned patients with this new tool, we observed EtG concentrations ranging between 3 and 70 mg/l and a time frame of detection of up to 80 h (Alt et al., 1997). EtG has also proved its usefulness in the expert assessment for judging driving ability in 151 cases (Seidl et al., 1998). The purpose of the present investigation was to evaluate EtG in different patient groups, using GC/MS and liquid chromatography (LC/MS-MS), as a relapse marker occupying an intermediate position on the time axis with regard to other markers.

PATIENTS AND METHODS

The first two patient groups described (n = 33 and 30 respectively) were hospitalized at the Alcohol and Drug Unit of the Department of Psychiatry II at the University of Ulm in Günzburg, Germany. The blood samples were obtained in the course of routine laboratory control. The third group (n = 43) consisted of patients from the Department of Neurorehabilitation at the Fachklinik Ichenhausen, Bavaria, Germany. In most of these patients, alcohol abstinence is important for both treatment and prognosis.

Patients in group 1 were 33 male alcoholics hospitalized for detoxification at the acute alcohol withdrawal clinic. They were all tested for BEC, which averaged (in mg/dl, mean ± SD) 183 ± 86, with a median of 180 and a range of 39–360. One hundred and eighty-one urine samples were also obtained both initially and subsequently every 4–12 h up to 96 h after hospitalization. The mean ± SD patient age (years) was 36.7 ± 9.3; median: 40.5; range: 26–57.

Patients in group 2 were 25 recently detoxified alcoholics and five poly-drug-abusing in-patients at a ‘motivation station’ (27 males, 3 females), all of whom had been treated under hospital conditions for at least 4 weeks. Their mean age (years) was 41.7 ± 11.3 with a median of 39.5 and a range of 23–66. In none was there any evidence of relapse as judged by the clinical impression of the therapist, routine laboratory tests for CDT, MCV, GGT, or randomly performed breath-ethanol tests. All 30 subjects were additionally rated by (a) their therapist and (b) the co-therapist, as to whether they considered the patients to be abstaining or relapsing, despite the above-mentioned findings. Urine and serum samples were obtained at a given day in the course of routine laboratory control.

The patients in group 3 were 43 neuro-rehabilitation patients (33 males, 10 females) from the Fachklinik Ichenhausen, Bavaria. Their mean age (years) was 59.9 ± 14.7 with a median of 63 and a range of 19–84. Urine samples from 18 of these patients were obtained on three occasions (Friday, Monday, and Wednesday), whereas samples from the remaining 25 were obtained on the Wednesday only.

For quantitative analysis by GC/MS, we used d5-EtG as the internal standard. For extraction purposes, 100 to 300 µl of either urine or serum were placed in a tube. After addition of d5-EtG and 1 ml of methanol, the tubes were capped and mixed for 1 min. The liquid layers were separated, transferred into GC vials and evaporated at 30°C with a mild nitrogen flow. Following evaporation, 50 µl of pyridine and N,O-bis(trimethyl silyl) tri-fluor acetamide (BSTFA) were added. The samples were vortex-mixed and then placed in a 90°C oven for 15 min. After removal of the derivation mixture, the residue was dissolved in 50 µl of ethyl acetate for GC/MS analysis. Identification was performed by the mass-fragments 160, 261, and 405, and evaluation was by the ion-mass pairs 261/266 and 405/410. The detection limit for ethyl glucuronide was 0.1 mg/l.
Additionally, urine and BECs were determined by head-space gas chromatography (Machata, 1962, 1964).

For LC/MS-MS analysis, 1 μg of the internal standard d₅-EtG was added to 100 μl of urine. An aliquot of this mixture (10 μl) was analysed by LC/MS-MS without further sample preparation. The samples were chromatographed on a Hewlett Packard 1100 HPLC using a 5 micron C-18, 125 mm by 2 mm column. Column flow rate was 0.18 ml/min. The analytes were eluted using a 1% aqueous formic acid and methanol (with 0.1% formic acid) gradient. The injection volume was 10 μl. A triple-quadrupole mass spectrometer API 365 (Sciex, Thornhill, Canada) equipped with a nebulizer-assisted electrospray (TurbIonSpray™) source was used for detection. Data were acquired in the multiple reaction monitoring mode. Analyte quantification was by peak area ratio. The MS–MS ion transitions monitored were m/z 221–75 for EtG and 226–75 for d₅-EtG. The detection limit for EtG was 0.1 mg/l.

RESULTS

A total of 286 urine and 30 serum samples from 106 patients in the three study groups were determined. In the first group of patients (n = 33, 181 urine samples) we had previously observed EtG concentrations ranging between 3 and 70 mg/l and a time frame of detection up to 80 h (Alt et al., 1997). In addition to these results, we focused on correlations of EtG with other indicators of alcohol consumption and predictive parameters. We found no correlation between the concentration of EtG in urine (344 ± 242 mg/l; range: 3.6–710) at t = 0 (hospitalization) and BEC (183 ± 86 mg/dl; median: 180; range 39–360; r = 0.17) (Fig. 1), time frame of detection (mean 57.7 ± 16.9 h; range: 12–80 h; r = 0.5), or total amount of clomethiazole required for the treatment of withdrawal symptoms (mean 32.9 g; median 32.5; r = 0.28). In a pilot study with 10 patients from this group, we also found no correlation (Pearson’s test) between the concentration of EtG in urine at t = 0 and CDT (r = −0.06), GGT (r = 0.28), MCV (r = 0.1), and cholinesterase (r = 0.28).

In four out of the 30 in-patients in group 2, neither clinical impression nor routine laboratory findings gave an indication for relapse, yet EtG concentrations in urine from these four patients ranged between 4.2 and 196.6 mg/l. Only in the patient with the highest EtG concentration in the urine sample (196.6 mg/l), was the ethanol metabolite also detected in serum, at a concentration of 4.8 mg/l. This is a ratio of about 40 (urine EtG/serum EtG). In this patient alone urine-ethanol concentration and BEC were found to be positive. One out of the four relapsing patients was rated correctly by the therapist. Table 1 gives a summary of results. These results are of particular

![Fig. 1. Correlation of ethyl glucuronide (EtG) concentrations in urine and blood-ethanol concentrations (BEC) at hospitalization.](image-url)

There was no correlation between the concentration of EtG in urine (mg/l) (mean 344, SD 242, range 3.6–710) at t = 0 (hospitalization) and BEC (g/l) (mean 183; SD 86; median: 3.9–360; r = 0.17).
of interest, as they reflect the relapsing behaviour of a single night and include one forensic polytoxicomanic patient. In non-relapsing patients, EtG was not detectable (data not shown). In the remaining 18 subjects in group 2, all parameters were negative and the ratings of therapist and co-therapist were in accord. Altogether the therapist’s and the co-therapist’s rating was correct in 70% of the cases.

A total of 75 urine samples were obtained from the 43 patients in group 3 on three occasions (Friday, Monday, Wednesday). On Wednesday, samples from patients 3, 4, 7, and 15 were missing. Six of 18 patients (33.3%) who had been screened three times were positive for EtG in urine. In seven patients EtG could be detected in urine.

Most of the neuro-rehabilitation patients found to be EtG positive had suffered a stroke with paresis partly also hemianopsia or aphasia, one patient had suffered a traumatic brain injury some weeks earlier and one had an arachnoidal cyst. Most of the EtG-positive patients were observed on Monday (4 patients = 66.7% of the positive ones). The pattern of distribution is shown in Table 2. On Wednesday, in only 4.7% (n = 2) of the patients EtG in urine was detectable. This discrepancy (4.7 vs 33.3%) clearly shows the usefulness of repeated screenings for EtG in urine.

The results have been reproduced independently at the University of Stuttgart with LC/MS-MS with the advantage of no derivatization being required. The results of the GC/MS and the LC/MS-MS method (75 samples each) show a correlation of 0.98 using Pearson’s correlation test and 1.0 using Spearman’s correlation test.

### Table 1. Proof of relapse with ethyl glucuronide (EtG)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>BEC (mg/dl)</th>
<th>UEC (mg/dl)</th>
<th>Serum EtG (mg/l)</th>
<th>Urine EtG (mg/l)</th>
<th>Therapist rating</th>
<th>Co-therapist rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>rel.</td>
<td>rel.</td>
</tr>
<tr>
<td>4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>11.1</td>
<td>abs.</td>
<td>abs.</td>
</tr>
<tr>
<td>7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>rel.</td>
<td>abs.</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>rel.</td>
<td>rel.</td>
</tr>
<tr>
<td>11</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4.2</td>
<td>abs.</td>
<td>abs.</td>
</tr>
<tr>
<td>18</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>abs.</td>
<td>rel.</td>
</tr>
<tr>
<td>19</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4.3</td>
<td>rel.</td>
<td>rel.</td>
</tr>
<tr>
<td>20</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>abs.</td>
<td>rel.</td>
</tr>
<tr>
<td>21</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>rel.</td>
<td>rel.</td>
</tr>
<tr>
<td>23</td>
<td>18</td>
<td>37</td>
<td>4.8</td>
<td>196.6</td>
<td>abs.</td>
<td>abs.</td>
</tr>
<tr>
<td>24</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>rel.</td>
<td>abs.</td>
</tr>
<tr>
<td>27</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>rel.</td>
<td>rel.</td>
</tr>
</tbody>
</table>

Blood and urine ethanol concentrations (BEC, UEC), serum and urine ethyl glucuronide (EtG) concentrations and therapist/co-therapist rating (abs., abstinent; rel., relapsing); shown for patients with either relapse proved by EtG or rating of the therapists, who considered a relapse as very probable on the given day.

### Table 2. Urine ethyl glucuronide (EtG) concentration in neuro-rehabilitation patients

<table>
<thead>
<tr>
<th>Sampling days</th>
<th>Urine EtG concentrations (mg/l) for patient no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>1 (Friday)</td>
<td>17.49</td>
</tr>
<tr>
<td>2 (Monday)</td>
<td>0</td>
</tr>
<tr>
<td>3 (Wednesday)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Not investigated on that day.

Eight out of 75 urine samples taken on three days (Friday, Monday, and Wednesday) from 43 patients were positive for EtG. Most of the neuro-rehabilitation patients found to be EtG-positive suffered from stroke with paresis partly also hemianopsia or aphasia, one had a traumatic brain injury some weeks earlier and one had an arachnoidal cyst.
Furthermore we have investigated the stability of EtG in urine by measuring and remeasuring the ethanol metabolite in a sample at room temperature every hour for 140 h. As shown in Fig. 2, the results fall within a very narrow range. This stability will be particularly useful for storage and transport if use of the EtG test becomes widespread.

**DISCUSSION**

EtG, a direct metabolite of ethanol, is a specific marker of alcohol consumption that can be detected for an extended time period after the complete elimination of alcohol from the body (Wurst et al., 1995, 1996a,b; Schmitt et al., 1995, 1997a,b; Alt et al., 1997; Seidl et al., 1998). As we have shown, initially with t-butyl glucuronide and later with d₅-EtG as internal standards, EtG can be detected for up to 80 h in the urine of alcohol-withdrawn patients with BECs which averaged (mg/dl, mean ± SD) 183 ± 86 (Wurst et al., 1995, 1996a,b; Alt et al., 1997). d₅-EtG (available since 1997) must be considered to be the gold internal standard, as its chemical and physical parameters, with the exception of mass, are identical to those of EtG. Parameters influencing the results might be the patient groups as well as the drinking pattern (Wurst et al., 1996a,b; Alt et al., 1997; Sticht et al., 1997; Schmitt et al., 1998; Seidl et al., 1998). Using no (Schmitt et al., 1997a,b) or propyl glucuronide (Sticht et al., 1997) as the internal standard as well as using acetylation instead of silylation with BSTFA might also contribute to differences in results. A possible accumulation of EtG seems to be very probable from our own results and also in view of the half-life of EtG of 2 to 3 h as described in the literature (Schmitt et al., 1997b). As shown for the non-alcohol-consuming patients in this paper and those described elsewhere, no measurable EtG concentrations were observed in the serum or urine of non-relapsing patients, non-drinking drivers or teetotallers (Wurst et al., 1995, 1996a,b; Alt et al., 1997; Schmitt et al., 1997a,b; Seidl et al., 1998). From our data of several hundred samples and because of theoretical considerations, there is no good reason to contradict the idea of the extraordinarily high sensitivity and specificity of EtG as an alcohol marker.

In the present prospective study with different groups of patients at different hospitals, we have
demonstrated that EtG is a marker of alcohol consumption that can be detected for an extended time period after the complete elimination of alcohol from the body, and that it is capable of detecting relapse in patients thus enabling the therapist to intervene at an early stage of relapse.

The methodical approach via GC/MS presented is technically available at almost all departments of forensic medicine at a moderate cost. For an even more widespread use of EtG as an alcohol relapse marker, more simple and even less expensive methods (e.g. the LC/MS-MS method described) are necessary and will shortly be available. These characteristics show EtG to be a promising marker for both alcohol consumption in general and for relapse control. The only other marker that detects a single relapse — the 5-hydroxytryptophol/5-hydroxyindole-3-acetic acid ratio — gives a safe proof only in cases with values >50 (Helander et al., 1992a,b, 1997). EtG diminishes with its specific time spectrum of detection the clinically as well as forensically important gap between short- and long-term markers, such as ethanol, and GGT, MCV or CDT. Its specificity exceeds that of all other known ethanol markers.

Acknowledgements — We would like to express our gratitude to Dr Joachim Durner, Director of the Department of Neurology and Neurorehabilitation at the Fachklinik Ichenhausen for giving us the opportunity to use the urine samples from his patients. We wish to thank Mrs S. Steeb and Mrs A. Sander from the Department of Biometrics and Medical Documentation at the University of Ulm for statistical advice.

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