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CASE REPORT

Measurement of direct ethanol metabolites in a case of a former driving under the influence (DUI) of alcohol offender, now claiming abstinence

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Abstract A 37-year-old female subject had been convicted of driving under the influence of alcohol, and 19 months later, claimed abstinence after supervised disulfiram treatment. Our aim was to elucidate the value of direct ethanol metabolites as measures of abstinence. Ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEE) in hair, phosphatidylethanol in whole blood and EtG and ethyl sulphate in urine were measured. The results were compared with self-report of alcohol consumption and traditional blood biomarkers for chronically elevated alcohol consumption as carbohydrate deficient transferrin (CDT), gamma glutamyl transpeptidase, mean corpuscular erythrocyte volume, aspartate aminotransferase and alanine aminotransferase. EtG was found in distal parts of hair only, whereas the proximal parts were negative. Furthermore, FAEE concentrations were found in the typical distribution over the hair length and showed values typical for either moderate social drinking or abstinence. CDT was above cut-off in 9 out of 16 analyses with a decreasing tendency and the lowest values in the last 2 months before the end of sampling. The data suggest that in addition to traditional markers, a combination of direct ethanol metabolites can be useful in the expert assessment of judging driving ability. A careful individual interpretation of the results for the different markers, however, is an absolute necessity.

Keywords Alcoholism · Driving under the influence of alcohol · Direct ethanol metabolites · Hair analysis

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Introduction

Monitoring abstinence in convicted driving under the influence (DUI) of alcohol offenders is of significant importance for the individual and society. Besides traditional markers like carbohydrate deficient transferrin (CDT), gamma glutamyl transpeptidase (GGT), methanol, acetone/2-propanol and mean corpuscular volume (MCV) or combinations of them [1], mainly in the last decade, direct ethanol metabolites have been studied as biomarkers of ethanol intake and/or chronic ethanol abuse.

The most promising among them are fatty acid ethyl esters (FAEEs) [2–5], ethyl glucuronide (EtG) [6–10], phosphatidylethanol (PEth) [11, 12] and ethyl sulphate (EtS) [6, 13, 14].

Each of these indicators remains positive in serum and urine for a characteristic period of time after the cessation of ethanol intake—FAEEs in serum up to 24 h, EtG in urine up to 5 days, PEth in whole blood up to 2 weeks or more. In addition, EtG and FAEE can be detected in hair and allow a retrospective interpretation [15–18].

In drivers suspected of DUI of alcohol, blood ethanol concentration (BAC) of 0.1–3.9 g ‰, serum EtG (SEtG) 3.2–13.7 mg/l, urinary ethanol (UAC) 0.1–2.0 g/l and urinary EtG (UEtG) 3.0–130 mg/l have been found [19]. EtG also demonstrated the utility in the expert assessment for judging the driving ability [20]: 13 out of 151 cases tested positive for UEtG, whereas none of the traditional markers including GGT, MCV and CDT had evidenced for chronically elevated alcohol consumption in these cases.

PEth is a phospholipid formed only in the presence of ethanol via the action of phospholipase D [21, 22]. It has been measured in whole blood and proposed as a marker of ethanol abuse due to its high specificity and low degradation rate. The mean half-life of PEth in blood from alcoholics was found to be 4 days, and PEth was still measurable after 2 weeks of sobriety [23]. Two recent studies found no false negatives in active alcoholics [24] and no false positives in sober subjects with a previous history of addiction [25].

FAEEs in hair have also been suggested as markers of chronically elevated ethanol intake. They are incorporated into hair and can be analysed there even after several months [17]. In these investigations, the sum of the FAEE concentrations (C_{FAEE}) of the four esters ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate was used as a marker for excessive alcohol consumption. The incorporation was found to occur mainly from sebum into the keratinised hair [18]. For this reason, in hair samples generally, an increase of C_{FAEE} by accumulation from proximal to distal hair sections was found. From previous investigation of hair samples from fatalities with known excessive alcohol consumption, patients in withdrawal treatment, social drinkers and teetotalers, a cut-off value for C_{FAEE} of 1.0 ng/mg for the proximal segment (0–6 cm from the skin) was suggested, above which with a very high probability of heavy alcohol misuse must be assumed. Values ≤0.4 ng/mg are not stringently in contradiction to the claim of abstinence, as teetotalers also show low FAEE concentrations in hair [18, 26].

Since 2000, several groups have reported on the determination of EtG in hair [15, 27, 28]. Alt et al. determined EtG in 16 hair samples, taken at autopsies from persons with a known history of alcoholism, and found EtG concentrations between 218 and 4,025 pg/mg in 14 hair samples. In four hair samples taken from alcohol withdrawal patients, EtG concentrations between 119 and 388 pg/mg hair were detected. However, measurable concentrations of EtG were not detected in hair from six social drinkers with a self-reported daily ethanol consumption of up to 20 g and five hair samples from children. Recently, Yegles et al. [29] compared EtG and FAEE concentrations in the hair of alcoholics, social drinkers and teetotalers. Their results strengthened the importance of EtG and FAEEs in hair as suitable qualitative markers of chronic harmful ethanol intake. They suggest that a positive EtG result and/or a value of C_{FAEE} above 1 ng/mg hair (proximal segment 0–6 cm from the skin) must be considered to be a strong evidence for excessive drinking behaviour.

Our aim was to investigate the utility of using these direct ethanol metabolites, particularly FAEEs and EtG in hair and PEth in whole blood, in judging the claim of abstinence for more than 1.5 years in a 37-year-old DUI offender.

Materials and methods

Subject

A 37-year-old female (weight=53 kg; height=153 cm; hair colour brown, hair not bleached or dyed) was found to be DUI of alcohol. She reported a daily ethanol intake in the previous 20 months of 50 g of ethanol (wine) per day. She claimed to have stopped drinking after being convicted for DUI and started regular monthly blood analyses for the markers of chronic ethanol abuse such as CDT, ASAT, ALAT, GGT and MCV 3 months after the incident. As intermittently CDT was above the cut-off value, she was put on disulfiram (supervised intake of 400 mg, three times a week) 15 months after DUI. Having heard of the possibility to assess ethanol intake by hair analysis for direct ethanol metabolites, she requested the determinations. Urine, blood and hair samples (16 cm total length) were collected after the subject gave written informed consent. The hair sample was analysed for EtG and FAEEs, blood was analysed for PEth and the urine sample was analysed for UEtG and urinary EtS (UEtS).

Methods

Ethyl glucuronide in hair

EtG in hair was determined with gas chromatography–mass spectrometry (GC–MS) in negative chemical ionisation (NCI) mode and d_5-EtG as internal standard as described by

Yegles et al. [29]. The limit of detection and the limit of quantitation were 2.0 and 6.7 pg/mg hair, respectively.

Fatty acid ethyl esters in hair

The analytical determination of the four FAEEs (ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate) was performed by external decontamination of the hair with *n*-heptane, liquid extraction with a dimethylsulphoxide/*n*-heptane mixture after the addition of deuterated standards of all four esters, separation and evaporation of the *n*-heptane layer, solid phase micro-extraction (SPME) of the residue and gas chromatography–mass spectrometry (GC–MS) as described previously [17, 26]. For the sum of the four major FAEEs (C_{FAEE}), a cut-off of 1.0 ng/mg was used to exclude excessive alcohol consumption. The limit of detection was 0.07 ng/mg.

Phosphatidylethanol in whole blood

PEth was measured in heparinised whole blood as described elsewhere with high performance liquid chromatography combined with an evaporative light-scattering detector [23].

EtG and EtS in urine

UEtG and UEtS have been determined using liquid chromatography/tandem mass spectrometry (LC/MS–MS) and deuterated standards as described elsewhere [13, 30]. The limit of detection was 0.05 mg/l.

CDT, GGT, ASAT, ALAT, MCV

These tests were performed on a monthly basis in a commercial lab. Quantitation of CDT (cut-off=2.6%) was performed with the CDT kit from BioRad Laboratories, Philadelphia, PA, USA according to manufacturer's instruction.

Results

The hair strand of a total length of 16 cm was analysed in five segments (from proximal to distal: 0–3, 3–6, 6–9, 9–12 and 12–16 cm). In the proximal hair segments (0–3, 3–6 and 6–9 cm), EtG was below the limit of detection, whereas in the distal segments (9–12 and 12–16 cm), EtG was positive. The C_{FAEE} showed the typical course with

Table 1 Synopsis of the results for direct ethanol metabolites in hair and blood, traditional biomarkers and self-report about drinking behaviour

	Segment 5(12–16 cm)				Segment 4 (9–12 cm)			Segment 3 (6–9 cm)			Segment 2 (3–6 cm)			Segment 1 (0–3 cm)		
Hair marker[a]																
EtMy (ng/mg)	0.02				0.04			0.03			0.03			0.02		
EtPa (ng/mg)	0.40				0.48			0.39			0.17			0.09		
EtOl (ng/mg)	0.14				0.16			0.14			0.14			0.07		
EtSt (ng/mg)	0.06				0.07			0.10			0.05			0.02		
C_{FAEE} (ng/mg)	0.62				0.75			0.66			0.39			0.20		
EtG (pg/mg)	54				45			<LOD			<LOD			<LOD		
Blood marker																
Months	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
CDT (%)	3.0	3.1	2.8	2.7	2.5	3.0	2.7	2.8	2.6	3.3	2.4	2.3	2.3	2.7	2.2	2.1[b]
ASAT (U/l)	10	12	10	11	10	10	9	10	10	12	11	13	9	9	10	22[b]
ALAT (U/l)	15	17	14	12	12	10	10	12	10	15	11	20	15	15	15	21[b]
GGT (U/l)	5	7	6	6	6	7	6	5	6	7	6	6	5	6	5	9[b]
MCV (fl)	92	91	96	93	92	92	97	96	93	93	93	92	92	92	93	–
PEth	No data available														<LOD	
UEtS	No data available															<LOD
UEtG	No data available															<LOD
Self-report	Abstinent															

EtMy: ethyl myristate, *EtPa*: ethyl palmitate, *EtOl*: ethyl oleate, *EtSt*: ethyl stearate, C_{FAEE}: concentration sum of the FAEE EtMy, EtPa, EtOl and EtSt, *EtG*: ethyl glucuronide, *Months*: months before taking hair sample, *CDT*: carbohydrate deficient transferrin, *ASAT*: aspartate aminotransferase, *ALAT*: alanine aminotransferase, *GGT*: gamma glutamyl transpeptidase, *MCV*: mean corpuscular volume, *PEth*: phosphatidylethanol, *UEtS*: urinary ethyl sulphate concentration, *UEtG*: urinary ethyl glucuronide concentration. Reference values: C_{FAEE}: see the "Discussion" section, EtG (hair)<limit of detection (LOD), CDT<2.6%, ASAT<26 U/l, ALAT<31 U/l; GGT<38 U/l, MCV 82–100 fl, PEth, UEtG and UEtS<LOD.

[a] The arrangement of the segmental hair concentrations over the time scale of the blood markers does not imply a reliable chronological correspondence (see the "Discussion" section).

[b] Independent laboratory, different from the one doing the previous analyses; all values below laboratory cut offs.

increasing concentrations from proximal to distal with a maximum in the fourth segment (9–12 cm from the skin). Building the average FAEE concentration for the segment 0–6 cm, a C_{FAEE} of 0.30 ng/mg results.

GGT, ASAT, ALAT and MCV as markers with a relatively low sensitivity were below the cut-off in all 16 serum samples. In contrast, CDT was found higher than the cut-off in 9 out of 16 samples. Seven out of eight CDT values were positive in the first half of the time period of monthly blood sampling and only two out of eight in the second half.

EtG and EtS in urine and PEth in blood (samples for these markers were taken only once at the same time as the hair sample) were below the limits of detection. The results of the hair analysis and of the monthly performed blood tests are shown in Table 1.

Discussion

Although it seems that no clear correlation exists between self-report of alcohol consumption and the chronologically corresponding concentrations in segmental hair analysis for EtG [29], the negative proximal hair segments suggest that there was no recent excessive ethanol consumption. Taking into account the telogen and catagen portion of the hair and assuming an average monthly hair growth of approximately 1 to 1.5 cm, the positive EtG results in the distal hair segments (9–12 and 12–16 cm) could originate from heavy alcohol consumption 12 to 15 months before sampling and are, therefore, in contradiction to the subject's claim of abstinence straight after the DUI incidence. On the other hand, moderate drinking in the time period before the start of the disulfiram treatment cannot be excluded either.

The C_{FAEE} of 0.30 ng/mg calculated for the 0–6 cm segment is clearly under the suggested cut-off of 1 ng/mg for chronic excessive alcohol consumption. It was seen in a former study that a cut-off of 1.0 ng/mg, particularly in clinical cases, could lead to too many false negative results [24]. Therefore, the use of 0.4 ng/mg as cut-off based on data from a ROC curve analysis was suggested. The measured FAEE concentrations are below this cut-off, too. In previous investigations, concentrations of FAEEs were also found in hair of teetotalers, but the C_{FAEE} was never higher than 0.4 ng/mg [18]. The origin of the FAEEs in these cases is not yet clear. As a rule, social drinkers also have $C_{FAEE}<0.4$ ng/mg. Therefore, the results are not necessarily in contradiction to the stated claim of abstinence, although the method is not suitable to differentiate between moderate social drinking and complete abstinence. However, heavy drinking in the months before sampling can definitely be excluded.

For the last month before hair sampling, the claim of abstinence is plausible because PEth was not detected in the blood sample drawn at the same time as the hair sample. However, even in this time period, the intake of minor amounts of ethanol cannot be excluded, as PEth is found to be positive only if more than 40–60 g of ethanol per day is consumed for a longer period of time. The negative findings for EtG and EtS in urine proved the abstinence at least for the last days before sampling.

With the possibility to monitor substance use over longer periods of time, hair analysis confirmed to be an interesting tool for clinical and forensic applications. Both EtG and C_{FAEE} are promising markers in this field.

The data suggest that in addition to traditional markers, a combination of direct ethanol metabolites can be useful in the expert assessment of judging driving ability. A careful interpretation of the results for the different marker is absolutely necessary as incautious use of cut-off values may lead to wrong conclusions.

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