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Ethyl Glucuronide Concentration in Serum of Human Volunteers, Teetotalers, and Suspected Drinking Drivers

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ABSTRACT: The kinetic profile of ethanol and ethyl glucuronide (EtG) in serum was investigated in three subject groups: 1) Healthy, moderately drinking volunteers (daily intake less than 30 g ethanol) who ingested a single dose of ethanol. In this group the maximum of serum ethyl glucuronide concentration (SEtGC) and of serum ethanol concentration (SEC) did not exceed 3.7 mg/L and 1.5 g/L respectively. EtG peaked 2 to 3.5 h later than ethanol. EtG was eliminated with a terminal half-life of 2 to 3 h. EtG decreased slower than ethanol—the metabolite could still be determined in serum up to 8 h after complete ethanol elimination. 2) In serum samples of teetotalers neither ethanol nor EtG could be found. 3) In 37 of 50 serum samples of drivers suspected of driving under the influence of ethanol, SEtGC was found between the limit of detection (0.1 mg/L) and 20 mg/L. If the SEC is less than 1 g/L and the SEtGC is significantly higher than 5 mg/L, we assume alcohol misuse.

KEYWORDS: forensic science, forensic toxicology, ethyl glucuronide, human kinetic profiles, ethanol metabolism, gas chromatography–mass spectrometry

Ethyl glucuronide is a minor metabolite of ethanol whose formation and elimination in humans has not previously been investigated. EtG is formed from ethanol by conjugation with UDP-glucuronic acid and was first isolated in 1952 by Kamil et al. from rabbits' urine (1). In 1967, Jaakonmaki et al. detected the metabolite also in human urine (2). For quantitation and kinetic studies, EtG was synthesized and previously detected by us in human serum using GC/MS (3). EtG was also detectable in the clipped hair samples of ethanol consumers (4,5). This study investigates the time course and possible implications of this minor metabolite in humans.

Materials and Method

The three subject groups investigated are listed in Table 1. The approval for the drinking experiment and the testing of every group was given by the Ethics Committee of the Faculty of Medicine, University of Heidelberg. Before the drinking experiment was started and/or blood samples were drawn, informed consent was obtained from each subject.

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TABLE 1—*Subject groups in which ethanol and EtG were analyzed in serum. The conditions of ethanol consumption in group 3 were unknown.*

Group	Characterization	Subjects
1	teetotalers	10
2	moderate drinkers in drinking experiments	12
3	suspected drinking drivers	50

Subject Group 1—10 serum samples were taken from abstinent 20 to 40-year-old patients staying in a psychiatric hospital. The patients lived in closed wards and had no access to alcoholic beverages for at least six month.

Subject Group 2—In drinking experiments, 12 healthy volunteers (age: 20 to 35 years, daily ethanol consumption less than 30 g) were asked to consume 44 to 90 g of ethanol within 60 to 90 min. Serum samples were taken at regular time intervals until breath alcohol was below the limit of detection of the breath analyzer. In two cases all blood samples were taken after ethanol was completely degraded. The individual weight of the subjects was measured.

Subject Group 3—50 blood samples taken by police surgeons from 20 to 50-year-old drivers were examined. They were all suspected of driving under the influence of alcohol.

Chemicals and Reagents—Medichem Diagnostica (Stuttgart, F.R.G.): Serum ethanol standards. EtG was prepared from D-glucurono-3,6-lactone in a five step synthesis as previously described (3). All chemicals used were analytical grade.

Determination of Ethyl Glucuronide in Serum—The determination of EtG was accomplished according to (3) with little modification. 200 μL serum were mixed with 1 mL methanol. Precipitated proteins were removed by centrifugation. 900 μL of the supernatant were evaporated to dryness. The residue was derivatized by addition of 100 μL acetic anhydride and 25 μL pyridine at 80°C for 30 min. The solution was evaporated under a gentle stream of nitrogen. The residue was dissolved in 100 μL chloroform and 1 μL of the solution was injected into a GC/MS-system. A calibration curve with SEtGC of 0.2, 0.5, 1, 2, 10, 50 mg/L was established by addition of methanolic EtG solution (0.1 mg/mL) to blank serum. The relationship between signal and concentration was linear from 0.2 to 50 mg/L. The recovery was determined in a separate experiment as being approx. 70%. The correlation coefficients of the linear calibrations were above 0.990, the limit of

TABLE 2—*Time in hours and minutes (h:min) to achieve maximal values of SEC and SEtGC for subjects in a drinking experiment with 10 healthy subjects.*

Subject (Gender)	Max. SEC		Max. SEtGC		Ethanol Dose	Drinking Time	Body-Height	Body-Weight
	[g/L]	[h:min]	[mg/L]	[h:min]	[g]	[h:min]	[cm]	[kg]
1 (m)	1.02	1:30	1.06	3:34	60	1:00	174	76
2 (f)	1.48	2:28	3.70	5:48	80	1:00	170	57
3 (m)	1.42	2:10	2.33	4:40	72	1:00	170	70
4 (m)	0.46	2:09	0.32	5:13	51	1:00	180	73
5 (m)	0.99	2:15	0.10	4:10	69	1:00	180	72
6 (m)	0.79	2:24	0.66	4:19	80	1:15	189	89
7 (m)	1.32	3:20	1.40	5:25	60	1:15	173	70
8 (m)	0.55	2:20	0.65	4:10	60	1:15	185	110
9 (m)	1.32	3:20	0.73	4:22	44	1:15	175	63
10 (m)	0.92	2:20	0.73	4.15	64	1:15	176	67

detection was 0.1 mg/L. The accuracy was proven by means of a spiked serum standard containing 1 mg EtG/L (coefficient of variation (n = 7) within-day: ±8% and within day-to-day (n = 7): ±13.5%). Instrumental settings: GC/MS: Hewlett Packard HP5988A; CP-SIL5 column (12.5 m × 0.2 mm ID); injector: 250°C; ion source: 250°C; temperature program: initial temperature, 60°C for 1 min; rise to 320°C with an increase of 20°C/min; hold for 1 min; SIM (m/z): 115, 157, 183, 243; dwell time 50 ms; target ion for quantitation: m/z 243, RT 7.8 min (acetylated EtG, RI: 1920 (6)).

Headspace Determination of Ethanol in Serum—For direct analysis of serum, 200 µl were pipetted into 20 mL vials together with 1.8 mL of a 0.15% solution of tert-butanol as internal standard. Aqueous ethanol standards of 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 g/L sealed in glass ampoules were used for calibration. The relationship between signal and concentration was linear from 0.1 to 4.0 g/L with a correlation coefficient of 0.999, the limit of quantitation being approx. 0.05 g/L. Accuracy was proven using serum ethanol standards of 0.80 and 1.10 g/L. The coefficient of variation (n = 10) was within-day less than ±1% and between day (n = 20): approx. ±3%. Instrumental settings: GC-headspace: PE 8420 (Perkin Elmer) equipped with a flame ionization detector; column: Carbowax 1500 on Graphpac C80/100 mesh (Supelco); integrator HP 3396A (Hewlett Packard); temperature: 75°C isotherm; injector: 130°C; transfer line: 130°C; vial equilibration temperature: 66°C, vial equilibration time: 30 min.

Determination of Breath Alcohol—An automatic breath analyzer was used for breath alcohol determination (Alcomat M52052-A1, Siemens, F.R.G., infrared light absorption at 3.4 µm). The limit of detection was approx. 10 µg ethanol/L exhaled air.

Results

Subject Group 1—Neither ethanol nor EtG were detected in any of the serum samples.

Subject Group 2—In serum samples taken before the ethanol consumption was started, no EtG was detected. The maximum SEC was 1.48 g/L. The SEtGC did not exceed 3.7 mg/L (Table 2). Compared to SEC, the SEtGC peaked and started to decline with a delay of 2 to 3.5 h (Fig. 1). In 2 separate cases the maximal SEC's were calculated from the ethanol dose consumed using the Widmark formula (7). In these two cases EtG was detectable 15 and 17 h after the start of the experiment and up to 8 h after complete ethanol elimination (Fig. 2). There was an exponential decline of the SEtGC found with a half life of 2 to 3 h.

Subject Group 3—In all samples containing alcohol, EtG was also detectable. 13 samples contained neither ethanol nor EtG. Ethanol free serum samples (n = 7, SEC below 0.05 g/L) contained EtG in concentrations up to 20 mg/L. In Fig. 3 the SEC is plotted versus the SEtGC. The distribution of data shows no definite correlation between SEC and SEtGC.

Discussion

In neither the serum samples taken before the ethanol consumption nor those of the teetotalers ethyl glucuronide (EtG) was detected. From this one may conclude, that EtG is exclusively formed after consumption of ethanol.

In 10 testpersons with moderate alcohol consumption of less than 30 g per day, EtG was detected with a lag time of up to 45 min (Fig. 1). The maximum SEtGC was achieved 3.5 to 6 h after the subjects had stopped drinking and 2 to 3.5 h after the maximum of the SEC. In these drinking experiments the maximum SEC and SEtGC in one individual was 1.48 g/L with a peak SEtGC of 3.7 mg/L. The profiles in Fig. 1 indicate a considerable variation of the individual EtG formation. The individual variation of the EtG profile in serum probably depends on the resorption, distribution and elimination of ethanol and on the individual activity of UDP-glucuronyltransferases. Another reason could be assumed comparing the relatively small amount of EtG formation and the ethanol dose: As UDP-glucuronyltransferases are located in microsomal membranes (8), a limited but varying access of ethanol to the binding site may also be responsible for individual differences.

EtG showed a half-life of 2 to 3 h after complete alcohol elimination and was still detectable in serum samples for some hours (Fig. 2). In this period of time, testing for EtG offers a means of proving a previous ethanol consumption if in doubt. With respect to the proof of ethanol misuse, further investigations are in progress concerning the dose dependence of the EtG formation as well as half-life and duration of the excretion of EtG in urine.

In Fig. 3 the relation between SEC and SEtGC in serum samples of 50 individuals suspected of driving under the influence of drugs or ethanol (subject group 3) is shown. No definite correlation could be found. Obviously, the position of a set of values depended on the actual pharmacokinetic phase of ethanol. This can be explained by the temporal shift between the SEtGC curve and the SEC curve as shown in the concentration-time-diagrams (Fig. 1).

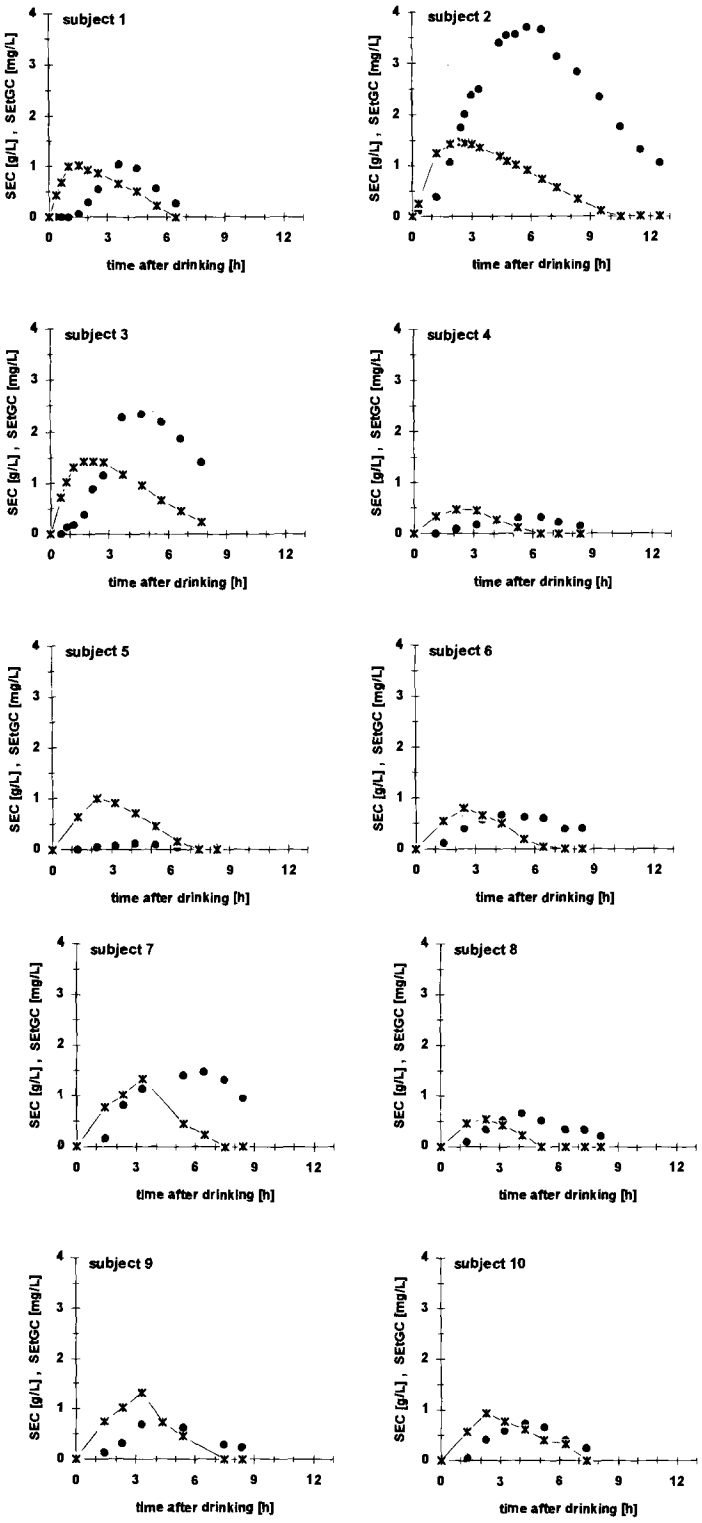

FIG. 1—*Serum ethanol concentration (SEC: ×, joined with straight lines) and EtG concentration in serum (SEtGC: ●) vs. time after intake of ethanol within 1 h (see also Table 2).*

In 7 samples alcohol could not be detected but the SEtGC was found at 1 to 20 mg/L. From the results of group 2 it can be concluded that SEtGC significantly higher then 5 mg/L may indicate alcohol misuse, especially if the SEC has dropped from higher concentration to below 1 g/L. However, for a final evaluation more experiments are needed with respect to possible dose dependence

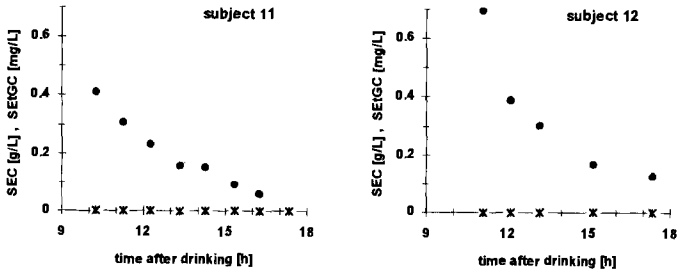

FIG. 2—*Elimination of EtG in serum (SEtGC: ●) vs. time of two male subjects after complete ethanol degradation (*subject 11, *weight: 78 kg, height: 174 cm, dose: 87 g ethanol, max. SEC calculated: 2.0 g/L;* subject 12, *weight: 63 kg, height: 172 cm, dose: 108 g ethanol, max. SEC calculated: 1.3 g/L). Times given correspond to the start of ethanol consumption.*

of EtG formation as well as to polymorphisms and/or possible inductors of UDP-glucuronyltransferases.

Liver enzyme activities, e.g., of gamma glutamyltransferase, alanine aminotransferase and aspartate aminotransferase or the concentrations of methanol in serum and carbohydrate deficient transferrin (CDT) are parameters whose efficiency as marker of ethanol is limited by their specificity and sensitivity. Additionally, a SEC above 2 g/L is regarded as an indicator of an unchecked drinking behavior (9). Possibly, the diagnostic means can be supplemented by the determination of the ethanol metabolite in serum. If ethanol abstinence is in question, a determination of EtG in serum, or rather in urine, can be recommended.

Conclusions

EtG is detectable in serum exclusively after alcohol consumption. EtG peaks later and decreases slower as compared with ethanol. The formation of EtG depends on the serum ethanol concentration. After ethanol elimination, the SEtGC declines exponentially with a half-life of 2 to 3 h. The kinetic profile explains why the SEC and SEtGC do not seem to correlate in 50 samples of drivers suspected of being under alcohol influence. If the SEC is less than 1 g/L and the SEtGC is much higher than 5 mg/L, an alcohol misuse seems probable. The current markers of alcohol misuse like methanol, CDT and enzyme activities can be supplemented by the determination of the highly ethanol specific EtG. Testing for EtG is restricted to a period of about 6 to more than 18 h after drinking, being dependent on the ethanol dose and

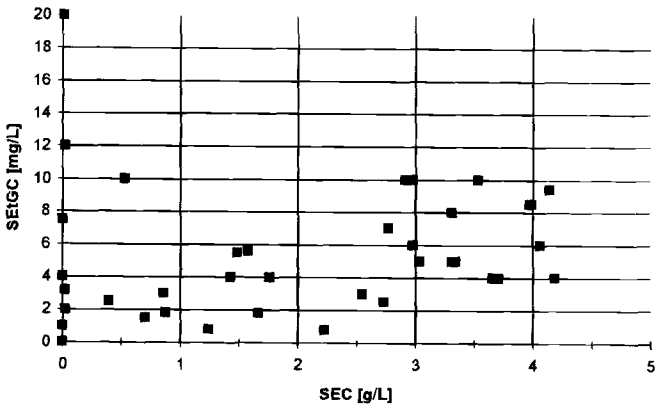

FIG. 3—*Serum ethanol concentration (SEC) vs. EtG concentration in serum (SEtGC) of drivers suspected to be under the influence of ethanol,* n = *50.*

individual metabolism. It is predominantly indicated when the ethanol concentration has become zero or low. The use of EtG as a marker of ethanol misuse, however, requires the clarification of the variability of this metabolic pathway with respect to the conditions of ethanol consumption.

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