Research article

Drug Testing and Analysis

Received: 31 January 2013 Revised: 17 April 2013 Accepted: 21 April 2013 Published online in Wiley Online Library

(www.drugtestinganalysis.com) DOI 10.1002/dta.1498

Pharmacokinetics of GHB and detection window in serum and urine after single uptake of a low dose of GBL – an experiment with two volunteers

Alexandra Schröck,[a]* Yvonne Hari,[a] Stefan König,[a] Volker Auwärter,[b] Stefan Schürch[c] and Wolfgang Weinmann[a]

During the last few years γ-hydroxybutyric acid (GHB) and γ-butyrolactone (GBL) have attracted much interest as recreational drugs and knock-out drops in drug-facilitated sexual assaults. This experiment aims at getting an insight into the pharmacokinetics of GHB after intake of GBL. Therefore Two volunteers took a single dose of 1.5 ml GBL, which had been spiked to a soft drink. Assuming that GBL was completely metabolized to GHB, the corresponding amount of GHB was 2.1 g. Blood and urine samples were collected 5 h and 24 h after ingestion, respectively. Additionally, hair samples (head hair and beard hair) were taken within four to five weeks after intake of GBL. Samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) after protein precipitation with acetonitrile. The following observations were made: spiked to a soft drink, GBL, which tastes very bitter, formed a liquid layer at the bottom of the glass, only disappearing when stirring. Both volunteers reported weak central effects after approximately 15 min, which disappeared completely half an hour later. Maximum concentrations of GHB in serum were measured after 20 min (95 μg/ml and 106 μg/ml). Already after 4–5 h the GHB concentrations in serum decreased below 1 μg/ml. In urine maximum GHB concentrations (140 μg/ml and 120 μg/ml) were measured after 1–2 h, and decreased to less than 1 μg/ml within 8–10 h. The ratio of GHB in serum versus blood was 1.2 and 1.6.
Copyright © 2013 John Wiley & Sons, Ltd.

Supplementary information may be found in the online version of this article.

Keywords: LC-MS/MS; GHB; GBL; pharmacokinetics

Introduction

γ-hydroxybutyric acid (GHB) is an endogenous neurotransmitter and is present in mammalian brain tissue.[1–3] In the early 1960s, GHB was synthesized for medical purposes[4] as an analogue to the neurotransmitter γ-aminobutyric acid (GABA). Initially it was used as an anaesthetic, but GHB lacked analgesic properties and its duration of action was unpredictable.[5,6] Starting in the 1980s, GHB was used for non-medical purposes, for example in body-building and as recreational drug.[7] In the USA, it was available for many years as a dietary supplement.[8] Currently, GHB is used for the treatment of narcolepsy and cataplexy under the name Xyrem®,[9] and its use might be helpful in alcohol withdrawal.[8,10]

When consumed at low doses (20 to 30 mg per kg body weight), the effects are euphoria, disinhibition, drowsiness, and impairment of a person's driving ability.[11,12] Higher doses of GHB have sedative or comatose effects and can cause memory loss.[7,13] Lethal mono-intoxications with GHB have been reported. GHB causes depression of cardiovascular and respiratory systems.[14–16] Frequent users, however, are in great danger of getting addicted to GHB.[1]

Due to endogenous GHB concentrations and due to its fast elimination after oral intake, the interpretation of obtained GHB levels can be difficult. Various studies showed inter-individual differences in endogenous GHB concentrations in blood and urine. Blood concentrations up to 5 mg/l (in fresh blood samples) and urine concentrations up to 10 mg/l are regarded as normal endogenous GHB levels.[17,18] Dietary effects on endogenous GHB production[19] and effects of storage temperature that would correspond with exogenous GHB intake were not observed either.[20]

GHB is a controlled substance in many European countries. Since γ-butyrolactone (GBL) is used as a solvent in the chemical industry and is not strictly controlled in some countries, the consumption of GBL is getting more common, and it is rapidly converted *in vivo* to GHB.

In the last few years, GHB and GBL have received much negative attention in the media, as these substances are used as recreational drugs or as knock-out drops for drug-facilitated sexual assaults. The aim of this study was to get an insight into the pharmacokinetics and detectability of GHB after ingestion of a single dose of GBL in a spiked drink.

* *Correspondence to: Alexandra Schröck, Institute of Forensic Medicine, Forensic Toxicology and Chemistry, University of Bern, Bühlstrasse 20, 3012 Bern, Switzerland. E-mail: alexandra.schroeck@irm.unibe.ch*

a *Institute of Forensic Medicine, Forensic Toxicology and Chemistry, University of Bern, Switzerland*

b *Institute of Forensic Medicine, University Medical Center, Freiburg, Germany*

c *Department of Chemistry and Biochemistry, University of Bern, Switzerland*

Copyright © 2013 John Wiley & Sons, Ltd.

Experimental

Reagents

GHB and GHB-D_6 were supplied by Lipomed (Arlesheim, Switzerland). Formic acid (49–51 %) and GBL were obtained from Fluka (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). HPLC-grade water was produced in-house with a Milli-Q water system from Millipore (Billerica, MA, USA). Acetonitrile and methanol were purchased from Biosolve (Chemie Brunschwig, Basel, Switzerland). Ammonium hydroxide solution (25 %) and ammonium acetate were obtained from Merck (Darmstadt, Germany). Blank blood and serum were supplied by the blood donation center (SRK Bern AG, Switzerland). Blank urine and hair samples for calibration were collected from volunteers at the Institute of Forensic Medicine Bern.

Study design

Two volunteers (male, 47 and 42 years, 78 kg and 75 kg, respectively) took a single dose of 1.5 ml GBL each, which had been spiked to a soft drink (Coca-Cola®). Blank urine and blood samples were obtained from both subjects before starting the experiment, which was performed at the Institute of Forensic Medicine, University Medical Center, Freiburg, Germany.

Two blood samples were taken every 20 min from the cubital vein within 5 h of GBL consumption using lithium-heparin sampling tubes for whole blood analysis and serum sampling tubes (Sarstedt, Nümbrecht, Germany) to isolate serum through centrifugation. Urine samples were collected during 24 h. All samples were stored at -20 °C prior to analysis. Additionally, hair samples (head hair and beard hair) were taken within four to five weeks after intake of GBL. Beard hair was shaved every other day and had a length of 1 mm.

Analytical methods

Preparation of calibration samples

Working solutions were prepared from the GHB stock solution (1 mg/ml in methanol). Blank blood, serum, and urine were spiked with these solutions at concentration levels of 1.0, 5.0, 10, 25, and 50 μg/ml.

For hair analysis seven calibration samples containing 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10 ng GHB per mg hair were prepared. Before spiking, the hair material used for calibration was extracted with water by ultrasonication for 2 h to remove endogenous GHB.

Sample preparation

Hundred microlitres of blood/serum/urine (calibrators and samples) were transferred to an Eppendorf tube and 800 μl of internal standard solution (10 μg/ml GHB-D_6 dissolved in mobile phase B) were added. After 5 min of mixing and 10 min of centrifugation (13 000 rpm) the supernatant solutions were transferred into autosampler vials, 5 μl were injected.

Head hair samples were cut between two adjoining hair clips, which lead to segments of a constant length of 7 mm. The segments were washed with water, acetone and dichloromethane. The hair samples were left to dry and cut into small pieces (1–2 mm). The same washing procedure was applied to beard hair samples. Twenty mg of hair were placed into an Eppendorf tube and 1 ml water and 10 μl of internal standard solution (4 μg/ml GHB-D_6 in methanol) were added. After shaking for 5 min and centrifuging for 1 min, the samples were placed into an ultrasonic bath for 2 h. The samples were centrifuged for 5 min and the extracts were transferred to 2-ml glass vials. The solutions were evaporated in a vacuum concentrator at 55 °C. The samples were reconstituted in 100 μl reconstitution solution (99:1 water: acetonitrile with 0.1 % formic acid). 20 μl of the sample were injected.

Instrumentation

For serum, whole blood, and urine samples the system consisted of a CTC PAL autosampler, an Agilent 1200 series HPLC and a QTrap 3200 mass spectrometer (AB Sciex) controlled by Analyst 1.5.1 software.

Analytical separation was performed by a Hilic Kinetex column, 50 mm x 2.1 mm, 2.6 μm (Phenomenex, Brechbühler AG, Schlieren, Switzerland) with a flow rate of 0.3 ml/min. Mobile phase A was water with ammonium acetate (10 mM) and mobile phase B consisted of acetonitrile, mobile phase A (95/5) and 0.2 % NH_3. The following 9 min gradient was used: 0 to 1.5 min, 100 % B; 1.5 to 2 min, 100 to 10 % B linear; 2 to 3 min, 10 % B; 3 to 3.1 min, 10 to 100 % B linear; 3.1 to 9 min, 100 % B.

To detect GHB (retention time 4.0 min), the mass spectrometer was operated in ESI negative MRM mode, with an ion spray voltage of -4250 V and a source temperature of 450 °C. The mass spectrometric parameters are shown in Table 1.

For hair analysis, the system consisted of a Dionex 3000 system with an autosampler, two binary pumps, one isocratic pump and a column oven. As mass spectrometer an AB Sciex 5500 QTrap was used, controlled by Analyst 1.5.2 software.

Analytical separation was performed by reversed-phase chromatography with a flow rate of 0.3 ml/min. Mobile phase A consisted of water with 0.1 % formic acid and mobile phase B was acetonitrile with 0.1 % formic acid.

The method included online SPE (solid-phase extraction) for sample purification. The set-up for online SPE consisted of two HPLC columns linked by a 10-port switching valve.

The sample was loaded onto a short trapping column (Thermo Scientific Hypercarb™, 30 x 2.0 mm). With the valve in 'loading' position, the trapping column was flushed with an aqueous mobile phase for 1 min to wash out salts and water-soluble impurities. The switching of the 10-port valve to 'eluting' position caused an inversion of the flow through the trapping column transporting the sample onto the analytical column (Thermo Scientific Hypercarb™, 100 x 2.1 mm). A two-step gradient was run that comprised a slow increase of the organic fraction in the mobile phase from 0 % to 30 % over 6.5 min, followed by a more rapid increase to 95 % acetonitrile. After 9.5 min the 10-port valve was returned to the 'loading' position and both columns were equilibrated with mobile phase A for 3 min before the next sample was injected.

To detect GHB in hair (retention time 4.6 min), the mass spectrometer was operated in ESI negative MRM mode, with an ion spray voltage of -4200 V and a source temperature of 650 °C. The mass spectrometric parameters are shown in Table 2.

Table 1. Mass spectrometric parameters QTrap 3200 mass spectrometer (AB Sciex) for the detection of GHB in serum, blood, and urine

Substance	Precursor Ion (Q1)	Product Ion (Q3)	DP (V)	EP (V)	CE (V)
GHB	103	57	-20	-8	-18
		85			-14
GHB-D6	109	61	-20	-8	-18
		90			-14

Copyright © 2013 John Wiley & Sons, Ltd.

Table 2. Mass spectrometric parameters QTrap 5500 mass spectrometer (AB Sciex) for the detection of GHB in head and beard hair

Substance	Precursor Ion (Q1)	Product Ion (Q3)	DP (V)	EP (V)	CE (V)
GHB	103	57	-25	-7	-20
		85	-30		-15
GHB-D6	109	61	-22	-8	-19
		90	-30		-16

Results and discussion

Observed effects

Spiked to a soft drink, GBL forms a liquid layer at the bottom of the glass until stirring. The taste of GBL was described as rather bitter but probably it would be hardly noticed in a spiked cocktail. Assuming that GBL was metabolized completely, the corresponding amount of ingested GHB was 2.1 g (27 mg/kg body weight and 28 mg/kg body weight, respectively), which is a therapeutic dose for the treatment of narcolepsy and heavy side effects are not reported. In this experiment also only weak central effects, like dizziness, slight dilation of pupils and delayed reaction to light were observed after approximately 15 min and disappeared half an hour later.

Serum and whole blood GHB concentrations

Maximum concentrations of GHB in serum and blood were measured after 20 min. For test person 1 the maximum concentration in serum was 95 µg/ml and 58 µg/ml in whole blood. For test person 2 the maximum concentration in serum was 106 µg/ml and 83 µg/ml in whole blood. For both volunteers the GHB concentrations in serum were clearly higher than those in blood samples.

Blood had been taken every 20 min, and in the samples taken at 3 h the GHB concentrations in serum were still 4.3 µg/ml and 4.9 µg/ml, respectively. In blood the concentrations were 3.2 µg/ml and 2.3 µg/ml, respectively. After 4–5 h (240–300 min) the GHB concentrations in serum, and also in blood, decreased below the analytical limit of 1 µg/ml. No endogenous GHB in serum and blood was detected in either test person before ingestion of GBL. Therefore, the recommended cut-off for endogenous GHB of 5 µg/ml seems to be too high for these two test persons; also the detection window would be even shorter (only about 3 h).

The mean serum-to-blood distribution coefficients of 1.6 and 1.2 were determined for test persons 1 and 2, respectively, by comparing the obtained serum concentrations to the corresponding blood concentrations. As expected, GHB was detectable in serum and blood soon after ingestion of GBL, but was also rapidly eliminated (Figures 1 and 2).

GHB elimination with a first order kinetic was found for both test persons with serum elimination half-life times of 26 min (test person 1) and 24 min (test person 2).

GHB concentrations in urine

Maximum GHB concentrations (140 µg/ml and 120 µg/ml) were measured after 1–2 h.

Five hours after GBL intake, the GHB concentration in the urine sample of test person 1 was still 38 µg/ml, but after the same time only 4 µg/ml GHB were detectable in the urine sample of test person 2. After 8 h the GHB concentration of test person 1 had

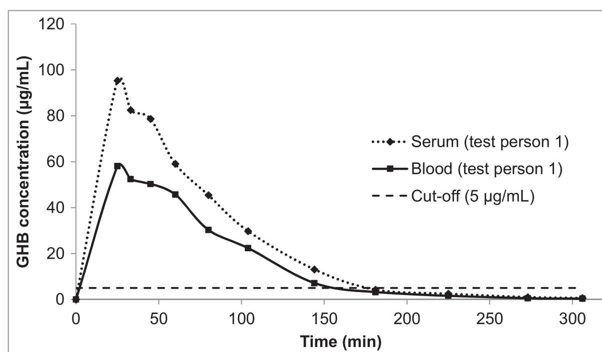

Figure 1. Serum and whole blood GHB concentrations of test person 1 (data are provided in supplementary information).

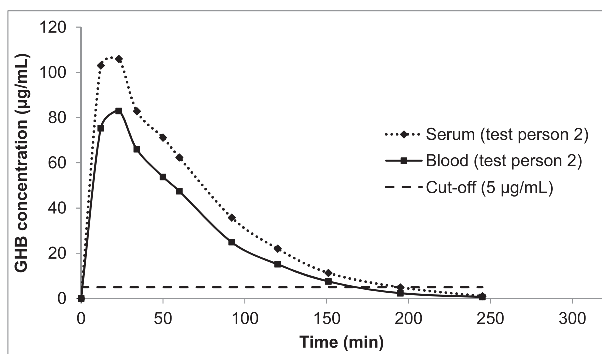

Figure 2. Serum and whole blood GHB concentrations of test person 2 (data are provided in supplementary information).

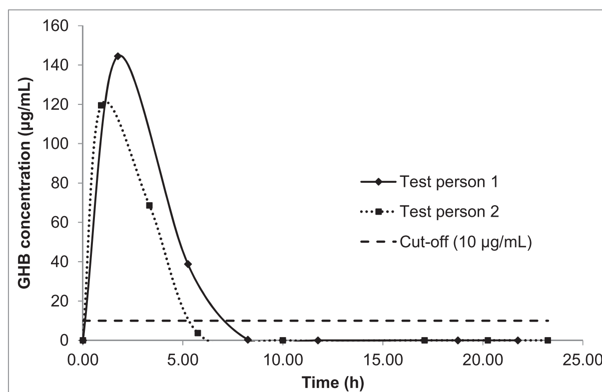

Figure 3. Urine GHB concentrations of both test persons (data are provided in supplementary information).

decreased below the detection limit. For test person 2 this level was reached after 10 h (Figure 3).

In forensic cases, for differentiation of endogenous versus exogenous GHB concentrations in urine, a cut-off of 10 µg/ml is usually used. Since no endogenous GHB in urine was detected in both test persons prior to the ingestion of GBL, the recommended cut-off level seems to be too high for these two test persons. These results are in agreement with suggestions for lower cut-off levels for endogenous GHB from other studies.[21–23]

Hair analysis

The GHB concentrations measured in head hair were in the range of 0.1 to 0.6 ng GHB per mg hair. No increase of the GHB content

 Copyright © 2013 John Wiley & Sons, Ltd.

of the hair samples was detected after a single intake of GBL (samples were collected from test person 1 on days 9, 14, 25, and 28 and from test person 2 on days 8, 17, 25, 37).

Control hair samples of 27 GHB-free individuals were analyzed. The endogenous GHB concentration of these samples was found to range from 0.1 to 1.3 ng/mg. But in 8 of the 27 hair samples the GHB concentrations were below the LOQ of 0.1 ng/mg hair. The GHB concentrations measured in the test persons' hair after the GBL intake lie well within this endogenous range. These results are in agreement with measurements of endogenous GHB concentrations in head hair samples recently presented by Martz *et al.*[24] Segmental analysis showed that the endogenous GHB content of the hair samples is consistent. However, significant differences between the five hair samples collected from test person 1 on five different days were noted.

In beard hair the measured GHB concentrations were up to 20 times higher than the concentrations measured in head hair. For both test persons, the range of measured concentrations in beard was rather large (0.2–3.1 ng/mg and 0.7–3.5 ng/mg, respectively), but no increased GHB content of the samples was observed for the first days after the intake of GBL.

A possible explanation for the elevated GHB concentrations and the large range measured in beard hair might be external contamination by sweat. Washing and substance incorporation from sweat may also cause the deviation of the concentrations measured in hair samples collected from one individual on different days. Although all GHB concentrations measured in head hair samples compare well to the endogenous range, further investigation of intra-individual day-to-day variation is advisable.

Conclusion

Single uptake of GBL (1.5 ml) yields measurable concentrations of GHB in blood/serum and urine. Detection windows are 4–5 h in blood or serum, and up to 10 h in urine.

With the recommended cut-off levels for endogenous GHB of 5 µg/ml in serum and blood and 10 µg/ml in urine the detection windows would be even shorter, about 3 h in serum and blood and up to 8 h in urine. Since no endogenous GHB was detected in both test persons before ingestion of GBL and since all samples were freshly taken and stored at -20 °C prior to analysis the cut-off levels for differentiation of endogenous or exogenous GHB under these circumstances might be lowered.

Beard or head hair analysis for GHB was not successful in detection of a single GBL intake, since GHB levels did not exceed the endogenous levels and no significant increase was found along the hair shaft in head hair by segmental analysis. Day-to-day variations of GHB in beard hair were noticed, however, no significant increase of the GHB concentration was detected after GBL intake in beard hair either. Hair analysis for the detection of single uptake of GBL therefore cannot be recommended.

Supplementary information

Supplementary information may be found in the online version of this article.

Acknowledgement

We would like to thank PD Dr. med. Annette Thierauf (Institute of Forensic Medicine, University Medical Center, Freiburg, Germany) for blood sampling.

References

[1] O.C. Snead, K.M. Gibson. γ-Hydroxybutyric acid. *New Engl. J. Med.* **2005**, *352*, 2721.
[2] J.D. Doherty, S.E. Hattox, O.C. Snead, R.H. Roth. Identification of endogenous gamma-hydroxybutyrate in human and bovine brain and its regional distribution in human, guinea pig and rhesus monkey brain. *J. Pharmacol. Exp. Ther.* **1978**, *207*, 130.
[3] S.P. Bessman, W.N. Fishbein. Gamma-Hydroxybutyrate, a normal brain metabolite. *Nature* **1963**, *200*, 1207.
[4] H. Laborit. Sodium 4-hydroxybutyrate. *Int. J. Neuropharmacol.* **1964**, *3*, 433.
[5] L.J. Marinetti, D.S. Isenschmid, B.R. Hepler, S. Kanluen. Analysis of GHB and 4-methyl-GHB in postmortem matrices after long-term storage. *J. Anal. Toxicol.* **2005**, *29*, 41.
[6] M.E. Tunstall. Gamma-OH in anesthesia for caesarean section. *Proc. R. Soc. Med.* **1968**, *61*, 827.
[7] R.L. Chin, K.A. Sporer, B. Cullison, J.E. Dyer, T.D. Wu. Clinical course of gamma-hydroxybutyrate overdose. *Ann. Emerg. Med.* **1998**, *31*, 716.
[8] K.L. Nicholson, R.L. Balster. GHB: A new and novel drug of abuse. *Drug Alcohol Depend.* **2001**, *63*, 1.
[9] A.D. Brailsford, D.A. Cowan, A.T. Kicman. Pharmacokinetic properties of gamma-hydroxybutyrate (GHB) in whole blood, serum, and urine. *J. Anal. Toxicol.* **2012**, *36*, 88.
[10] F. Caputo, T. Vignoli, I. Maremmani, M. Bernardi, G. Zoli. Gamma hydroxybutyric acid (GHB) for the treatment of alcohol dependence: A review. *Int. J. Environ. Res. Public Health* **2009**, *6*, 1917.
[11] S. Dresen, J. Kempf, W. Weinmann. Prevalence of gamma-hydroxybutyrate (GHB) in serum samples of amphetamine, metamphetamine and ecstasy impaired drivers. *Forensic Sci. Int.* **2007**, *173*, 112.
[12] F.J. Couper, B.K. Logan. Addicted to driving under the influence-a GHB/GBL case report. *J. Anal. Toxicol.* **2004**, *28*, 512.
[13] G.P. Galloway, S.L. Frederick-Osborne, R. Seymour, S.E. Contini, D.E. Smith. Abuse and therapeutic potential of gamma-hydroxybutyric acid. *Alcohol* **2000**, *20*, 263.
[14] K.S. Kalasinsky, M.M. Dixon, G.A. Schmunk, S.J. Kish. Blood, brain, and hair GHB concentrations following fatal ingestion. *J. Forensic Sci.* **2001**, *46*, 728.
[15] C. Jones. Suspicious death related to gamma-hydroxybutyrate (GHB) toxicity. *J. Clin. Forensic Med.* **2001**, *8*, 74.
[16] S.D. Ferrara, L. Tedeschi, G. Frison, A. Rossi. Fatality due to gamma-hydroxybutyric acid (GHB) and heroin intoxication. *J. Forensic Sci.* **1995**, *40*, 501.
[17] A.A. Elian. Determination of endogenous gamma-hydroxybutyric acid (GHB) levels in antemortem urine and blood. *Forensic Sci. Int.* **2002**, *128*, 120.
[18] D.T. Yeatman, K. Reid. A study of urinary endogenous gamma-hydroxybutyrate (GHB) levels. *J. Anal. Toxicol.* **2003**, *27*, 40.
[19] S.P. Elliott. Gamma hydroxybutyric acid (GHB) concentrations in humans and factors affecting endogenous production. *Forensic Sci. Int.* **2003**, *133*, 9.
[20] M.A. LeBeau, M.L. Miller, B. Levine. Effect of storage temperature on endogenous GHB levels in urine. *Forensic Sci. Int.* **2001**, *119*, 161.
[21] F. Mari, L. Politi, C. Trignano, M.G. Di Milia, M. Di Padua, E. Bertol. What constitutes a normal ante-mortem urine GHB concentration? *J. Forensic Leg. Med.* **2009**, *16*, 148.
[22] R. Brenneisen, M.A. Elsohly, T.P. Murphy, J. Passarelli, S. Russmann, S.J. Salamone, D.E. Watson. Pharmacokinetics and excretion of gamma-hydroxybutyrate (GHB) in healthy subjects. *J. Anal. Toxicol.* **2004**, *28*, 625.
[23] H. Andresen, N. Sprys, A. Schmoldt, A. Mueller, S. Iwersen-Bergmann. Gamma-hydroxybutyrate in urine and serum: Additional data supporting current cut-off recommendations. *Forensic Sci. Int.* **2010**, *200*, 93.
[24] W. Martz, D. Rutledge. Intraindividual variation of levels of endogenous GHB in hair. Annual meeting of the Society of Hair Testing, Toronto, June 2012. *J. Popul. Ther. Clin. Pharmacol.* **2012**, *19*, e311.

Copyright © 2013 John Wiley & Sons, Ltd.