VOLUME 34 (3) PAGES 189–280 ISSN 0306-5251

British Journal of Clinical Pharmacology

September 1992

PUBLISHED FOR
THE BRITISH PHARMACOLOGICAL SOCIETY BY
BLACKWELL SCIENTIFIC PUBLICATIONS LTD

British Journal of Clinical Pharmacology

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WCK1013

Br. J. clin. Pharmac. (1992), **34**, 231–235

Pharmacokinetics of γ-hydroxybutyric acid in alcohol dependent patients after single and repeated oral doses

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1 The pharmacokinetics of γ-hydroxybutyric acid (GHB) were studied in 10 alcohol dependent subjects after single and repeated therapeutic oral doses (25 mg kg^{-1} every 12 h for 7 days).

2 GHB was readily absorbed and rapidly eliminated (t_{max} = 20–45 min; mean $t_{½z}$ 27 ± 5 s.d. min). Urinary recovery of unchanged GHB was negligible (< 1% of the dose). γ-butyrolactone was not detected in either plasma or urine, indicating that lactonization of GHB does not occur *in vivo*.

3 The multiple-dose regimen resulted neither in accumulation of GHB nor in time-dependent modification of its pharmacokinetics.

4 In five subjects, the data were consistent with nonlinear elimination kinetics of GHB. Administration of a 50 mg kg^{-1} dose to these subjects resulted in significant increases in dose-normalized AUC, $t_{½z}$ and mean residence time.

5 Doubling of the dose also resulted in a significant increase in t_{max} with little change in C_{max}.

6 At the administered doses, GHB did not accumulate in the plasma and caused no serious side effects.

Keywords γ-hydroxybutyric acid pharmacokinetics alcohol dependence

Introduction

γ-hydroxybutyric acid (GHB) is present in the mammalian brain with highest concentrations in the hypothalamus and basal ganglia (Snead & Morley, 1981). It appears to function as a neurotransmitter or a neuromodulator rather than as an incidental metabolite of γ-aminobutyric acid (Vayer *et al.*, 1987). GHB has been used as an intravenous anaesthetic agent (Laborit *et al.*, 1960) and in the treatment of sleep disorders (Mamelak *et al.*, 1986). Following the demonstration of its effectiveness in inhibiting voluntary ethanol consumption and suppressing the ethanol withdrawal syndrome in rats physically dependent on ethanol (Fadda *et al.*, 1983, 1989), GHB has been used in oral, non-hypnotic doses to treat the effects of alcohol withdrawal in man (Gallimberti *et al.*, 1989). Of the various mechanisms proposed for this therapeutic effect, inhibition of dopamine release (Gessa *et al.*, 1966; Walters *et al.*, 1973), increase in acetylcholine release (Stadler *et al.*, 1974), GABAergic actions (Anden & Stock, 1973; Roth & Nowycky, 1977), and interaction with GHB specific receptors (Vayer *et al.*, 1987), none has been established conclusively.

Following intravenous administration of high doses of GHB to dogs, evidence of nonlinear elimination kinetics has been obtained, with apparent half-lives of 1–2 h (Shumate & Snead, 1979; Van der Pol *et al.*, 1975). Both absorption and elimination have been shown to be capacity-limited in rats (Arena & Fung, 1980; Lettieri & Fung, 1979). Few data are available on the pharmacokinetics of GHB in man. Thus, there is an anecdotal report of dose-dependent elimination kinetics ($t_{½}$ = 0.5–5 h) (Vree *et al.*, 1976).

The aim of this study was to characterize the kinetics of GHB after oral administration to alcohol dependent patients and to assess any accumulation or time-dependent changes on multiple dosing.

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Methods

Patients

The study was carried out in 10 male subjects attending the 3rd Medical Division of Padova General Hospital for treatment of alcohol withdrawal syndrome and alcohol dependence. After the protocol of the study was approved by the University of Padova Medical School Ethics Committee, and after the purpose and the procedures of the study were fully explained, all subjects gave informed and written consent to participate.

A complete preliminary clinical examination, routine biochemical and haematological screening, and laboratory tests of kidney and liver functions were performed before the study. All subjects were in good nutritional state, not suffering from decompensated liver diseases or other severe organic illnesses. All patients had normal kidney function as assessed from the levels of serum creatinine (<120 μmol l^{-1}) and blood urea nitrogen (<7.5 mmol l^{-1}). Physical characteristics of the patients, results of liver function tests and concomitant medications are shown in Table 1. Subject 6 suffered from the manic type of manic-depressive psychosis, but was free from psychotic symptoms on admission to the hospital and during the course of the study. Subjects 7 and 8 had biopsy-proven liver cirrhosis in a compensated stage (grade A according to Child's classification; Conn, 1981). Apart from subject 8, all subjects were smokers (6 to 20 cigarettes per day) but they abstained from smoking during the preceding week and the whole period of study.

Study protocol

At 07.00 h after an overnight fast, GHB dissolved in a black cherry syrup (CT, Sanremo, Italy) was administered to each patient at a dose of 25 mg kg^{-1} every 12 h for a minimum of 7 days. Venous blood samples were collected through an indwelling catheter into heparinized plastic tubes at 0, 10, 15, 20, 30, 45 min and 1, 1.5, 2, 3, 4, 6, 12 h after the first dose and after the 13th dose on the seventh day. Urine was collected before dosing and at 0 to 4, 4 to 8 and 8 to 12 h after the 1st and 13th doses. Five of the 10 subjects were given a single 50 mg kg^{-1} dose of GHB on the 10th day and plasma and urine samples were taken as on days 1 and 7. Plasma and urine samples were stored at −40° C for 1 day prior to assay. Preliminary experiments showed the GHB was stable during this time.

Analytical methods

Plasma and urine samples (2 ml) acidified with perchloric acid 0.8 N (plasma) and hydrochloric acid 6 N (urine), were heated at 80° C for 20 min to convert GHB to butyrolactone (GBL) (Lettieri & Fung, 1979; Van der Pol *et al.*, 1975). Omission of this step indicated that no GBL was present in the samples as a metabolite of GHB. After adjusting the pH to 6.5 and adding internal standard (δ-valerolactone), plasma and urine samples were extracted with benzene, centrifuged and concentrated under a stream of nitrogen. Aliquots (3 μl) of the final solutions were injected into a Hewlett Packard (HP) 5790 gas chromatograph coupled to an HP 5970 A Mass Selective Detector (MSD), equipped with an HP ULTRA 1 (Part. N. 1A-101) bonded phase capillary column (12 m × 0.20 mm i.d.; 0.3 μ). Detection was by electron impact mass spectrometry in the Selected Ion Monitoring mode programmed to detect the characteristic ionic species at m/z 41, 42, 56, 86, 100 for GHB and δ-valerolactone.

The assay was linear over the clinically relevant concentration range (2–200 μg ml^{-1}), with correlation coefficients of 0.999 and 0.998 for plasma and urine, respectively. The intra- and inter-assay coefficients of variation (n = 5) determined at 5 μg ml^{-1} were always below 5%. The limits of determination were 1 μg ml^{-1} and 0.2 μg ml^{-1} for plasma and urine, respectively.

Pharmacokinetic and statistical analyses

Peak plasma GHB concentrations (C_{max}) and the time of their occurrence (t_{max}) were noted directly from the data. Terminal half-lives ($t_{½z}$) were estimated by log-linear regression of the terminal 2–4 data points. The area under the plasma drug concentration-time curve (AUC) and the area under the first moment of the plasma drug concentration-time curve (AUMC) were

Table 1 Patient demographic data, results of liver function tests and concomitant medication

Patient	*Age (years)*	*Weight (kg)*	*Serum albumin (g l^{-1})*	*Serum bilirubin (μmol l^{-1})*	*Prothrombin level (% normal)*	*AST*[a] *(iu)*	*ALT (iu)*	*γ-GT (iu)*	*Concomitant medication*
1	53	84	47	9.1	96	22	27	60	1,2,3
2	47	75	45	13.0	92	15	13	10	1,2,3
3	45	72	45	10.5	81	13	19	19	
4	56	92	45	12.5	100	25	26	28	1,2,4,5,6
5	48	74	40	31.3	86	122	74	448	1,2,3,6
6	47	60	43	15.2	99	70	104	34	7,8
7	41	76	48	32.5	63	114	124	629	1
8	34	75	49	13.6	78	90	156	215	1,2,3,6,9,10
9	56	57	54	10.5	100	66	51	180	1,2,3,6
10	39	67	55	9.1	87	138	81	343	1,2,3,6
Normal range			35–55	5–17	70–100	15–45	15–50	3–65	

[a]AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; γ-GT = γ-Glutamyltransferase.
Medication: 1 = thiamine; 2 = pyridoxine; 3 = cyanocobalamin; 4 = cetirizine; 5 = chlorphenamine; 6 = folinic acid; 7 = haloperidol; 8 = orphenadrine; 9 = lactulose; 10 = ranitidine.

estimated using the linear trapezoidal rule, with extrapolation to infinity using $C(\text{last})/\lambda_z$ (Gilbaldi & Perrrier, 1982). The extrapolated portion was always less than 10% of the total area. Mean residence time (MRT) was calculated from AUMC/AUC. Oral clearance (CL_o) was calculated from D/AUC. Urinary recovery was calculated as the cumulative amount excreted within the 12 h collection period and expressed as a percentage of the administered dose. The renal clearance (CL_R) of GHB was calculated from the ratio of the total amount recovered in the urine to the AUC.

The two-tailed Wilcoxon signed rank test was used to compare the parameters obtained after the 1st and 13th doses, as well as the parameters obtained after administration of different doses. The two-tailed Wilcoxon rank-sum test was used to evaluate differences between subgroups of patients. Other statistical analyses are specified in the text. A P value <0.05 was considered statistically significant.

Results

The individual and mean values of the pharmacokinetic parameters of GHB obtained after the 1st and 13th doses are shown in Table 2. Values of t_{max} and $t_{½z}$ suggest that GHB was readily absorbed after oral administration and rapidly eliminated. The drug was essentially removed from plasma by 2 to 4 h after dosage as indicated by values of CL and MRT. GHB was not excreted unchanged to any significant extent. In all cases, urinary recovery was virtually complete within 8 h of any administration. Consistent with the short terminal half-life, no accumulation occurred on repetitive dosing (the mean ratio between the AUC values after the 13th and the 1st administration was 1.03 ± 0.20 s.d). No statistically significant differences were observed between the pharmacokinetic parameters determined after the 1st and the 13th dose.

In five of the 10 subjects examined (patients 1, 2, 3, 4, and 8) the shape of the plasma concentration-time curve of GHB was consistent with first-order elimination kinetics, whereas in the other five subjects the decay phase exhibited a downward curvature suggestive of capacity-limited elimination (Figure 1a, b). In each of the 10 subjects, similar curves were obtained after the 1st and 13th doses. Four of the five subjects exhibiting linear kinetics (patients 1 to 4) had apparently normal liver function (Table 1), whereas in all patients exhibiting nonlinear kinetics, two to five values of the liver function tests were abnormally elevated. Analysis by the Fisher exact probability test showed that the occurrence of nonlinear kinetics was significantly more frequent in patients with abnormal liver function tests ($P = 0.024$). In the group exhibiting nonlinear decay kinetics, values of AUC and MRT were somewhat higher, but the differences did not reach statistical signficance. To confirm capacity-limited elimination of GHB, patients 5, 6, 7, 9 and 10 were given a single dose of 50 mg kg^{-1}

Table 2 Pharmacokinetic parameters of GHB following oral administration of 25 mg kg^{-1} GHB every 12 h to 10 alcohol dependent patients. Data obtained after the 1st and the 13th dose (values in brackets)

Patient	C_{max} (μg ml^{-1})	t_{max} (min)	$t_{½z}$ (min)	*MRT* (min)	*AUC* (μg ml^{-1} min)	CL_o (ml min^{-1} kg^{-1})	*Urinary recovery* (% dose)	CL_R (ml min^{-1} kg^{-1})
1	51 (72)	20 (20)	22 (19)	37 (34)	2410 (2616)	10.4 (9.6)	0.33 (0.37)	0.04 (0.04)
2	48 (52)	30 (30)	27 (29)	57 (48)	1663 (1984)	15.0 (12.6)	0.85 (1.05)	0.13 (0.13)
3	35 (32)	20 (30)	24 (24)	41 (45)	1577 (1750)	15.8 (14.3)	1.06 (0.63)	0.17 (0.09)
4	65 (54)	45 (20)	33 (29)	65 (50)	4485 (4440)	5.6 (5.6)	0.84 (0.54)	0.05 (0.03)
5	24 (35)	45 (45)	33 (26)	74 (82)	1631 (1701)	15.3 (14.7)	0.09 (0.17)	0.01 (0.02)
6	61 (71)	30 (20)	35 (39)	79 (81)	4363 (4038)	5.7 (6.2)	0.27 (0.31)	0.02 (0.02)
7	76 (72)	20 (30)	20 (23)	48 (54)	3360 (3397)	7.4 (7.4)	1.03 (1.12)	0.08 (0.08)
8	45 (32)	30 (30)	25 (25)	52 (55)	2482 (2708)	10.1 (9.2)	0.42 (0.35)	0.04 (0.03)
9	53 (48)	30 (30)	25 (22)	77 (73)	3950 (3513)	6.3 (7.1)	1.50 (1.45)	0.09 (0.09)
10	88 (85)	30 (30)	23 (29)	60 (54)	5303 (5102)	4.7 (4.9)	0.87 (1.30)	0.04 (0.06)
Mean	54 (55)	30[a] (30)[a]	27 (26)	59 (58)	3122 (3125)	9.6 (9.2)	0.73 (0.73)	0.07 (0.06)
± s.d.	±19 (±19)		±5 (±5)	±15 (± 16)	±1356 (±1171)	±4.4 (±3.6)	±0.44 (±0.46)	±0.05 (±0.04)
P value[b]	NS	NS	NS	NS	NS	NS	NS	NS

[a]Median value.
[b]13th *vs* 1st dose.

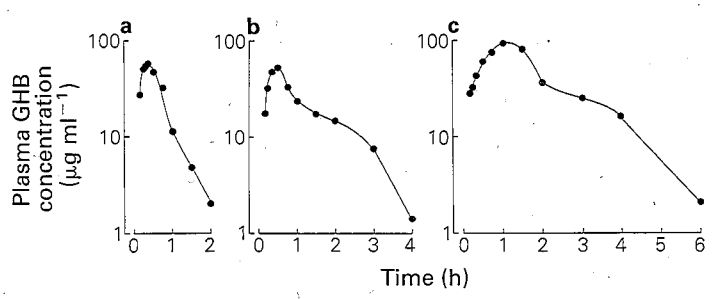

Figure 1 Plasma concentrations of GHB after oral administration of 25 mg kg^{-1} GHB to representative patients exhibiting linear (a) and nonlinear (b) elimination kinetics (subjects 1 and 9, respectively). (c) Plasma GHB concentrations after administration of 50 mg kg^{-1} GHB to subject 9.

Table 3 Dose dependency of GHB pharmacokinetic parameters. Mean values ± s.d. from five patients (5, 6, 7, 9 and 10) after administration of 25 mg kg^{-1} (1st and 13th doses) and 50 mg kg^{-1} GHB, on 1st, 7th and 10th days, respectively, of multiple dose regimen

	Dose (mg kg^{-1})				
	25			P *value*[a]	
	1st dose	*13th dose*	*50*	*1st dose*	*13th dose*
C_{max} (μg ml^{-1})	60 ± 24	62 ± 20	45 ± 17[b]	NS	NS
t_{max} (min)	30 (20–45)[c]	30 (20–45)[c]	45 (30–60)[c]	<0.01	<0.005
$t_{½z}$ (min)	27 ± 6	28 ± 7	35 ± 7	<0.01	<0.05
MRT (min)	68 ± 13	69 ± 14	96 ± 16	<0.05	<0.05
AUC (μg ml^{-1} min)	3721 ± 1366	3550 ± 1234	5419 ± 1637[b]	<0.005	<0.005
CL_o (ml min^{-1} kg^{-1})	7.9 ± 4.3	8.1 ± 4.8	5.3 ± 2.2	<0.05	<0.05
Urinary recovery (% dose)	0.75 ± 0.57	0.87 ± 0.59	1.33 ± 0.62	<0.05	<0.05
CL_R (ml min^{-1} kg^{-1})	0.05 ± 0.04	0.05 ± 0.03	0.08 ± 0.04	NS	<0.05

[a]50 mg kg^{-1} dose *vs* 1st and 13th 25 mg kg^{-1} doses.
[b]Normalised to 25 mg kg^{-1}.
[c]Median value (range).

of GHB on the 10th day. This doubling of the dose resulted in dose-disproportionate increases in AUC and MRT (Table 3, Figure 1b, c).

No side effects were recorded, with the exception of a slight transient drowsiness around the time of peak drug concentration in subjects 3 and 8 after the first 25 mg kg^{-1} dose, and subjects 7 and 9 after administration of the 50 mg kg^{-1} dose. C_{max} values in these subjects (35 to 97 μg ml^{-1}) were similar to those observed in the other subjects at corresponding doses.

Discussion

Bessman & Skolnik (1964) postulated that GBL is formed from exogenously administered GHB and considered the lactone to be the pharmacologically active species. However, subsequent investigations failed to confirm this, since only GHB could be detected in biological fluids and tissues after administration of GHB, GBL or precursors of the former (Giarman & Roth, 1964; Lettieri & Fung, 1978; Snead *et al.*, 1989). Therefore, GBL, rather than GHB, can be classified as a prodrug (Arena & Fung, 1980). Our observations are in accordance with this and confirm that analytical procedures involving preliminary conversion of GHB to GBL can be used to study the pharmacokinetics of GHB.

Our results suggest that both the oral absorption and the elimination of GHB are fast processes, but that clearance becomes capacity-limited as the dose is raised.

The observation that, following administration of the 25 mg kg^{-1} dose, evidence of nonlinear kinetics was apparent exclusively in patients with abnormal values of liver function tests, suggests that a relationship exists between liver function and saturation of the elimination pathway(s) of GHB. Nevertheless, this may be of limited therapeutic relevance, since no accumulation of GHB in plasma was observed at therapeutic doses irrespective of whether there was evidence of nonlinear kinetics.

Oral administration of increasing doses of GHB to rats has been shown to result in a dose-dependent increase in t_{max}, suggestive of a slower rate of absorption. Concomitant increases in C_{max} were much less than expected from first-order absorption kinetics (Lettieri & Fung, 1979). These dose-related effects have been shown to reflect capacity-limited absorption of GHB (Arena & Fung, 1980). Similar results were obtained in this study on doubling the dose (Table 3), suggesting that GHB absorption is capacity-limited also in humans.

Two further findings of clinical relevance have emerged from this study: firstly, the pharmacokinetic parameters of GHB are time-invariant. This suggests that neither GHB nor its metabolites cause auto-induction or auto-inhibition of metabolism. Secondly, GHB is rapidly cleared such that no accumulation occurs in the plasma at the usual maintenance doses. Even after administration of 50 mg kg^{-1} the drug is completely eliminated within 4 to 6 h. On the basis of our clinical observations, a daily dose of 100 mg kg^{-1} of GHB may be needed in certain cases of severe alcohol dependence. In the light of the present results, this daily dosage may be safe if appropriately divided.

The authors thank Dr G. Guidetti for his assistance with the statistical analysis, and Mr G. Volpato for technical help.

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(Received 15 October 1991, accepted 16 March 1992)