CASE REPORT

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Blood, Brain, and Hair GHB Concentrations Following Fatal Ingestion*+

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ABSTRACT: Despite the increasing incidence of illicit use of gamma-hydroxybutyrate (GHB), little information is available documenting levels of the drug in GHB fatalities. We measured GHB levels in postmortem blood, brain and hair specimens from a suspected overdose case by gas chromatography/mass spectrometry (GC/MS) following solid phase extraction (SPE) and derivatization with bis(trimethyl-silyl) trifluoroacetamide (BSTFA). Examination found 330 μg/mL GHB in femoral blood and 221 ng/mg GHB in frontal cortex brain tissue, values higher than those typically reported in the literature. The hair shaft was negative for GHB whereas the plucked root bulbs with outer root sheath attached (2221 ng/mg) and root bulbs after washing and removal of the outer root sheath (47 ng/mg) contained the drug. Our results are consistent with an acute single dose of GHB and, as the toxicology screen was negative for other drugs of abuse, emphasize the significant danger of this drug.

KEYWORDS: forensic science, forensic toxicology, death, gamma-hydroxybutyrate, blood, brain, hair, gas chromatography/mass spectrometry, solid phase extraction

GHB is an endogenous substance found in the body (1). This CNS depressant has been used for induction of anesthesia (1), treatment of narcolepsy (2), and for alcohol and opiate withdrawal (3,4). It has been used for a food supplement and by bodybuilders for its supposed growth hormone release action (5). Recently, GHB has also been used as a sexual assault drug and is becoming a widely used drug of abuse (6,7). Illicit use of GHB typically involves doses of 35 mg/kg (1). Doses of 10 mg/kg cause amnesia; 20–30 mg/kg cause sleep; and doses of 50 mg/kg or higher produce anesthesia (1). In controlled human studies where 25 mg/kg doses were administered, peak plasma concentrations were 24–88 μg/mL (8), and 50 mg/kg doses resulted in peak plasma concentrations of 48–125 μg/mL (9). As little information is available on levels of the drug in GHB fatalities, especially in brain, we measured levels of GHB in postmortem blood, brain and hair of a subject suspected of having taken an acute dose of the drug.

Methods

Autopsied brain, heart and femoral blood, and scalp hair were taken from a 22-year-old Caucasian female who allegedly had never previously used GHB and who had taken a single dose of liquid GHB. The estimated intervals between consumption of GHB and death and between death and autopsy were approximately 7 h or less and 24 h, respectively. The blood was preserved in fluoridated tubes and the brain was frozen at −80°C. No urine was available for this study. Autopsy findings were negative. Drug screens of the blood were negative for ethanol, methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA), opiates (morphine/codeine), phencyclidine (PCP), methamphetamine and cannabinoids. GHB screened by LC/MS was positive and confirmation was performed by GC/MS. Based upon the toxicology findings (see below), the suspected cause of death was GHB toxicity.

Sample Preparation

Hair was plucked from the scalp. The root bulbs were removed for individual analysis and the remainder of the hair shafts were segmented into ½ in. lengths beginning with the root end. Segmented samples and root bulbs were washed with 1 × 5mL 1% sodium dodecyl sulfate followed by 5 × 5mL deionized water. Sample was rinsed with 3 mL of methanol and collected for GC analysis to determine that the rinse was negative for the drug of interest and no repeat of the wash procedure was required. Sample was allowed to air dry and then weighed in approximately 10 mg aliquots for extraction. In each test tube with the 10 mg hair sample, 100 μL of internal standard, GHB-d6 (10 μg/mL in methanol), was added along with 100 μL of 100 mM phosphate buffer pH6 and 100 μL of 0.1 N KOH. Samples were then heated in a water bath at 40°C for 2 h. Samples were removed from the water bath after 2 h and an additional 100 μL of 100 mM phosphate

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buffer pH6 was added. Samples were then ready for extraction procedure described below.

Brain was weighed out in 100 mg aliquots. Samples were placed in 16 × 125 mm test tubes along with 100 μL of internal standard, GHB-d6 (10 μg/mL in methanol), and 1 mL of 100 mM phosphate buffer pH6. Samples were homogenized and 100 μL aliquoted into clean test tubes for the extraction process. Alternatively, a second sample preparation suggested by United Chemical Technologies (UCT) was performed for comparison where 100 mg of the tissue was aliquoted to a test tube and 1:4 dilution weight/volume of acetone was added to the test tube. Samples were homogenized and the entire sample was extracted by the procedure below.

Blood (200 μL) was aliquoted into test tubes along with 200 μL of internal standard, GHB-d6 (10 μg/mL in methanol), and 1 mL of acetone. Samples were ready for extraction procedure below.

Extraction Procedure

Extraction columns (United Chemical Technologies ZSGHB020) were conditioned with successive addition of 3 mL methanol, 3 mL deionized water, and 0.5 mL 100 mM phosphate buffer pH6. Sample was loaded onto the column. Extraction method followed the UCT recommended procedure for the columns and GHB (10). Elution solvent (99/1) methanol/ammonium hydroxide was added to the sample tubes and then transferred to the column. All samples loading and elution solvent was collected. Samples were evaporated to dryness at 60°C under N2. Dimethylformamide (200 μL) was added to each tube followed by 1 mL of hexane saturated with dimethylformamide; mixed, centrifuged, then transferred to a clean test tube. Samples were evaporated to dryness under N2 at 50°C. Samples were derivatized with 50 μL ethyl acetate and 50 μL BSTFA with 1% TMCS for hair, 100 μL ethyl acetate and 100 μL BSTFA with 1% TMCS for brain, and 300 μL ethyl acetate and 100 μL BSTFA with 1% TMCS for blood. Samples were vortexed and transferred to injection vials for GC/MS analysis. Percent recovery ranged from 41–86% depending on the concentration used.

Instrumental Conditions

Analysis was performed on a Varian Saturn 3 ion trap mass spectrometer interfaced with a Varian 3400 gas chromatograph equipped with a Zebron ZB-5 15 m × 0.25 mm ID capillary column with 0.25 mm film loading (Phenomenex, Torrance, CA). The instrument was used in full scan electron ionization mode, scanning *m/z* in the range 230–245 with a 3 min solvent delay. The oven temperature was programmed with an initial temperature of 70°C with a hold time of 1 min. Oven temperature was increased to 100°C at 8°C/min, to 175°C at 25°C/min, and to 280°C at 35°C/min. Injector temperature was set at 250°C and the detector was also set at 250°C. The constant flow pressure was set at 10 psi with the 1077 injector using helium as the carrier gas. One microliter of sample was injected into the injector in splitless mode. The ions of interest are 233, 234, 235 and 239, 240, 241 for GHB and GHB-d6, respectively. Each instrumental analysis batch contained 3 calibrators at concentrations appropriate for the matrix, as well as one positive and one negative control. GHB standards yielded a linearity range of 10–100 000 ng with an $r^2 = 0.995$. Detection limit was 1 ng/μL for blood and 1 ng/mg for brain and hair.

Results and Discussion

Blood, brain, and hair specimens were extracted, derivatized then analyzed by GC/MS. Quantitation was based on internal standardization using GHB-d6. Samples were quantified by using the ions *m/z* 233, 234, 235 for GHB versus 239, 240, 241 for GHB-d6. The retention time for GHB and GHB-d6 was approximately 3.89 and 3.91 min, respectively.

The submitting unit had previously determined that 648 μg/mL GHB was present in the heart blood. The femoral blood specimen that was submitted contained 330 μg/mL GHB as determined by the method described above. Control postmortem blood contained an average of 7.7 μg/mL GHB (range, 3.0–14.0 μg/mL, $n = 12$). Control antemortem blood contained an average of 1.4 μg/mL GHB (range, 1.0–2.0 μg/mL, $n = 4$). The high level of GHB in the femoral blood posed difficulty running the sample on the ion trap mass spectrometer; therefore, it was necessary to dilute the sample with ethyl acetate before injection for analysis. Fatalities with GHB have reported postmortem blood values at 27–121 μg/mL (1).

Brain from the frontal cortex region was analyzed and results were determined to be 204.8 ng/mg by the described method and 236.5 ng/mg by the UCT suggested method for an average of 220.7 ng/mg. The methods differ in sample preparation only. This variance could be due to the different brain sections that were used for the analysis. Control brain contained no quantifiable amounts of GHB. A previous fatality reports a brain value of 40 ng/mg GHB (5).

Segmental hair analysis showed no detectable amounts of GHB. A previous study (11) of hair shaft samples collected from patients receiving long-term oral daily doses (3.5–14.0 g) of GHB for therapeutic purposes showed a level of 3–5 ng/mg of GHB in 50 mg of hair. This previous study also investigated endogenous amounts of GHB in hair, and showed an average of 0.09 ng/mg from the investigation of 300 mg of hair per analysis (11). This amount of material is 30 times the amount of starting material that was available for this case study and could explain why no detectable amounts of endogenous GHB were found in this study. The absence of detectable GHB, due to ingestion of the drug, in the hair segments of this case is also explained by the lack of chronic use of the drug, as suggested by the medical examiner investigation report. Hair root bulbs results were as follows: 47.4 ng/mg and 2221 ng/mg washed versus unwashed respectively, indicating that GHB readily distributes into the hair follicle. Unpublished work from other studies in this laboratory have also indicated that most drugs distribute readily into the hair follicle but some types of drugs are lost from the hair upon keratinization while others remain bound within the shaft when it exists the scalp.

Tests were performed to determine whether GHB was converting to GBL during the preparation process; however, running the samples in the region of *m/z* 35–100 did not demonstrate significant amounts of the ions associated with GBL. The hair segments were also analyzed for cocaine and opiates and found to be negative, suggesting no history of cocaine or opiate use.

GHB is known to be produced postmortem (12) but the endogenous levels both postmortem and antemortem are far below those found in this case study. The effect of endogenous production in the levels reported here can be considered negligible relative to the amounts found in these specimens. However, it is warned that the endogenous levels and postmortem production need to be considered in evaluating each case.

Conclusions

The findings from this study are consistent with a single acute fatal dose of GHB ingestion. The blood and brain values from this case are markedly above those of previously reported GHB fatalities, indicating that the range of GHB levels for acute overdose

cases should be revised upwards. Our toxicology data also indicate, for the first time, that the drug distributes into the hair root bulb. Finally, this case reinforces the finding, generally not appreciated in the drug abuse community, that life-threatening effects of this abused drug can occur following acute GHB exposure.

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