Pharmacokinetics and Excretion of Gamma-Hydroxybutyrate (GHB) in Healthy Subjects

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Abstract

In Europe and the United States, the recreational use of gamma-hydroxy butyric acid (GHB) at dance clubs and "rave" parties has increased substantially. In addition, GHB is used to assist in the commission of sexual assaults. The aim of this controlled clinical study was to acquire pharmacokinetic profiles, detection times, and excretion rates in human subjects. Eight GHB-naïve volunteers were administered a single 25-mg/kg body weight oral dose of GHB, and plasma, urine, and oral fluid specimens were analyzed by using gas chromatography–mass spectrometry (GC–MS). Liquid–liquid extraction was performed after acid conversion of GHB to gamma-butyrolactone. Limits of quantitation of 0.1 (oral fluid), 0.2 (urine), and 0.5 µg/mL (plasma) could be achieved in the selected ion monitoring mode. GHB plasma peaks of 39.4 ± 25.2 µg/mL (mean ± SEM) occurred 20–45 min after administration. The terminal plasma elimination half-life was 30.4 ± 2.45 min, the distribution volume 52.7 ± 15.0 L, and the total clearance 1228 ± 233 µL/min. In oral fluid, GHB could be detected up to 360 min, with peak concentrations of 203 ± 92.4 µg/mL in the 10-min samples. In urine, 200 ± 71.8 and 230 ± 86.3 µg/mL, were the highest GHB levels measured at 30 and 60 min, respectively. Only 1.2 ± 0.2% of the dose was excreted, resulting in a detection window of 720 min. Common side-effects were confusion, sleepiness, and dizziness; euphoria and change of vital functions were not observed. GHB is extensively metabolized and rapidly eliminated in urine and oral fluid. Consequently, samples should be collected as soon as possible after ingestion.

Introduction

Gamma-hydroxybutyric acid (GHB) is an endogenous compound present in most mammalian tissues at nanomolar concentrations and a minor metabolite or precursor of gamma-aminobutyric acid (GABA) (1–3). It has potential as a neurotransmitter and/or neuromodulator with binding to $GABA_B$ receptors. It is a weak agonist at $GABA_B$. Recently, it has been shown that GHB also binds to a G-protein-coupled presynaptic receptor that is distinct from $GABA_B$ (4). GHB was first synthesized in 1960 by the French biochemist H.-M. Laborit who was searching for analogues of GABA (5). Later it was introduced in Europe as a sleep aid, to treat narcolepsy, and as a surgical anesthetic, but disappeared from the market because of insufficient anesthetic potency and unpredictable anesthetic duration. In the United States, GHB was freely available in health food stores and very popular in the body-builder community as an anabolic steroid alternative. GHB was also used as a tryptophan replacement for weight control and sedation (6). The increasing abuse as a party drug, often together with 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) (7–10) and alcohol, and the use in crimes of drug-facilitated sexual assaults ("date-rape drug") (1,11–14) resulted in the ban of GHB by the U.S. Food and Drug Administration (FDA) in the late 1990s. At the recommendation of the World Health Organization (WHO), the Commission on Narcotic Drugs (CND) of the United Nations added GHB to Schedule IV of the 1971 Convention of Psychotropic Substances in March 2001. Since July 2002, GHB is in the U.S. under the product name Xyrem®, an approved drug for treatment of catalepsy associated with narcolepsy (15). In most European countries, GHB is allowed only for research purposes or limited clinical use (e.g., as anesthetic adjuvant) (1). In view of the alcohol-mimicking effects on the central nervous system, GHB has also been tested in clinical practice for alcoholism management because it is very effective in suppressing the alcohol withdrawal syndrome and the following alcohol craving (16).

Gas chromatography (GC) with flame-ionization (11) or mass spectrometric (MS) detection (11,17–22) and capillary electrophoresis with UV or MS detection (9,23) have been used for GHB determination in biological fluids. Extraction can be performed by acidic conversion of GHB to gamma-butyrolactone (GBL) followed by liquid–liquid or solid-phase extraction of GBL (20,21,23). Alternatively, GHB can be analyzed without prior lactonization as the di-TMS derivative (17,18,22,24,25).

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Solid-phase microextraction (SPME) has also been used with subsequent detection of the hexyl derivative of GHB (19).

The pharmacokinetics of GHB were studied in alcohol dependent (26) or narcoleptic patients (27); the dose-dependent absorption, elimination, and urinary excretion (single-subject study) were measured in healthy male volunteers (10,28). So far, no controlled clinical study was conducted where blood, urine, and oral fluid specimens were collected for analysis.

Reviews of the history, pharmacology, clinical use, and analysis of GHB have been published elsewhere (1–3,6).

It was the aim of the present study to acquire pharmacokinetic profiles of GHB and to compare its excretion in urine and oral fluids of eight GHB-naïve healthy volunteers after a single oral dose of 25 mg/kg body weight (b.wt.). In addition, psychological and somatic side-effects of GHB were monitored.

Experimental

Materials

GHB was supplied as the sodium salt (gamma-hydroxybutyrate, sodium oxybate) by Fluka (Buchs, Switzerland) with a purity of 99.5% (HPLC). Gamma-valerolactone (GVL) was aquired from Sigma-Aldrich (St. Louis, MO). All other chemicals were of ACS analytical grade.

Table I. Limit of Detection, Limit of Quantitation, and Linearity of the GHB Determination in Different Matrices

	LOD (µg/mL)	LOQ (µg/mL)	ULOL (µg/mL)	Correlation Coefficient (r^2)†
Plasma	0.2	0.5	200*	1.000
Oral fluid	0.1	0.1	200*	1.000
Urine	0.1	0.2	900*	1.000

* Maximum concentration tested.
† Based on a calibration curve from 10 to 50 µg/mL.

Methods

Subjects and clinical study. Eight healthy, GHB-naïve volunteers (4 male, 4 female; 37 ± 10 years old; 71 ± 12 kg b.wt.; mean ± sd) participated in the pharmacokinetic study, conducted at the Clinical Investigation Unit (CIU) of the University Hospital of Bern. The subjects were informed about the risks of the study and gave their written informed consent. The study was approved by the Regional Ethics Committee and the Swiss Agency for Therapeutic Products (Swissmedic). Each subject received a single oral dose of 25 mg GHB per kg b.wt. (1778 ± 298 mg) that was administered on an empty stomach as a freshly prepared solution of GHB sodium salt in water and by using a small drinking tube to avoid oral cavity contamination. The subjects were not allowed to consume any alcohol, drugs, or medications 48 h before, during, and for 24 h after the trial. Blood (10 mL) was collected through a peripheral vein catheter shortly before GHB dosing (baseline), and 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 240, and 360 min after dosing. The heparinized blood specimens were centrifuged for 10 min at 2000 rpm, and the plasma samples instantly deep-frozen and stored at –20°C until analysis. Total urine was collected 10 min before and 120, 240, 360, 480, 720, and 1440 min after dosing and stored at –20°C until analysis. Oral fluid, collected up to 360 min using Sarstedt Salivettes (Sevelen, Switzerland), was centrifuged and stored at –20°C. The pharmacodynamic measurements, performed at the blood sample collection times, consisted of monitoring vital functions (e.g., blood pressure, heart rate, hemoglobin oxygen saturation, etc.) and psychological and somatic side-effects.

Sample extraction. The extraction of GHB from 2 mL urine (clinical and calibration samples) was performed by adding 50 µL of the internal standard solution (GVL, 1.0 mg/mL) and 0.5 mL of 20% trifluoroacetic acid to a 15-mL centrifuge tube. After vortex mixing, the tubes were capped and heated in an oven at 75°C for 1 h. The tubes were then removed from the

Table II. Plasma Concentrations of GHB in GHB-Naïve Subjects (n = 8) after an Oral Dose of 25 mg GHB per kg b.wt.

	Plasma concentration of GHB (µg/mL)*													
	Time (min)													
Subject	0	10	15	20	25	30	45	60	90	120	150	180	240	360
A	< 0.2	0.82	1.72	5.23	10.4	31.3	23.3	18.1	2.46	1.19	0.89	0.70	< 0.2	< 0.2
B	< 0.2	0.52	0.25	0.56	0.86	0.62	1.45	1.25	3.05	4.70	2.36	1.02	0.66	1.30
C	< 0.2	< 0.2	10.8	8.58	7.17	9.71	16.9	12.1	9.94	3.32	1.84	< 0.2	< 0.2	< 0.2
D	< 0.2	32.6	62.0	65.8	61.9	54.3	41.2	31.5	16.4	9.54	4.03	1.81	< 0.2	< 0.2
E	< 0.2	1.44	7.00	18.8	26.8	42.6	44.6	32.4	19.7	10.6	2.51	0.76	0.64	0.29
F	< 0.2	6.30	11.0	16.2	21.4	18.2	11.5	8.92	4.05	2.50	1.52	0.58	< 0.2	< 0.2
G	< 0.2	19.2	30.5	41.4	56.2	52.7	33.7	22.9	10.2	1.45	< 0.2	< 0.2	< 0.2	<0.2
H	< 0.2	21.3	67.8	76.3	72.8	61.9	38.4	24.6	14.6	4.39	2.00	< 0.2	< 0.2	< 0.2
Mean		10.3	23.9	29.1	32.2	33.9	26.4	19.0	10.1	4.71	1.89			
SEM		3.93	8.54	9.11	8.73	7.15	4.92	3.48	2.06	1.12	0.38			

* Quantitation of all values less than 10 µg/mL was based on a single-point calibration at 10 µg/mL.

oven and allowed to cool down to room temperature. To each tube was then added 0.55 mL of 2M NaOH to adjust the pH to 6.5 followed by 3 mL of chloroform. The tubes were capped and shaken for 1 min. Approximately 0.5 mL of the organic layer (bottom) from each tube was transferred to a GC vial for injection.

The extraction procedure for plasma samples was the same as for urine except that 0.5 mL of plasma that was diluted with 1.5 mL of deionized water before proceeding with the extraction process was used.

The extraction of oral fluid samples was carried out by measuring 1 mL of the oral fluid from the collection device or whatever volume collected after centrifugation of the device and dilution with enough volume of deionized water to equal 2 mL. The mixture was then extracted following the same procedure as for urine.

GC–MS analysis. An HP-5890 GC interfaced with an HP-5970B MSD operated in the selected ion monitoring (SIM) mode was used. Chromatographic separation was achieved on a J&W Scientific 25-m × 0.2-mm (0.33-µm film) DB-5 MS column operated from 60°C (1 min) to 90°C at 10°C/min; the temperature was then raised to 270°C at 35°C/min with hold time of 1 min; oven equilibrium time was 0.5 min. Helium at a velocity of 38 cm/s was used as carrier gas. The electron multiplier voltage was the same as the tune value. The injector (250°C) was operated in the splitless mode (split open at 0.2 min), and 4 µL of each sample was injected. Ions were monitored at *m/z* 42, 56, and 86 for GBL and 41, 56, and 85 for GVL. The retention times for GBL and GVL were 4.35 and 4.80 min, respectively. Quantitation of GBL was based on the peak area of the ion *m/z* 42 versus the peak area of the GVL ion *m/z* 56.

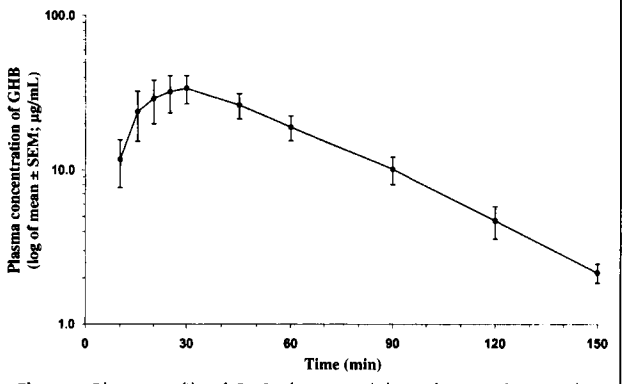

Figure 1. Plasma profile of GHB after an oral dose of 25 mg GHB per kg b.wt., GHB-naïve subjects (log of mean ± SEM, *n* = 8).

Pharmacokinetic data analysis. Based on the non-compartmental model, all pharmacokinetic parameters were assessed by use of standard calculation procedures performed by the TopFit Vers. 2.0 computer software. The linear trapezoidal rule was implemented for calculations of areas under the plasma concentration-time curves (AUC). The AUCs were extrapolated to infinity (AUC0 → ∞) by adding the last quantitated concentration (at 360 min) divided by the elimination constant (λ_z).

Results and Discussion

The determination of GHB by GC–MS was performed after lactonization to GBL, followed by liquid–liquid extraction, with GVL as the internal standard, resulting in a recovery of > 95%. A correlation coefficient r^2 of 1.000 was obtained for all calibration curves ofin the different matrices. The interday precision of the GHB quantitation was ± 8.7% determined with an 8 µg/mL control sample (n = 12). The limit of detection (LOD), limit of quantitation (LOQ), and upper limit of linearity (ULOL) are given in Table I.

After administration of a single oral dose of 25 mg GHB per kg b.wt. (1778 ± 94.3 mg; mean ± SEM) to 8 GHB-naïve volunteers GHB plasma levels of < LOD (not detected) to 76.3 µg/mL (0.16 ± 0.10 to 33.9 ± 7.15 µg/mL) were measured (Table II and Figure 1). Plasma peaks (C_{max}) of 4.70 to 76.3 µg/mL (39.4 ± 25.2 µg/mL), usually occurring between 20 and 45 min, and the terminal plasma elimination half-lifes ($t_{1/2z}$) of 17.4 to 42.5 min (30.4 ± 2.45 min) suggest that oral absorption and elimination of GHB are fast processes. The volume of distribution (V_d) was 19.1 to 155 L (52.7 ± 15.0 L), the mean residence time (MRT) 43.7 to 194 min (73.2 ± 15.7 min), and the total clearance (Cl_{tot}) 476 to 2520 mL/min (1228 ± 233

Table III. Pharmacokinetics of GHB in GHB-Naïve Subjects (n = 8) after an Oral Dose of 25 mg GHB per kg b.wt.

	Subject									
	A	B	C	D	E	F	G	H	Mean	SEM
Gender	f	f	m	m	f	f	m	m		
Body weight (kg)	58	64	89	80	61	59	80	78	71.1	3.77
Dose (mg)	1450	1600	2225	2000	1525	1475	2000	1950	1778	94.3
t_{max} (min)	30	120	45	20	45	25	25	20	41.3	10.6
C_{max} (µg/mL)	31.3	4.70	16.9	65.8	44.6	21.4	56.2	76.3	39.7	7.99
AUC (µg*min/mL)	1330	635	1209	4043	3207	1114	2682	3622	2230	414
MRT (min)	54.2	194	66.4	54.3	71.0	56.0	43.7	45.6	73.2	15.7
$t_{1/2z}$ (min)	17.4	42.5	32.4	33.1	36.8	30.5	26.0	24.6	30.4	2.45
V_d (L)	27.3	155	86.1	22.5	25.3	58.2	27.9	19.1	52.7	15.0
Cl_{tot} (mL/min)	1090	2520	1840	495	476	1320	746	538	1128	233
Urine recovery (%)	0.2	0.7	1.5	2.1	0.7	2.0	1.0	1.7	1.2	0.2

mL/min). Subject B displayed significantly higher MRT values (V_d and Cl) than the other subjects. Liver and kidney functions were checked with this individual, were within the normal range, and thus did not explain the variation. The semilog plot of the plasma concentrations (Figure 1) shows linear elimination kinetics. The pharmacokinetic data of GHB are summarized in Table III.

As shown by Table IV and Figure 2, the GHB concentration in oral fluid varied between < LOD (not detected) and 778 µg/mL (0.37 ± 0.21 to 203 ± 92.4 µg/mL). The highest values within a detection window of 360 min were usually observed in the 10- and 15-min samples. It cannot be excluded that the extremely high levels of subject B and H at 10 and 15 min are due to a contamination by improper use of the drinking tube. No GHB could be detected in the baseline samples except subjects A and F.

The GHB content in urine ranged from < LOD (not detected) to 840 µg/mL (Table V and Figure 3). Peak concentrations varied remarkably between 22.4 and 840 µg/mL (257 ± 82.2 µg/mL). With 230 ± 86.3 µg/mL, most subjects excreted the highest GHB concentrations in the 60-min urine. Recently it has been recommended to use a 10-µg/mL cutoff for the appropriate differentiation between endogenous and exogenous urinary GHB (29–31). Of the samples collected between 30 and 720 min, 33% showed GHB levels < 10 µg/mL. However, no GHB (LOD = 0.1 µg/mL) could be detected in the baseline urine and in the specimens collected at the end of the study (1440 min). The total amount of GHB recovered in urine within 24 h was 3.16 to 41.6 mg (22.7 ± 4.3 mg) corresponding to only 0.2 to 2.1% (1.2 ± 0.2%) of the dose administered. These data demonstrate the extensive metabolism of GHB.

At the dose administered, GHB did not produce any severe psychotropic side-effects, nor were the vital functions significantly altered. Confusion and sleepiness were the main side-effects observed. Less frequent was dizziness.

Conclusions

A pharmacokinetic study using GHB-naïve healthy subjects has revealed the extensive metabolism and rapid elimination of the drug in urine and oral fluids. The data indicate that there is a substantial interindividual variation among subjects in all three fluids collected. Only about 1% of the dose was recovered in urine, resulting in a detectability of 12 h. In oral fluid, the detection window was 150 min; thus, samples should be collected

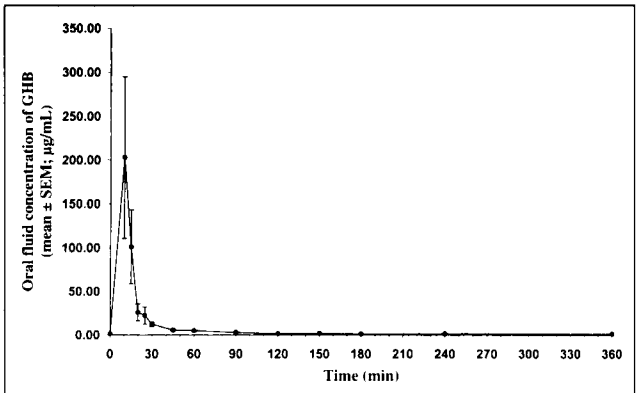

Figure 2. Oral fluid profile of GHB after an oral dose of 25 mg GHB per kg b.wt., GHB-naïve subjects (mean ± SEM, n = 8).

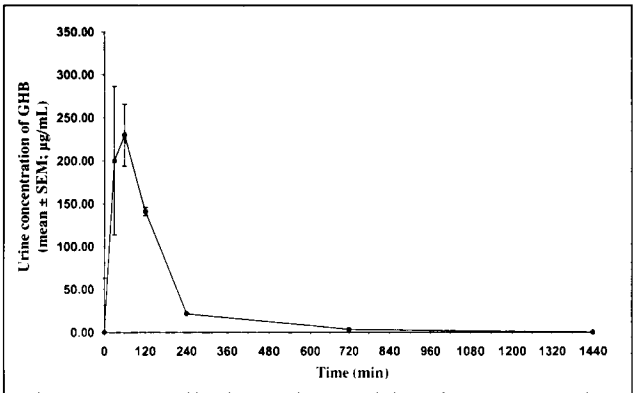

Figure 3. Urine profile of GHB after an oral dose of 25 mg GHB per kg b.wt., GHB-naïve subjects (mean ± SEM, n = 8).

Table IV. Oral Fluid Concentrations of GHB in GHB-Naïve subjects (n = 8) after an Oral Dose of 25 mg GHB per kg b.wt.

	Oral Fluid Concentration of GHB (µg/mL)*													
	Time (min)													
Subject	0	10	15	20	25	30	45	60	90	120	150	180	240	360
A	1.52	45.2	45.8	28.8	22.3	9.59	9.46	6.19	3.88	1.56	2.53	2.31	3.36	2.34
B	< 0.1	421	317	90.8	93.0	20.3	1.32	3.62	< 0.1	1.80	2.39	< 0.1	< 0.1	< 0.1
C	< 0.1	26.9	2.78	4.25	1.70	6.44	5.60	3.23	2.48	1.33	0.94	0.60	0.59	0.52
D	< 0.1	< 0.1	4.36	1.87	2.56	4.10	1.65	3.76	2.47	1.55	1.54	1.12	1.03	0.70
E	< 0.1	29.2	16.8	13.7	12.4	10.7	8.46	5.01	4.59	2.72	2.68	1.55	1.65	1.06
F	1.40	2.27	< 0.1	5.78	0.71	< 0.1	< 0.1	2.30	0.77	0.65	1.03	0.78	1.61	1.46
G	< 0.1	118	48.3	15.4	10.7	8.85	3.68	3.04	3.68	1.00	1.25	1.22	1.20	1.99
H	< 0.1	778	269	48.5	36.1	25.9	8.99	12.4	2.49	1.69	2.14	< 0.1	1.21	0.88
Mean		203	101	26.1	22.4	12.3	5.59	4.94	2.91	1.54	1.81	1.26	1.52	1.28
SEM		92.4	42.2	9.59	9.78	2.49	1.10	1.03	0.40	0.19	0.22	0.19	0.28	0.22

* Quantitation of all values less than 10 µg/mL was based on a single-point calibration at 10 µg/mL.

Table V. Urine Concentrations of GHB in GHB-Naïve Subjects (n = 8) after an Oral Dose of 25 mg GHB per kg b.wt.

	Urine Concentration of GHB (µg/mL)						
	Time (min)						
Subject	0	30	60	120	240	720	1440
A	< 0.1	45.7	48.4	8.79	< 0.1	< 0.1	< 0.1
B	< 0.1	< 0.1	15.6	111	20.5	3.24	< 0.1
C	< 0.1	184	232	129	53.1	2.56	< 0.1
D	< 0.1	654	840	311	13.8	4.96	< 0.1
E	< 0.1	5.01	22.4	18.4	8.19	< 0.1	< 0.1
F	< 0.1	26.7	132	253	19.4	< 0.1	< 0.1
G	< 0.1	181	194	62.7	25.4	< 0.1	< 0.1
H	< 0.1	303	358	236	11.0	2.11	< 0.1
Mean		175	230	141	18.9	1.61	
SEM		70.1	86.3	35.9	5.04	0.60	

in as short time as possible from the time of ingestion. Recently, it has been demonstrated that a single exposure to GHB in a case of sexual assault can be documented by hair analysis when collected about one month after the crime (32). However, it is strongly advised to determine GHB basal levels (33). Concentrations of < 10 µg/mL were observed in a significant number of specimens throughout the 1440-min collection period. This suggests that a recommended 10-µg/mL cutoff in urine (29–31) may be too high to be optimally sensitive for forensic purposes.

Acknowledgments

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