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Short Communication

Running title: Home-Based DBS sampling of GHB in Xyrem®-Treated Narcoleptic Patients

Feasibility of Following up Gamma-Hydroxybutyric Acid Concentrations in Sodium Oxybate (Xyrem®)-Treated Narcoleptic Patients Using Dried Blood Spot Sampling at Home

An Exploratory Study

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Abstract

Background: Gamma-hydroxybutyric acid (GHB), well known as a party drug, especially in Europe, is also legally used (sodium oxybate, Xyrem®) to treat a rare sleep disorder, narcolepsy with cataplexy. This exploratory study was set up to measure GHB concentrations in dried blood spots (DBS) collected by narcoleptic patients treated with sodium oxybate. Intra- and inter-individual variation in clinical effects following sodium oxybate administration has been reported. The use of DBS as a sampling technique, which is stated to be easy and convenient, may provide a better insight into GHB concentrations following sodium oxybate intake in a real-life setting.

Objective: The aim was two-fold: evaluation of the applicability of a recently developed DBS-based gas chromatography-mass spectrometry (GC-MS) method, and of the feasibility of the sampling technique in an ambulant setting.

Methods: Seven narcoleptic patients being treated with sodium oxybate at the Department for Respiratory Diseases of Ghent University Hospital were asked to collect DBS approximately 20 min after the first sodium oxybate (Xyrem®; UCB Pharma Ltd, Brussels, Belgium) intake on a maximum of 7 consecutive days. Using an automatic lancet, patients pricked their fingertip and, after wiping off the first drop of blood, subsequent drops were collected on a DBS card. The DBS cards were sent to the laboratory by regular mail and, before analysis, were visually inspected to record DBS quality (large enough, symmetrically spread on the filter paper with even colouration on both sides of the filter paper).

Results: Of the seven patients, three patients succeeded to collect five series of DBS, one patient decided to cease participation because of nausea, one was lost during follow-up and two patients stated falling asleep almost immediately after the intake of sodium oxybate. Analysing the DBS in duplicate resulted in acceptable within-DBS card precision. DBS with acceptable quality were obtained by patients without supervision.

Conclusion: Our results demonstrate the acceptable precision of the complete procedure, from sampling at home to quantitative analysis in the laboratory. Given the intra- and inter-individual variability in clinical effects seen with sodium oxybate, the easy adaptation of DBS sampling opens the possibility of following up GHB concentrations in patients in real-life settings in future studies.

1 Introduction

Gamma-hydroxybutyric acid (GHB) is a short-chain fatty acid synthesized in the early 1960s as a structural analogue of gamma-aminobutyric acid. GHB is also naturally present in blood, urine and peripheral and brain tissue.[1,2] The sodium salt of GHB, which is a popular club drug, is used as an orphan drug (sodium oxybate, Xyrem®) in the treatment of narcolepsy (with cataplexy or with excessive daytime sleepiness [EDS]), a chronic neurological sleep disorder.[3] This orphan disease is characterized by EDS, cataplexy (a sudden loss of muscle tone provoked by emotional stimuli), disturbed nocturnal sleep, hypnagogic hallucinations and sleep paralysis. It has been demonstrated that GHB administration in narcoleptic patients with cataplexy increases slow-wave sleep duration, improves EDS and reduces the number of awakenings at night.[4-6] Therefore, sodium oxybate has been approved in 2002 by the US Food and Drug Administration (FDA) for the treatment of cataplexy in narcolepsy patients, and subsequently in 2005 for the treatment of EDS in narcolepsy patients. Also, in 2005, the European Medicines Agency (EMA) approved sodium oxybate for the treatment of narcolepsy with cataplexy. A maximum of 9 g can be administered each night, split in two equal doses because of the short half-life (plasma and whole blood half-life less than 1 h). The first dose should be taken at bedtime and the second 2.5–4 h later.[4-6] Intra- and interindividual variation in clinical effect has been seen with sodium oxybate; however, it is not known whether this correlates with variation in obtained GHB concentrations. Therefore, the dried blood spot (DBS) sampling technique, stated to be easy and minimally invasive, may be useful to obtain patient samples in a non-hospital-based setting.

The present study was designed to determine the GHB blood concentration obtained after the first intake of sodium oxybate by the use of the DBS sampling technique. The first objective was to evaluate the applicability of a recently developed and validated DBS-based gas chromatography-mass spectrometry (GC-MS) method.[7] Whereas DBS sampling has been used for decades in newborn screening, more recently, this alternative sampling strategy is increasingly gaining interest in the context of therapeutic drug monitoring, (pre-) clinical studies and toxicology. Having advantages such as being easy to perform and minimally invasive, DBS sampling renders blood sampling by the patient at home a feasible option, allowing better insight to be gained into GHB concentrations following sodium oxybate administration.[8-11] However, only a few studies have evaluated true home-based sampling; therefore, a second objective was to evaluate DBS collection in a real-life setting.[12,13]

2 Methods

Since narcolepsy with cataplexy is an orphan disease and sodium oxybate may only be prescribed in selected cases, there is very limited access to patients. In Belgium, around 80 patients of the approximately 200 patients diagnosed with narcolepsy are currently using sodium oxybate (Xyrem®; UCB Pharma Ltd, Brussels, Belgium), of whom seven are treated in the Department for Respiratory Diseases of Ghent University Hospital. Those seven patients were included in this study, approved by the local medical ethical board. The patients, taking sodium oxybate on a daily basis, were asked to fill a maximum of four pre-printed circles (8-mm diameter) on a DBS card approximately 20 min after the first sodium oxybate intake on 7 consecutive days. They received DBS cards (Whatman 903 filter paper; reference no. WHA10334885, Dassel, Germany), single-use automatic lancets for capillary blood collection (Becton Dickinson; reference no. VAC366594, Franklin Lakes, NJ, USA), disinfection tissues, zip-closure plastic bags to store the DBS cards and a pre-paid envelope to send the material back to the laboratory. Each patient provided informed consent and was given a 30-min explanation concerning the aim of the study and the DBS collection, together with an illustrating folder. Also, a questionnaire, with questions concerning ease of sample collection and pain, as well as inconvenience experienced with this collection technique, was provided. Patients were asked to complete and return this questionnaire at the end of the collection period.

2.1 Dried Blood Spots (DBS) Collection

To obtain a DBS, the hand was first cleaned and held down or warmed for a few minutes. With the help of an automatic lancet, the fingertip was pricked. While the first drop was wiped off with a sterile piece of cloth because of the presence of tissue fluid, the subsequent drops were collected on the DBS card.[7] After overnight drying (horizontal on a clean and empty glass), the card was placed in a zip-closure plastic bag and, finally, all the cards were sent to the laboratory by regular mail.

2.2 DBS Analysis

Upon arrival at the laboratory, DBS were visually inspected according to Edelbroek et al.[10] DBS were considered of acceptable quality when these were large enough, symmetrically spread on the filter paper and had even colouration on both sides of the filter paper.[10] The number of well collected DBS for each day was recorded and DBS analysis was performed in duplicate if possible using a previously developed and fully validated GC-MS method. Briefly, a 6-mm disc was punched out from a DBS and placed in a test tube. The internal standard GHB-d6 was added directly on the punch (5 µL of a 25-µg/mL methanolic solution), and subsequently dried under nitrogen at 25°C. Derivatization took place by adding ‘on spot’ 50

µL of a freshly prepared mixture of trifluoroacetic acid anhydride and heptafluorobutanol, and by placing the test tube in a heating block at 60°C for 10 min. The sample was cooled down during centrifugation, dried under nitrogen and then redissolved in 100 µL of ethyl acetate. Finally, after centrifugation, the supernatant was transferred to a vial. One µL of the derivatized extract was injected into the GC-MS, operating in selective ion monitoring (SIM) mode for quantification.[7] The method used has a lower and upper limit of quantification of 2 and 100 µg/mL GHB, respectively. Of those DBS with a GHB concentration above 100 µg/mL, the final ethylacetate extracts were diluted ten-fold according to the previously validated dilution technique. Furthermore, in our previous work we demonstrated that similar GHB concentrations are found in capillary DBS, in DBS prepared from venous blood and in venous blood, collected simultaneously from GHB-intoxicated patients. Based on that study, it can be concluded that DBS from capillary blood can be used as a suitable alternative for venous blood.[7]

3 Results

In total, five series of DBS were collected by three different patients. One patient sent back a single series, the two other patients two series. An overview of the GHB concentrations (µg/mL) in DBS collected by the three patients is depicted in Fig. 1. The tables in Online Resource 1 give a detailed overview of the sodium oxybate dose, which was taken at bedtime, the measured GHB concentrations, the time between sodium oxybate intake and DBS collection, the number of usable DBS and remarks. Table 1 gives an overview of the within-card precision, which was calculated as the percentage relative standard deviation (% RSD, standard deviation/mean × 100) of two DBS, obtained at the same time points. Upon evaluation of the day-to-day GHB concentrations found in the collected DBS (Fig. 1), it becomes clear that patient 2 had more intra-individual variation than patients 1 and 3. When this patient performed a second sampling 5 months later, day-to-day variation was again observed. On the other hand, analysis of DBS of the second series of patient 3 resulted in similar GHB concentrations as compared with series 1, with the exception of one day. To rule out variation in analytical performance as a cause for this exception, the two remaining DBS were also analyzed, resulting in similar GHB concentrations (mean: 8.8 µg/mL, % RSD: 5.9 %, $n = 2 \times 2$).

Concerning the DBS sampling technique, the three patients reported that DBS were easy or quite easy to obtain. According to the first and third volunteer, no pain or inconvenience was experienced; patient 2 reported inconvenience of the finger prick itself. Despite the positive evaluation, visual inspection at the laboratory (using guidelines from Edelbroek et al.[10]) revealed that not all DBS fulfilled the requirements (Fig. 2). Since a maximum of four DBS was requested, DBS cards with at least two suitable DBS were considered to have acceptable quality.[14] For the first collection period, this criterion was fulfilled by 100

% of total cards collected by patient 1, none of the cards by patient 2 (analysis of single spots was however possible on 6 out of 7 days) and 83 % of the cards by patient 3. When repeating the study (series 2), the number of well collected DBS increased, to 71 % by patient 2 and to 100 % by patient 3 (Fig. 2).

4 Discussion

Of the seven patients, one patient decided to cease participation after 3 days because of nausea, one was lost in follow-up and two stated falling asleep almost immediately after the intake of sodium oxybate. The GHB concentrations found in the three patients that completed the study are in line with those previously reported in pharmacokinetic studies using plasma samples of narcoleptic patients ingesting twice nightly a 3-g dose.[15] In addition, no interference is to be expected from sodium oxybate intake on the previous day, since in all cases there were more than 21 h between the intake of two first doses on subsequent days. Scharf et al.,[15] using a dosing scheme similar to the one in our report (two doses some 4 h apart), reported a decrease to endogenous levels in <11 h, consistent with the reported rapid metabolism of GHB in, and elimination from, the human body.[16]

With a single exception, the variation in GHB concentrations in DBS collected at the same time (within-DBS card precision) was acceptable for all duplicate measurements (<11.5 % RSD, Table 1). Besides confirming the precision of the analytical procedure, this also demonstrates the suitability of the collection technique. Patients were able to send back representative and independent samples collected in an ambulant setting, leading to valid measurements.

The intra-individual variation found in the narcoleptic patients of this study is in line with reports describing variation of serum levels following ingestion of low GHB doses in healthy subjects.[17,18] Also, variation in clinical effects has been reported;[17,19] however, it is not known whether this relates to differently obtained GHB concentrations. Although not within the scope of this exploratory study, our results demonstrate that in future studies, it may be possible to explore the relationship between GHB blood levels and sleep quality in a real-life setting using DBS sampling. In addition, although here, we opted for the most challenging scenario, in which sampling was performed shortly after the intake of the first dose (which may pose a problem for some patients), the sensitivity of our method[7] also renders sampling before or several hours after intake of the second dose a feasible option. Introduction of DBS-based sampling at home may not only allow better insight to be gained into the intra-individual variations in GHB concentration from day-to-day, but may also give relevant information about the concentrations attained at a certain time point. Indeed, in addition to sampling shortly after the first sodium oxybate dose, as was done here, it may be relevant to know the GHB concentration at the time of awakening at night (just before the second dose) or in the morning (e.g. when the patient wakes up or leaves to work).

Interesting to mention in this respect is the fact that hitherto it is not known whether the spontaneous awakening at night of sodium oxybate-treated patients is associated with the drop of GHB levels below a certain threshold (and, if so, whether this threshold is similar in different patients). The most representative insights into this matter may be obtained in a home setting, rather than in a hospital environment. Also, from a legal perspective, it is relevant to know whether at the time a sodium oxybate-treated patient gets into a car to drive, the GHB concentrations have dropped below 4–5 µg/mL, the cut-off value used in forensic toxicology.

Overall, the sampling technique was positively evaluated by the patients, and when patients 2 and 3 repeated the collection, improvement in DBS quality was seen. This improvement supports the idea that if in future studies adequate training is provided, the DBS collection technique may be used as an alternative to venipuncture in an ambulant setting.[12-14] No guidelines are available on the percentage of spots that should fulfill a certain quality standard. As we asked the patients in this study to generate four DBS and as we wished to perform duplicate analysis (i.e., analysis on two different spots on every time point), we put forward an acceptance criterion of 50 % (i.e., there should be at least two DBS with acceptable quality: large enough, symmetrically spread on the filter paper, both sides of the filter paper being evenly coloured). In fact, for any given DBS-based method, we would recommend to strive for at least two suitable DBS, in order to allow sample re-analysis, when deemed necessary.

5 Conclusions

This study shows that the DBS sampling technique may be easily adapted in a real-life setting, since DBS cards with acceptable quality are obtained by non-medically trained patients without any supervision or aid of a trained person. In addition, our results demonstrate the acceptable precision associated with execution of the complete procedure, from patient self-sampling to analysis in the laboratory. Given the nature of the medication (requiring intake just before going to bed and having a very short half-life), monitoring of GHB concentrations was hitherto only possible in a hospital setting. Our study is the first to demonstrate that unsupervised sampling by sodium oxybate-treated patients at home is not only feasible, but also leads to samples with acceptable quality. Therefore, in future studies, this minimally invasive sampling technique, in which samples can be obtained in an easy and convenient way for the patient, may be used to acquire additional information on the relationship between GHB blood concentrations, the corresponding effects and adverse effects and sleep quality.

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Table I. Within-DBS card precision (% RSD), calculated for each duplicate measurement

Day	Patient 1	Patient 2		Patient 3	
		Series 1	Series 2	Series 1	Series 2
1	5.4	ND	11.0	ND	7.7
2	1.7	ND	2.0	4.5	11.4
3	3.2	ND	ND	2.2	0.1
4	24.6	ND	8.5	2.2	1.0
5	[a]	ND	7.9	-	7.6
6	7.5	ND	10.1	5.6	6.5
7	10.4	ND.	ND	1.4	-

a Two usable DBS, but only one was analyzed, together with one DBS collected in the morning

DBS dried blood spot, *ND* no duplicate measurements possible, *RSD* relative standard deviation, - indicates no DBS collected

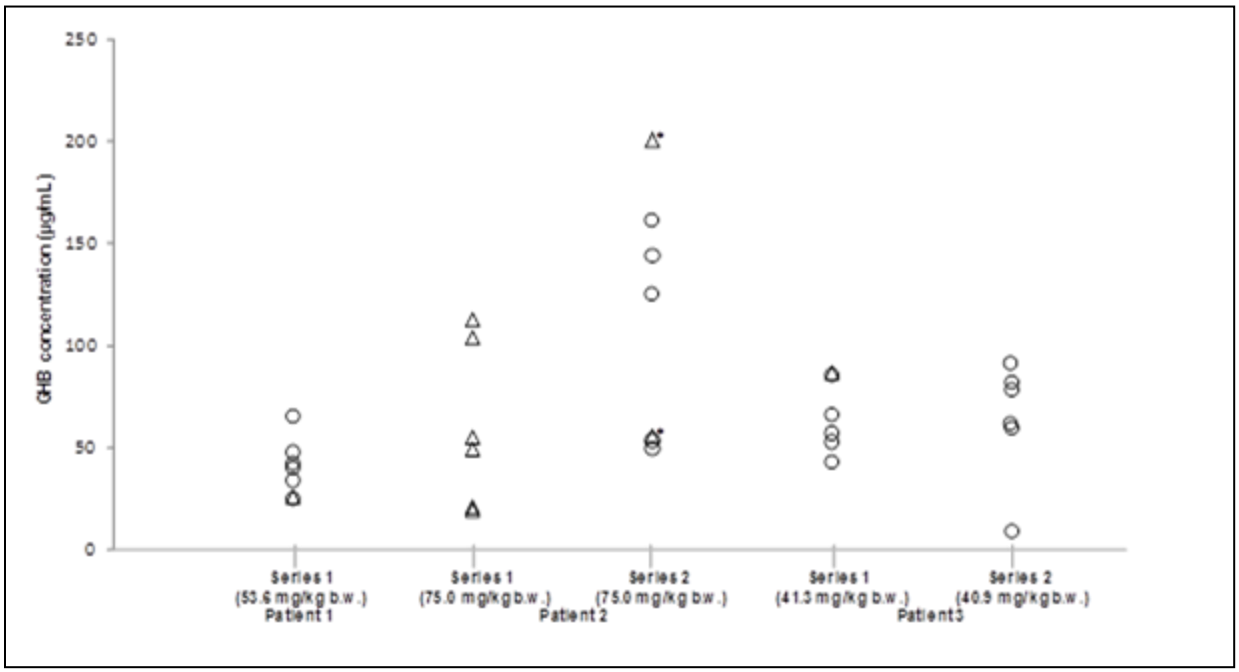

Fig. 1 Overview of the measured gamma-hydroxybutyric acid (GHB) concentrations (µg/mL, analysis of 1 spot (◯) or mean of analysis of 2 spots (△) on one card) in dried blood spots (DBS) collected by three narcoleptic patients approximately 20 min after the intake of their sodium oxybate dose during a maximum period of 7 days. Patients 2 and 3 collected two series with an interval of 5 and 9 months between series, respectively. DBS analysis was performed using a gas chromatography-mass spectrometry (GC-MS) method with 'on spot' derivatization. *b.w.* bodyweight, * DBS collected after the second dose of Xyrem®.

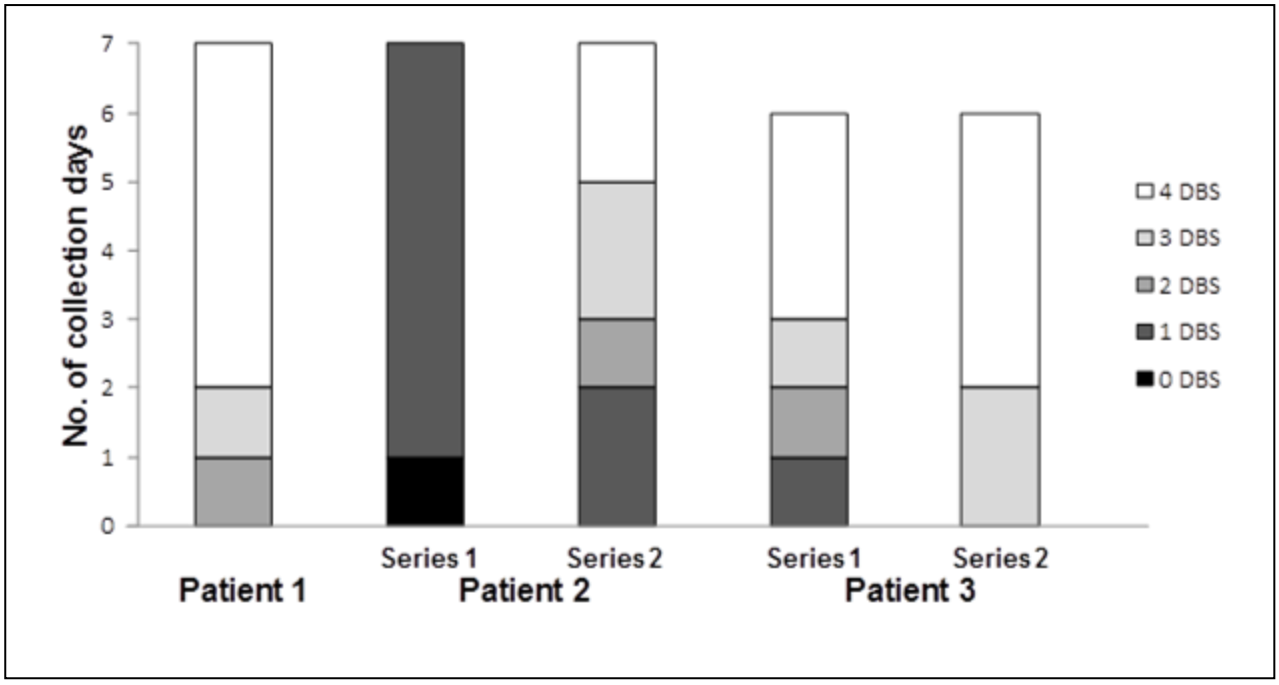

Fig. 2 Overview of the number of well collected dried blood spots (DBS) per day per patient

Supplementary Table I

Overview per patient of the sodium oxybate dose which was taken at bedtime, the measured GHB concentrations, the time between sodium oxybate intake and DBS collection, the number of usable DBS and remarks. If at least 2 DBS on a DBS card had acceptable quality (№ usable DBS ≥ 2), analysis was performed in duplicate (n= 2 DBS) and the result of the 2 measurements is reported (table GHB conc (µg/ml)). The remaining DBS were kept for possible re-analysis. If only one DBS was considered suitable for analysis (№ usable DBS =1), that DBS was analyzed, and only that single result is given (table GHB conc (µg/ml)). If there were no DBS with acceptable quality provided (№ usable DBS =0), no analysis could be performed.

Patient 1 4.50 g (53.6 mg/kg b.w.)	GHB conc (µg/ml)	Δ time (min) sodium oxybate intake-DBS collection	№ usable DBS	Remarks
Day 1	32.6 35.2	20	4	
Day 2	42.9 41.9	15	3	
Day 3	39.1 40.9	20	4	
Day 4	19.9 28.3	30	4	
Day 5	25.7	20	2	At morning: 20.2 µg/ml
Day 6	68.1 61.2	20	4	
Day 7	51.0 44.0	20	4	

Patient 2	GHB conc (µg/ml)	Δ time (min) sodium oxybate intake-DBS collection	№ usable DBS	Remarks
Series 1 **3.75 g** **(75.0 mg/kg b.w.)**				
Day 1	54.9	17	1	
Day 2	20.7	25	1	
Day 3	103.8	20	1	
Day 4			0	
Day 5	49.1	17	1	
Day 6	19.2	15	1	
Day 7	112.7	18	1	
Series 2 **3.75 g** **(75.0 mg/kg b.w.)**				
Day 1	49.2 57.5	20	4	Long sleep, slept well 1.16 am until 4.53 am
Day 2	48.7 50.1	25	4	Slept normal 0.30 am until 3.25 am
Day 3	201	15	1	Short sleep, tired again shortly after – 5.00 am until 6.30 am **DBS collected after 2nd dose**
Day 4	132 117	20	3	Slept not enough and badly 0.20 am until 2.45 am
Day 5	152 136	20	3	Slept not enough and badly 1.10 am until 3.50 am
Day 6	150 173	20	2	Slept not enough and badly 0.05 am until 2.30 am
Day 7	55.5	15	1	Slept not long, but good 3.50 am until 6.30 am **DBS collected after 2nd dose**

Patient 3	GHB conc (µg/ml)	Δ time (min) sodium oxybate intake-DBS collection	№ usable DBS	Remarks
Series 1 **4.50 g** **(41.3 mg/kg b.w.)**				
Day 1	86.7	30	1	
Day 2	88.1 82.7	30	2	
Day 3	51.8 53.4	30	4	
Day 4	43.0 41.7	30	4	
Day 5				Stayed unexpectedly with his parents
Day 6	68.2 63.0	30	3	
Day 7	56.4 57.5	30	4	
Series 2 **4.50 g** **(40.9 mg/kg b.w.)**				
Day 1	82.7 74.2	20	3	
Day 2	64.0 54.5	20	3	
Day 3	81.8 81.7	20	4	
Day 4	61.9 61.1	20	4	
Day 5	9.10 8.17	20	4	Unexplainable low GHB conc
Day 6	86.5 94.9	20	4	
Day 7				Used all the material