Unusually High Concentrations in a Fatal GHB Case

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Abstract

The first case in France involving a fatal overdose resulting from the ingestion of gamma-hydroxybutyrate (GHB) is presented. GHB was tested by gas chromatography-mass spectrometry (GC–MS) after precipitation. Briefly, 20 μL of body fluids (blood, bile, urine, gastric contents, or vitreous humor) was pipetted in a glass tube, followed by 20 μL GHB-d6 and 45 μL acetonitrile. After vortex mixing and centrifuging, the supernatant was collected and evaporated to dryness. The residue was derivatized with BSTFA with 1% TMCS for 20 min at 70°C. After injection on a 30-m HP5 MS capillary column, GHB (m/z 233, 204, and 147) and GHB-d6 (m/z 239) were identified by MS. GHB was also tested in pubic hair after incubation in 0.01N NaOH, neutralization, acidification, extraction in ethyl acetate and derivatization with MTBSTFA, using GC–MS–MS. GHB was positive in all the tested specimens, with the following concentrations 2937, 33,727, 1800, and 2856 mg/L in femoral blood, urine, bile, and vitreous humor, respectively. This seems to be the highest blood concentration ever observed. Postmortem redistribution appears weak, as the concentration in cardiac blood was 3385 mg/L (cardiac blood/femoral blood ratio of 1.15). Oral route was suggested with GHB at 7.08 g in 100 mL of gastric contents. Pubic hair analysis clearly indicated chronic GHB abuse, with concentrations along the shaft in the range 19.4 to 25.0 ng/mg (in comparison with physiological concentrations < 2 ng/mg). Methyleneoxymethamphetamine was present in femoral blood at 144 ng/mL. These results are consistent with an acute fatal overdose of GHB.

Introduction

Although considered a drug of abuse, gamma-hydroxybutyrate (GHB) has been used clinically since the 1960s as an intravenous anesthetic. It was also investigated for treatment of insomnia, alcohol and opiates withdrawal syndrome, and cerebro-vascular disorders.

GHB is a substance naturally present within mammalian species (1,2). It is a white crystalline material and is available as the sodium salt in the form of a white powder, capsules, or tablets and also as a colorless, odorless liquid. It is taken orally and rarely injected.

Doses of 10 mg/kg cause amnesia; 20–30 mg/kg induce sleep; and doses of 50 mg/kg or higher produce anesthesia (1). Illicit use of GHB typically involves doses of 35 mg/kg (3). GHB may be taken in doses of one-half to three teaspoons and produces drowsiness, euphoria, dizziness, amnesia, and visual disturbances about 15 min later, lasting, on average, about 3 h (1).

People who may use GHB include bodybuilders who believe that the drug stimulates the release of growth hormone, although whether it does effect or cause increased muscle mass or growth is still under debate (4); ravers or club attendees for its intoxicating effects such as euphoria, reduced inhibitions, sedation, and muscle relaxation that can be beneficial after ecstasy abuse (5); drivers as a result of recreational abuse (6); and victims of drug-facilitated sexual assault (7).

The purported enhancement of sexuality, coupled with a possible abrupt coma-inducing effect, ease of administration in spiked drinks, and potential amnesia have resulted in the use of GHB as an assault-related drug. GHB is also attractive to rapists as it is readily available (e.g., on the Internet, on the street, in dance clubs or fitness centers).

Overdoses with GHB are known to cause nausea and vomiting, random clonic muscle movements, convulsions, coma, and respiratory depression (1,3).

The levels of GHB obtained in casework require critical interpretation because GHB is a naturally occurring substance in the body. In 1998, Fieler et al. (8) demonstrated that GHB can be detected in postmortem blood in very substantial concentrations, ranging from 3.2 to 168 mg/L, in cases not known to be GHB-related. These authors suggested the analysis of GHB in urine to differentiate endogenous production from exposure. The advantage of urine over blood in documenting GHB-involved death was confirmed by Stephens et al. (9) in 1999. However, in 2001, Elliott (10) indicated that the analysis of urine specimens (range 0 to 217 mg/L) from 13 non-GHB-related fatalities would indicate GHB ingestion and potential intoxication, complicating any interpretation of suspected GHB-related fatalities. These findings are still under debate.

In order to establish a scientific basis to discriminate between endogenous formation and exposure, Kintz et al. (11) re-

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cently proposed simultaneously testing for GHB in femoral blood and vitreous humor.

This reported fatal case, which appears to be the first fatality in France involving GHB, confirms the place of vitreous humor in demonstrating GHB exposure. Moreover, the concentrations that were observed in this case were unusually high.

Case History

A 43-year-old man was found unconscious by 2 relatives at his home. Despite rapid emergency aid, he was pronounced dead soon after. The decedent was a Caucasian, 1.81-m tall, with a weight of 82 kg. He was known to be a drug abuser, particularly for club drugs. Autopsy was unremarkable, no particular morphological changes were noted, except for pulmonary congestion. There was no evidence of violence and no needle marks were found. Specimens of body fluids, including cardiac and femoral blood, urine, bile, vitreous humor, gastric contents, and pubic hair were collected during autopsy. Specimens were stored at +4°C before analysis, except pubic hair, which was stored at ambient temperature.

Materials and Methods

Chemicals and reagents

Acetonitrile was HPLC grade (Merck, Darmstadt, Germany). Pyridine and ammonium iodide (NH$_4$I) were purchased from Prolabo (Paris, France). BSTFA, 1% N,O-bis( trifluoroacrylyl) trifluoroacetamide + 1% trimethylchlorosilane (TMCS), MTBSTFA, and 1% N-(tert-butyldimethylsilyl)-N-methyl trifluoroacetamide + 1% t-butyldimethylchlorosilane (TBDMCS) were purchased from Fluka (Saint-Quentin Fallavier, France). The derivatization reagent for pubic hair was a mixture of MTBSTFA/TBDMCS/NH$_4$I/acetonitrile/pyridine (891:9:5:70:30, v/v/m/v/v). GHB and GHB-d$_6$ were purchased from Promochem (Molsheim, France).

Analysis of GHB in biological fluids

The procedure was identical for all biological matrices analyzed, blood, urine, bile, and vitreous humor (12). Gastric contents were previously centrifuged and the supernatant used. Linearity was established for each matrix separately. Typical $r^2$ values were > 0.99.

Twenty microliters of each fluid (previously diluted 1:20 with water) was treated with 45 $\mu$L of acetonitrile, in the presence of 20 $\mu$L of GHB-d$_6$ (25 mg/L) used as internal standard (IS). After agitation and centrifugation for 15 min, the supernatant was collected and evaporated to dryness under nitrogen flow. The residue was derivatized by adding 35 $\mu$L BSTFA with 1% TMCS, then incubated for 25 min at 70°C.

A 1-$\mu$L aliquot of the derivatized extract was injected into the column of a Hewlett-Packard (Palo Alto, CA) gas chromatograph (GC) (6890 series). The flow of carrier gas (helium, purity grade N55) through the column (HP5-MS capillary column, 5% phenyl-95% methylsiloxane, 30 m $\times$ 0.25-mm i.d., 0.25-µm film thickness) was 1.0 mL/min.

The injector temperature was 270°C and splitless injection was employed with a split valve off-time of 1.0 min. The column oven temperature was programmed to rise from an initial temperature of 70°C, hold for 1 min, increase to 100°C at 10°C/min, then increase to 295°C at 30°C/min, and hold at 295°C for the final 1 min.

The detector was a Hewlett-Packard 5973 operated in the electron ionization mode. The electron multiplier was operated at 1900 V. Data were recorded in full scan mode and ions monitored were m/z 233, 204, and 147 and m/z 239 for GHB and GHB-d$_6$, respectively (the underlined ions were used for quantitation).

Responses for GHB were linear in the range 1 to 200 mg/L, regardless of the analyzed fluid. Limit of quantification was 1 mg/L. Derivatives were stable at least for 24 h.

Analysis of GHB in pubic hair

Pubic hair was analyzed for GHB according to our previous paper (13). Briefly, the hair was decontaminated twice using 5 mL of methylene chloride for 2 min at room temperature, and then it was cut into three segments of 8 mm each. About 5–10 mg of decontaminated hair was incubated in 0.5 mL 0.01N NaOH, 16 h at 56°C, in presence of 10 ng of GHB-d$_6$ used as internal standard. After cooling, the homogenate was neutralized with 50 µL of 0.1N HCl, and 2 mL ethyl acetate was added together with 50 µL of 0.01M H$_2$SO$_4$.

After agitation and centrifugation, the organic phase was evaporated to dryness under nitrogen flow. The residue was derivatized by adding 25 µL of the derivatization mixture, then incubated for 75 min at 75°C.

A 2-$\mu$L aliquot of the derivatized extract was injected into the column of a Hewlett-Packard (Palo Alto, CA) GC (6890 series). The flow of carrier gas (helium, purity grade N55) through the column (HP5-MS capillary column, 5% phenyl-95% methylsiloxane, 30 m $\times$ 0.25-mm i.d., 0.25-µm film thickness) was 1.0 mL/min.

The injector temperature was 270°C, and splitless injection was employed with a split valve off-time of 1.0 min. The column oven temperature was programmed to rise from an initial temperature of 70°C, hold for 1 min, increase to 295°C at 15°C/min, and hold at 295°C for the final 1 min.

The detector was a Finnigan TSQ 700 operated in the electron ionization mode and in selected reaction monitoring. The precursor ions, m/z 275 and 281 for GHB and the IS, respectively, were selected in the first quadrupole. The product ions for GHB and IS, m/z 275 and 281, were selected in the third quadrupole after collision with argon at a cell pressure at 0.40 mTorr. The collision offset voltage was –8 V. The electron multiplier was operated at 1900 V. Limit of quantification was 0.1 ng/mg.

Results

GHB is currently circulating as a drug-of-choice within the
dance music scene (i.e., raves and nightclubs). At high doses, its ability to produce muscle relaxation has been exploited to minimize some side-effects of ecstasy. It is predominantly used for its perceived desirable effects, including the ability to heighten sexual desire and induce euphoria and hallucinations. In France, there seems to be some controversy between media coverage of GHB use and the results of toxicological analyses. In the current context, this issue was addressed by evaluation of both femoral blood and vitreous humor to facilitate interpretation (11). It is therefore confirmed that, in a case of authentic GHB ingestion, these two specimens will be positive for the drug and will allow discrimination.

Conclusions

The toxicological findings from this study are consistent with an acute fatal overdose of GHB. The blood concentration from this case is markedly above those of previous reported GHB fatalities, indicating that the potential range of GHB levels for acute overdose cases should be revised upwards.

Acknowledgments

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References

11. P. Kintz, M. Villain, V. Cirimele, and B. Ludes. GHB in postmortem

Table I. GHB Concentrations in the Autopsy Specimens

<table>
<thead>
<tr>
<th>Specimen</th>
<th>GHB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral blood</td>
<td>2937 mg/L</td>
</tr>
<tr>
<td>Cardiac blood</td>
<td>3385 mg/L</td>
</tr>
<tr>
<td>Urine</td>
<td>3372 mg/L</td>
</tr>
<tr>
<td>Bile</td>
<td>1800 mg/L</td>
</tr>
<tr>
<td>Vitreous humor</td>
<td>2836 mg/L</td>
</tr>
<tr>
<td>Gastric contents</td>
<td>7.08 g in 100 mL</td>
</tr>
<tr>
<td>Pubic hair, segment 1</td>
<td>25.0 ng/mg</td>
</tr>
<tr>
<td>Pubic hair, segment 2</td>
<td>22.6 ng/mg</td>
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<tr>
<td>Pubic hair, segment 3</td>
<td>19.4 ng/mg</td>
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Table II. Reported GHB Blood Concentrations in the Literature

<table>
<thead>
<tr>
<th>GHB in Blood (mg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>77 (femoral)</td>
<td>15</td>
</tr>
<tr>
<td>330 (femoral)</td>
<td>16</td>
</tr>
<tr>
<td>345 (unknown)</td>
<td>17</td>
</tr>
<tr>
<td>303 (heart)</td>
<td>18</td>
</tr>
<tr>
<td>n = 4, 74 to 2160 (unknown)</td>
<td>19</td>
</tr>
<tr>
<td>538 (unknown)</td>
<td>20</td>
</tr>
</tbody>
</table>


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