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Rate of elimination of γ-hydroxybutyrate from blood determined by analysis of two consecutive samples from apprehended drivers in Norway

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ABSTRACT

Aim: Gamma-hydroxybutyrate (GHB) is a common drug of abuse with an elimination half-life of 20–45 min. However, there is some evidence that GHB might exhibit saturation kinetics after ingesting high recreational doses. The aim of this study was to investigate the elimination kinetics of GHB from blood in people apprehended by the police for impaired driving and secondary to describe concentrations in all GHB-positive drivers.

Methods: Two consecutive blood samples were taken about 30–40 min apart from N = 16 apprehended drivers in Norway. GHB was determined in blood by an Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS) method. The changes in GHB between the two consecutive blood samples allowed estimating GHB's elimination half-life, assuming first-order and zero-order elimination kinetics. GHB concentrations are also reported for N = 1276 apprehended drivers with GHB in blood.

Results: The median time interval between collecting the two blood samples was 36 min (range 20–56 min). The median concentration of GHB in the first blood sample was 56.5 mg/L (range 14.1–142 mg/L) compared with 47.8 mg/L in the second sample (range 9.75–113 mg/L). The median elimination half-life was 103 min (range 21–187 min), and GHB's median zero-order elimination rate constant was 21.0 mg/L/h (range 6.71–45.4 mg/L/h). Back-calculation to the time of driving resulted in GHB concentrations up to 820 mg/L assuming first-order kinetics and up to 242 mg/L assuming zero-order kinetics. In all drivers (N = 1276), the median GHB concentration was 73.7 mg/L and highest was 484 mg/L.

Conclusion: The elimination half-life of GHB in blood samples from apprehended drivers was longer than expected compared with results of controlled dosing studies. Zero-order kinetics seems a more appropriate model for GHB when concentrations are back-calculated, and the median elimination rate was 21 mg/L/h.

1. Introduction

Gamma-hydroxybutyrate (GHB) is an endogenous substance, a prescription medicine and a recreational drug of abuse [1]. It was originally tested as a potential anesthetic agent, but this clinical application was abandoned, due to a lack of an analgesic effect and instances of vomiting [1,2]. Today GHB is a registered pharmaceutical used mainly for the treatment of cataplectic symptoms in narcoleptic patients [3,4] and in some countries to treat alcohol withdrawal syndrome and addiction [5]. GHB acts as a depressant of the central nervous system and has similar dose-dependent effects to that of ethanol, barbiturates and benzodiazepines, including euphoria, loss of inhibitions, sedation, unconsciousness and death [6,7]. It has been popular as a recreational drug of abuse since the early 1990s, and in doses ranging from 2–5 g [8,9] it acts on GABA-A, GABA-B and postulated GHB-specific receptors [10].

GHB is a drug that is occasionally detected in blood samples from apprehended drivers in many countries [11,12], and is a problem for traffic safety, owing to impairment effects on performance and behavior [6,13,14]. When GHB is detected in drugged driving or other forensic cases, there is sometimes a need

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to back-calculate the blood concentration from time of sampling to the time of driving, which is often several hours earlier [15].

GHB is oxidized by GHB dehydrogenase to succinic semialdehyde and further by succinic semialdehyde dehydrogenase to succinic acid, which enters the Krebs' cycle to produce carbon dioxide and water [16,17]. The terminal half-life for GHB according to controlled human dosing studies mainly is reported to be 20–45 min [18–26], however, there are studies presenting a slightly longer half-life of GHB especially with high concentrations [27–29]. Previous publications have also questioned whether GHB elimination is better described using a zero-order kinetic model [26,30]. In a case report [30] in which a person was intoxicated with alcohol and GHB, back-calculation to determine ingested amount of GHB using first-order kinetics gave an improbable high dose, that if ingested probably would have proven fatal.

Many papers have reported concentrations of GHB in blood samples from drugged drivers [11,12,31,32], but there are no published data on its elimination half-life in this population. The primary aim of this study was to calculate both a first-order half-life and a zero-order elimination rate constant for GHB in apprehended drivers based on the concentrations determined in two consecutive blood samples. We also used these results to back-calculate GHB concentrations in the actual forensic cases. A secondary aim of this study was to describe GHB concentrations in all GHB positive drivers apprehended in Norway over the same study period. This allowed evaluating whether the cases used to estimate elimination kinetics were representable of the larger population of GHB users. To further describe the population of drivers influenced by GHB, we investigated the prevalence of other psychoactive substances present in the same blood samples.

2. Methods

2.1. Study population

Information was extracted from a toxicology database belonging to the Department of Forensic Medicine, Oslo University Hospital. This database includes results of toxicological analyses of blood and other biological specimens from apprehended drunk and drugged drivers. The study included 16 cases from the period between 01.01.2008 – 05.11.2018 in which two consecutive blood samples were submitted for toxicological analysis. These samples were taken 20–56 min apart and both specimens contained measurable amounts of GHB.

In all 16 cases, GHB concentrations in blood decreased between the two sampling times, which was a necessary requirement for making a kinetic evaluation. In the following analysis, the first blood sampling time point is referred to as T_1 while the second time point is referred to as T_2, and the time interval between them is ΔT_1.

For the secondary aim of our study, we compared GHB concentrations in blood from these 16 special cases with the concentrations found in all GHB positive apprehended drivers (N = 1276) from the same study period. During this time approximately 60,000 impaired driving cases underwent toxicological analysis and GHB was detected in about 2% of these.

2.2. Blood samples and analysis of GHB

All blood samples were taken from a cubital vein and the blood was collected in 5 mL Vacutainer tubes, containing 20 mg of sodium fluoride (a final concentration of 0.4 %), as a preservative, in addition to 143 IU of heparin (BD Vacutainer Systems, Belliver Industrial Estate, Plymouth, UK). The samples were routinely screened for presence of ethanol and a large number of licit and illicit drugs including GHB.

The analytical method used for quantitative analysis of GHB both screening and confirmation analysis was previously described elsewhere [33]. In brief, this involved using ultra high pressure liquid chromatography (UPLC) and tandem mass spectrometry (MS/MS). The method is fully validated for routine use in forensic toxicology. Until 2011, the laboratory used a cut-off level for GHB of 10.4 mg/L, and after that a cut-off level of 8.3 mg/L. Only values above this were reported as positive.

2.3. Pharmacokinetic calculations

The blood samples from apprehended drivers were analysed at least once at each time point for GHB content. When multiple determinations were made, a mean concentration from each time point was calculated. The concentration of GHB at T_1 is referred to as C_1, while the concentration at T_2 is referred to as C_2.

For a drug eliminated from blood according to first-order kinetics, the following equation can be used to calculate elimination rate constant:

$$\text{First-order slope} = -K_1 = -[\ln(C_1) - \ln(C_2)] / \Delta T_1$$

$$\text{Half-life } (T_{1/2}) = \ln 2 / K_1 = 0.693 / K_1$$

For a drug that obeys zero-order kinetics, the elimination rate from blood is calculated as follows:

$$\text{Zero-order slope} = -K_0 = -[C_1 - C_2] / \Delta T_1$$

To back-calculate GHB concentrations to the time of driving, referred to as "C_0 (first-order)" and "C_0 (zero-order)", respectively, we used the time elapsed from time of driving according to the laboratory police report form and time when the first blood sample was taken, referred to as ΔT_0.

For the post-absorptive elimination phase it is given that $C_t = C_0 - k_0 \times t$

To back-calculate the concentrations to the time of driving assuming zero-order elimination we therefore use

$$C_0 \text{ (zero-order)} = C_1 + \Delta T_0 \times K_0$$

Assuming first-order elimination kinetics, elimination is given by $\ln C_t = \ln C_0 - k_0 \times t$

For our back-calculations we therefore use

$$\ln C_0 = \ln C_1 + K_1 \times \Delta T_0$$

Which can be expressed as $C_0 = C_1 \times e^{K_1 \times \Delta T_0}$

This further simplifies to $C_0 = C_1 \times 2^{(\Delta T_0 / T_{1/2})}$

2.4. Statistical analysis

Microsoft Excel software version 2010 and IBM SPSS® Software version 22.0 were used for the statistical analyses. The mean, median and highest and lowest values were used as descriptive statistics. The toxicological results were assessed retrospectively and no new determinations of GHB or other drugs were undertaken.

3. Results

During the study period, sixteen cases matched our inclusion criteria and were used in the pharmacokinetic analysis (Table 1). A summary of GHB concentrations and calculated first-order and zero-order elimination rate constants are shown in Table 2. In one subject (No. 16), the calculated half-life was exceptionally long, which suggests that in this case absorption of GHB was incomplete when the first blood sample was taken. We therefore present the

Table 1
Individual concentrations and calculated half-lives and zero-order elimination rate constants for GHB in blood of apprehended drivers.

Subject	C_1 (mg/L)[a]	C_2 (mg/L)[b]	ΔT_1 (min)[c]	$T_{1/2}$ (min)[d]	Zero-order rate constant (mg/L/h)[e]
1	26.7	12.3	24	21	36.0
2	38.1	25.0	32	53	24.6
3	46.3	34.5	23	54	30.9
4	14.1	9.75	39	73	6.71
5	72.9	60.5	21	78[f]	35.5
6	30.6	20.0	49	80[f]	13.0
7	36.9	29.6	29	91	15.2
8	46.9	39.1	27	103	17.3
9	129	99.9	41	109	43.7
10	142	111	42	115	45.4
11	142	113	40	122	43.3
12	76.0	56.4	56	130	21.0
13	28.9	26.4	20	154	7.50
14	90.8	77.2	41	176	19.9
15	66.1	59.1	30	187	13.9
16	95.7	93.2	42	1149[f]	3.42

[a] Concentration of GHB in first blood sample.
[b] Concentration of GHB in second blood sample.
[c] Time interval between first and second blood sample.
[d] Calculated terminal half-life.
[e] Zero-order kinetics elimination rate constant.
[f] Ethanol also detected in the sample.

Table 2
GHB concentrations, calculated elimination half-lives and zero-order rate constants.

	With outlier			Without outlier		
Parameter	Mean	Median	Highest and lowest values	Mean	Median	Highest and lowest values
C_1 (mg/L)[a]	67.7	56.5	14.1 - 142	65.9	46.9	141 – 142
C_2 (mg/L)[b]	54.2	47.8	9.75 – 113	51.6	39.1	975 – 113
$T_{1/2}$ (min.)[c]	168	106	21 – 1149	103	103	21 –187
Zero-order rate constant (mg/L/h)[d]	23.6	20.4	3.42 – 45.4	24.9	21.0	6.**71** – 45.4

[a] Concentration of GHB in first blood sample.
[b] Concentration of GHB in second blood sample.
[c] First-order kinetics elimination half-life.
[d] Zero-order kinetics elimination rate constant.

data with and without this aberrant case included. Excluding this case, the median calculated elimination half-life was 103 min and the median zero-order elimination rate constant was 21.0 mg/L/h. There was a significant correlation between the measured C_1 concentration and the elimination half-life (Spearman's rho = 0.56, p = 0.025).

The results of the back-calculations of GHB concentration to time of driving are presented in Table 3. Information on the time of consumption of GHB in relation to the time of driving was not available for this case series. This may lead to overestimating the true concentration, since some drivers might have been in the absorption phase when driving and would reach their maximum

Table 3
Back-calculated concentrations of GHB in blood to the time of driving using both first- and zero-order elimination kinetics.

Subject	C_0 (mg/L)[a] (first order)	C_0 (mg/L)[b] (zero-order)	ΔT_0 (min)[c]
1	820	90.4	106
2	812	133	232
4	37.5	25.6	103
5	151	121	82
6	113	63.1	150
7	146	82.5	180
8	78.1	68.8	76
9	151	147	24
10	202	186	58
11	312	242	139
12	127	110	97
13	42.3	39.4	84
14	102	101	30
16	104	104	144
Mean	229	108	108
Median	137	102	100
Highest and lowest values	37.5 – 820	25.6 – 242	24 - 232

[a] Concentration of GHB at the time of driving calculated assuming first-order kinetics.
[b] Concentration of GHB at the time of driving calculated assuming zero-order kinetics.
[c] Time interval between driving and first blood sample.

Table 4
Mean, median and highest/lowest GHB concentrations in blood of drivers apprehended in Norway.

Type of case	N[a]	Mean conc. (mg/L)	Median conc. (mg/L)	Highest and lowest values
GHB mono-drug[b]	110	104	98.7	9.26 – 251
GHB poly-drug[c]	1166	83.2	70.2	8.85 – 484
GHB all cases[d]	1276	85.0	73.7	8.85 – 484

[a] N = number of cases.
[b] GHB as the only drug identified in blood.
[c] GHB identified with one or more other psychoactive substances.
[d] All cases with GHB identified in blood.

Table 5
Most common drugs detected in combination with GHB.

Drug identified together with GHB	N[a]	% of all cases
Amphetamine	773	60.6
Clonazepam	631	49.5
Methamphetamine	542	42.5
N-desmethyldiazepam	414	32.4
Diazepam	368	28.8
THC	289	22.6
Alprazolam	145	11.4
Ethanol	112	8.78

[a] N: number of occurrences, although in many cases multiple drugs were identified, so the percentage of all cases exceeds 100 %.

concentration of GHB in blood at a later time point. For two subjects (No. 3 and 15), time of driving was not available. The median ΔT_0 (time interval between driving and blood sampling) was 100 min. By back-calculating using first-order kinetics, the results for the two subjects with shortest estimated half-lives are 820 and 812 mg/L, respectively, compared to 90.4 and 133 mg/L for the same subjects when applying zero-order elimination kinetics.

The concentrations of GHB in blood from N = 1276 apprehended drivers are summarized in Table 4. Of these only N = 110 were mono-drug cases. The drugs most often combined with GHB are shown in Table 5 with amphetamine topping the list. More than one additional drug was detected in N = 985 (77 %) of the cases.

4. Discussion

The present study indicates that the elimination half-life of GHB when calculated from two consecutive blood samples from impaired drivers is longer than previously reported. Additionally, it supports the idea that zero-order elimination kinetics seems more appropriate as a pharmacokinetic model for making a back-calculation in forensic casework.

To investigate the pharmacokinetic properties of a drug, the ideal method entails giving a bolus dose under controlled conditions and analysis of blood or plasma samples to determine a concentration-time-profile. The post-peak blood samples would then be used to determine the elimination rate constant with and without making a logarithmic transformation of the blood-drug concentrations. In forensic science it is obviously ethically and practically challenging to study the pharmacokinetics of drugs by controlled human dosing studies. Instead, useful information can be obtained from forensic practice casework when two or more blood samples at timed intervals apart are available, such as from apprehended drivers [34]. By making certain assumptions, such as that both specimens are taken on the post-absorptive phase, information can be gleaned about rate of drug elimination from blood in this population. This is previously performed for ethanol in a number of studies [35–38].

Our data (excluding the outlier) showed a median half-life of GHB of 103 min, which is considerably longer than the half-lives from previous studies of less than 60 min [18–26]. We calculated a zero-order elimination rate constant of 21.0 mg/L/h, which is very well in accordance with a previously published zero-order elimination rate constant for GHB of 18 mg/L/h [30]. Also, in two previously published studies [14,20]; we have been able to get detailed information about the GHB-concentrations, and permission to use the data to calculate a zero-order elimination rate constant. In an experimental study by Liakoni et al. [14] of 16 subjects who received 50 mg/kg GHB, the median GHB concentration in plasma after one and three hours was 83.1 mg/L and 24.4 mg/L respectively (blood plasma ratio for GHB is 0.8–1.2 [39]). The calculated median zero-order elimination rate constant for this population was 29 mg/L/h.

In the study of Brailsford et al. [20], 25 mg/kg GHB was given to 12 healthy volunteers. The concentration-time profile of GHB was used to determine a zero-order elimination rate constant of 23 mg/L/h when values from C_{max} to two hours post dosing were used. When a shorter time period was used, the elimination rate constant was somewhat higher. The median zero-order elimination rate constant from the present study was therefore in good agreement with values derived from these two controlled dosing studies.

The concentrations of GHB in blood from the 16 apprehended drivers agreed well with the concentrations determined for all drivers arrested in Norway with GHB in blood (Table 4). The mean and median concentrations in all drivers were 85.0 mg/L and 73.7 mg/L, respectively (N = 1276), being in good agreement also with impaired drivers from Sweden (mean 90 mg/L, median 84 mg/L, N = 473) [11] and Australia (mean 89 mg/kg, median 87 mg/kg, N = 160) [40]. The present study additionally shows that GHB impaired drivers, like other drugged drivers [41], are mostly poly-drug users, with a preference for amphetamines, but who also take other psychoactive substances (Table 5). It is especially interesting to observe the frequent combination of GHB with central stimulants, a combination that is also demonstrated for e.g. benzodiazepines and opioids [41]. The prevalence of GHB in the present population of apprehended drivers is comparable to a study done in Australia [40].

For drugs with first-order kinetics, the elimination rate from blood or plasma is directly proportional to the concentration present, whereas for drugs with zero-order kinetics a constant amount is eliminated per unit time (independent of concentration). The present back-calculations, especially in those cases with a calculated short elimination half-life, yield improbable high concentrations at the time of driving when applying first-order kinetics, higher than concentrations observed in our 1276 GHB positive cases over 10 years. This supports the notion that zero-order elimination kinetics is a more appropriate model when making a back-calculation in this population of drug users.

Our data therefore indicate a saturation of elimination capacity, as we see a significant correlation between concentration of GHB at the first time point and the first-order elimination half-life. The concentration at which saturation occurs cannot be determined from our study. In addition to saturation of the elimination pathway, there might be a degree of capacity-limited absorption as well, as previously documented for GHB [23]. Since we are dealing with recreational users of GHB who take high doses, longer T_{max}

(time interval from consumption to a maximum blood concentration is reached) and thereby increased possibility of ongoing absorption at C_1 cannot be excluded. If a precursor of GHB, gamma-butyrolactone or 1,4 butanediol was ingested in some cases, this could theoretically lead to prolonged half-life of GHB.

One strength of the present study is the ability to investigate pharmacokinetics in real life drugged driving cases. There is only scant information of drug pharmacokinetics in drug abuse and overdose cases in comparison with therapeutic drug monitoring cases. We also made use of a large background material of blood concentrations of GHB in the population of impaired drivers in Norway. The blood concentrations in cases with double blood samples agreed well with values in all apprehended drivers.

One limitation of the study is that we lack detailed information about the dose of GHB taken and the time of last use in relation to time of driving and time of sampling blood for toxicological analysis. The availability of only two blood samples separated in time by about 30 min is also open to critique as a way to calculate elimination rate constants. For long half-life drugs, such short time interval will not give a good estimate of the elimination, but for a short half-life drug like GHB this time span is more appropriate. One important question is also whether still ongoing absorption at the first time point might account for the longer estimated half-life in our study than in previous publications. It should also be noted that three of the 16 double blood sample cases included ethanol (as seen in Table 1) and interestingly enough, one of them was the one showing an extremely long half-life. The other two, however, showed relatively short half-lives. A pharmacokinetic interaction between ethanol and GHB has not been documented [27] and we are not able to throw any light on this question. In conclusion, this article reports GHB concentrations in blood from over 1000 apprehended drivers in Norway. In a sub-population of traffic offenders double blood samples were taken and used to determine rates of elimination from blood assuming both first-order and zero-order kinetics. For this population of drug users, the results support zero-order rather than first-order elimination kinetics.

Credit author statement

MÅ participated in planning the study, performed the statistical analyses and drafted the manuscript. LB, MAS and AWJ participated in planning the study and gave useful feedback on the manuscript. GH participated in planning the study and the design of the manuscript and supervised the project. All authors accepted the last version of the manuscript.

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Declaration

The study was conducted according to the data processing agreement with the Higher Prosecuting Authority, which stands as the owner of forensic materials in Norway. In accordance with this agreement, only anonymous data were used in the present study.

Declaration of Competing Interest

None of the authors have any conflicts of interests.

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