Int J Legal Med (2008) 122:507–509
DOI 10.1007/s00414-008-0273-z

CASE REPORT

Ethyl glucuronide and ethyl sulfate in autopsy samples 27 years after death

Lucia Politi · Luca Morini · Francesco Mari · Angelo Groppi · Elisabetta Bertol

Received: 24 April 2008 / Accepted: 27 June 2008 / Published online: 26 July 2008

Abstract The unique case of a 50-year-old known alcoholic whose corpse was exhumed 27 years after death is reported. The man apparently committed suicide by hanging, but many years later the case was questioned and homicide—linked to a long-lasting serial killer case—was suspected. Thus, the corpse was exhumed, and at the autopsy it was found to be naturally mummified. This fact permitted the analysis of body tissues with the aim to investigate the persistence of ethanol conjugates in the biological material 27 years after death. Fragments of liver and kidney, a blood clot, and a hair strand were collected and submitted to liquid chromatography tandem mass spectrometry analysis. Ethyl glucuronide (EtG) and ethyl sulfate (EtS) were identified and quantified in the liver, the kidney, and the blood clot. Hair analysis was found to be severely affected by ion suppression even after solid phase extraction. Consequently, EtG was identified in all hair segments (0–3 cm, 3–6 cm, and 6–10 cm), but no reliable quantification could be carried out. In summary, our findings demonstrate that, notwithstanding the expected conjugate degradation, EtG and EtS can be indicative of ante-mortem use of alcohol even many years after death.

L. Politi (✉) · F. Mari · E. Bertol
Department of Anatomy, Histology and Legal Medicine,
Division of Forensic Toxicology, University of Florence,
Viale Morgagni, 85,
I-50134 Firenze, Italy
e-mail: lucia.politi@unifi.it

L. Morini · A. Groppi
Department of Legal Medicine and Public Health,
University of Pavia,
Pavia, Italy

Keywords Ethyl glucuronide · Ethyl sulfate · Postmortem

Introduction

Ethyl glucuronide (EtG) has recently drawn attention, among its various applications, as a tool in the detection of antemortem alcohol ingestion and, in particular, as a valued means in the discrimination of antemortem ingestion and postmortem formation of ethanol [1]. In fact, it has been found by two different research groups that no EtG formation seems to occur in the corpse, even in the presence of putrefaction processes [1, 2]. On the contrary, it has been demonstrated that EtG undergoes hydrolysis under severe putrefaction conditions; therefore, an EtG-positive result probably verifies ingestion of ethanol, while a negative result must be interpreted with caution [3]. To date, at least to our knowledge, no parallel study has been performed on ethyl sulfate (EtS) that has been thoroughly shown to have at least the same applications of EtG (with the exception of hair) and higher stability in urine samples with reference to bacterial degradation [4, 5].

The aim of this paper was to report, for the first time, on the persistence of EtG and EtS in the body tissues of a known alcoholic 27 years after his death.

Case history

A 50-year-old man, a known alcoholic, died and the body was submitted for external examination only. The cause of death was not investigated further as it apparently was suicide by hanging and the case was filed. The body was entombed in a zinc coffin located in a cement loculus. Twenty-seven years after death the case was questioned due

to new findings in the investigations, and homicide was suspected. The conjecture was that the death could be linked to a long-lasting serial killer case. Hence, the corpse was exhumed and a complete autopsy was performed. Probably owing to the choice of entombment instead of inhumation, the body was found to be naturally mummified, and it was possible to identify internal organs, including liver and kidneys. The mummified liver and right kidney were collected during autopsy together with a blood clot found in the axillary artery and a head hair strand.

The investigations regarding the death revealed that the subject was commonly known to be an alcoholic and that 1 week before dying he was admitted at the local emergency department where the blood alcohol concentration was found to be 3.3 g/l.

Materials and methods

Sample preparation

All samples except for the hair strand were stored at −20°C until analysis, which was performed within 1 month after the autopsy. The hair strand was kept at room temperature.

Fragments of liver and kidney were weighed, a volume of deionized water four times greater than the sample weight was added and samples were thoroughly homogenized. Similarly, the blood clot was weighed and, after a volume of water four times the weight of the sample was added, it was carefully vortexed. Samples were ultrasonicated for 30 min.

After addition of 500 ng/g of the internal standard (D5-EtG, Promochem, Milan, Italy), liver, kidney, and blood clot were deproteinized with acetonitrile, diluted, centrifuged (13,000 rpm for 10 min) and 20 μl was injected in the liquid chromatography tandem mass spectrometry (LC-MS/MS) system as described for serum samples by Morini et al. [6]. Homogenized blank tissues (liver and kidney), from five different autopsies, spiked with EtG (Promochem) and EtS (TCI Europe, Zwijndrecht, Belgium) at 50, 100, 500, 1,000, and 5,000 ng/g and D5-EtG (100 ng/g), were used as calibrators together with a blank sample spiked with internal standard only. Quality controls at 50, 100, 700, and 2,000 ng/g were injected in the same batch.

The hair lock was initially washed using water and, after drying, it was divided into three segments: from 0 to 3 cm, from 3 to 6 cm, and from 6 to 10 cm. Samples were initially prepared following the LC-MS/MS method published elsewhere [7]. Briefly, after two washing steps (dichloromethane, methanol), hair samples were cut with scissors into 1- to 2-mm segments and incubated overnight at room temperature in deionized water, spiked with D_5-EtG (100 pg/mg). The following day, the samples were ultrasonicated for 2 h and, after centrifugation (13,000 rpm for 10 min), 8 μl was injected in the LC-MS/MS system. The same procedure was followed for blank and calibration curve (3, 5, 10, 20, 40, 100, 200, 1,000, and 2,000 pg/mg) and quality controls at 3, 5, 56, and 160 pg/mg.

Owing to ion suppression problems, hair samples were prepared by solid phase extraction (SPE): the procedure previously described was followed until overnight incubation. The following day, the samples were ultrasonicated for 2 h and, after centrifugation, were loaded on SPE aminopropyl cartridges (Bond Elut–NH_2, Varian, Milan, Italy), previously activated with methanol (1 ml), water (1 ml), and acetonitrile (1 ml). Afterward, 1 ml hexane was charged on the cartridge in order to remove non-polar residuals from the cartridges, and finally EtG was eluted by 700 μl deionized water. The three hair segments were extracted and injected in the same batch of the blank, the calibration curve, and the quality controls.

Instrumental analysis

Liquid chromatography tandem mass spectrometry analysis of liver, kidney, and the blood clot was performed as described elsewhere [6]. Identification was carried out monitoring two transitions for both analytes (EtG, EtS) and for the internal standard according to the method previously validated for serum.

Instrumental analysis for the detection of EtG in hair samples was reported before [7]. Identification was based on two transitions.

Results and discussion

The calibration curve prepared for tissues and blood clot was found to be linear for EtG and EtS (y=0.00392 × −0.116, R^2= 1.0000, for EtG, and y=0.00421 × −0.297, R^2=0.9990 for EtS), accuracy and precision (calculated as bias percent and relative standard deviation, respectively) of quality controls were always better than 13.8%. The method was previously validated for serum [6]. It was considered unnecessary to carry out further validation on the basis of the linearity of the curve, the acceptable accuracy and precision of quality, and

Table 1 EtG and EtS results in liver, kidney, and blood clot after LC-MS/MS analysis

	EtG (ng/g)	EtS (ng/g)	EtS/EtG molar ratio
Liver	141	535	6.68
Kidney	249	945	6.70
Blood clot	219	645	5.19

the limited meaning of exact quantification in a mummified tissue. Moreover, no significant difference was found in the absolute area of the internal standard, thus suggesting that ion suppression is unlikely to severely impact the results.

Both EtG and EtS were identified on the basis of the ratio between the two transitions monitored in liver, kidneys, and blood clot and quantitative analytical results are reported in Table 1. Data are referred to samples as collected at the autopsy, with no correction on tissues mummification and consequent dehydration. For EtG, quantitative results of liver (141 ng/g) and kidney (249 ng/g) were much lower than those reported in the literature, i.e., in liver samples, between 6.7–76.7 μg/g [8]. No quantitative published data were found for kidneys. Similarly, EtG concentration in the blood clot (219 ng/g of dehydrated sample) was considerably lower than EtG in postmortem blood reported before, i.e. 0.4–20.5 μg/ml [8], 0.11–55.6 μg/ml [1], and 0.2–34.9 μg/ml [3]. However, in consideration of the scarce stability of the molecule in postmortem samples, very low (if any) EtG concentrations were expected.

No information on EtS in postmortem samples was retrieved from the literature, and in this respect, it is interesting to note that the EtS-to-EtG molar ratio was much higher than the molar ratio in serum reported by a recent study (in the living), where it was found to be between 0 and 1.31 (mean: 0.9±0.35, median 1.01) [9]. EtS/EtG detected in the blood clot in our case was 5.19, which might be attributed to a higher stability of EtS to hydrolysis, or to an individual prevalence of EtS over EtG in the metabolization of ethanol, or to a different distribution of the two molecules in the body compartments. The very close values of molar ratios in the liver and in the kidney (6.68 and 6.70, respectively) might be indicative of a postmortem diffusion of the two molecules.

The three hair segments were initially analyzed by the published method [7], preceded by a brief water washing step, but no signal was found for either EtG or D5-EtG, probably owing to severe problems in ion suppression. Therefore, the SPE procedure described above was performed. The extraction permitted to identify both EtG and D5-EtG in samples, calibrators, and quality controls. The calibration curve was found to be linear ($y=0.00894\times+0.00541$, $R^2=1.0000$) and quality controls were acceptable (<11.4% for both bias and relative standard deviation). However, the absolute area of D5-EtG in the samples was always much lower than expected (about 15% of the area compared to calibrators and quality controls), thus suggesting the persistence of ion suppression. Consequently, no EtG quantification was performed for hair samples, even though it was possible to identify the molecule in all segments on the basis of the ratio between the two transitions monitored.

Conclusions

For the first time, at least to our knowledge, EtS was identified in postmortem samples. Moreover, EtG was identified in the liver, kidney, blood clot, and hair 27 years after death of a known alcoholic. The method developed and validated in the laboratory for serum was able to determine both molecules in the liver and kidney homogenate, whereas the method used for EtG hair analysis showed severe ion suppression problems. Even after the development of a solid phase extraction procedure, ion suppression allowed the identification, but not the quantification of EtG in the three hair segments analyzed. However, the aim of the study was not to achieve a reliable quantitative value for the two conjugates, as it would be meaningless so many years after death in mummified tissue. On the contrary, it was shown that EtG and EtS, notwithstanding the known degradation phenomena, can be used as proof of the use of alcohol even many years after death. Finally, the molar ratio between the two metabolites showed a higher proportion of EtS over EtG in all samples.

References

1. Høiseth G, Karinen R, Christophersen AS, Olsen L, Normann PT, Mørland J (2007) A study of ethyl glucuronide in post-mortem blood as a marker of ante-mortem ingestion of alcohol. Forensic Sci Int 165:41–45
2. Schloegl H, Dresen S, Spaczynski K, Stoertzel M, Wurst FM, Weinmann W (2006) Stability of ethyl glucuronide in urine, post-mortem tissue and blood samples. Int J Legal Med 120:83–88
3. Høiseth G, Karinen R, Johnsen L, Normann PT, Christophersen AS, Mørland J (2008) Disappearance of ethyl glucuronide during heavy putrefaction. Forensic Sci Int 176:147–151
4. Helander A, Dahl H (2005) Urinary tract infection: a risk factor for false-negative urinary ethyl glucuronide but not ethyl sulfate in the detection of recent alcohol consumption. Clin Chem 51:1728–1730
5. Helander A, Olsson I, Dahl H (2007) Postcollection synthesis of ethyl glucuronide by bacteria in urine may cause false identification of alcohol consumption. Clin Chem 53:1855–1857
6. Morini L, Politi L, Zucchella A, Polettini A (2007) Ethyl glucuronide and ethyl sulphate determination in serum by liquid chromatography-electrospray tandem mass spectrometry. Clin Chim Acta 376:213–219
7. Morini L, Politi L, Groppi A, Stramesi C, Polettini A (2006) Determination of ethyl glucuronide in hair samples by liquid chromatography/electrospray tandem mass spectrometry. J Mass Spectrom 41:34–42
8. Schloegl H, Rost T, Schmidt W, Wurst FM, Weinmann W (2006) Distribution of ethyl glucuronide in rib bone marrow, other tissues and body liquids as proof of alcohol consumption before death. Forensic Sci Int 156:213–218
9. Halter CC, Dresen S, Auwaerter V, Wurst FM, Weinmann W (2008) Kinetics in serum and urinary excretion of ethyl sulfate and ethyl glucuronide after medium dose ethanol intake. Int J Legal Med 122:123–128