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Monocarboxylate Transporter Inhibition with Osmotic Diuresis Increases γ -Hydroxybutyrate Renal Elimination in Humans: A Proof-of-Concept Study

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Abstract

Background and objective: The purpose of the current study was to demonstrate proof-of-concept that monocarboxylate transporter (MCT) inhibition with L-lactate combined with osmotic diuresis increases renal clearance of γ-hydroxybutyrate (GHB) in human subjects. GHB is a substrate for human and rodent MCTs, which are responsible for GHB renal reabsorption, and this therapy increases GHB renal clearance in rats.

Methods: Ten healthy volunteers were administered GHB orally as sodium oxybate 50 mg/kg (4.5 gm maximum dose) on two different study days. On study day 1, GHB was administered alone. On study day 2, treatment of L-lactate 0.125 mmol/kg and mannitol 200 mg/kg followed by L-lactate 0.75 mmol/kg/hr was administered intravenously 30 minutes after GHB ingestion. Blood and urine were collected for 6 hours, analyzed for GHB, and pharmacokinetic and statistical analyses performed.

Results: L-lactate/mannitol administration significantly increased GHB renal clearance compared to GHB alone, 439 vs. 615 mL/hr (P=0.001), and increased the percentage of GHB dose excreted in the urine, 2.2 vs. 3.3% (P=0.021). Total clearance was unchanged.

Conclusions: MCT inhibition with L-lactate combined with osmotic diuresis increases GHB renal elimination in humans. No effect on total clearance was observed in this study due to the negligible contribution of renal clearance to total clearance at this low GHB dose. Considering the nonlinear renal elimination of GHB, further research in overdose cases is warranted to assess the efficacy of this treatment strategy for increasing renal and total clearance at high GHB doses.

Keywords: γ-hydroxybutyrate; Pharmacokinetics; Renal clearance; Monocarboxylate transporter

Abbreviations: AUC: Area under the plasma concentration-time curve; Cl: Clearance; Cl_R: Renal clearance; CV: Coefficient of variation; F: Bioavailability; GHB: γ-hydroxybutyrate; MCT: Monocarboxylate transporter

Introduction

Overdose of γ-hydroxybutyrate (GHB) and its precursors, γ-butyrolactone and 1,4-butanediol, has recently been recognized as a significant issue in public health. From 1990-2000, over 7100 GHB overdoses including 65 deaths were reported in the U.S. [1], and in a recent publication, 209 GHB-associated deaths were reported in the U.S. from 1995-2005 [2]. Manifestations of GHB overdose include sedation, coma, hypothermia, bradycardia, and respiratory arrest [3-5]. Although abuse of GHB has been recognized, there currently exists no pharmacological treatment for the overdose of these compounds. Current treatment consists primarily of supportive care and mechanical ventilation in cases of significant respiratory depression [6,7].

GHB is currently used in the form of sodium oxybate (Xyrem®) for the treatment of excessive daytime sleepiness and cataplexy in patients with narcolepsy in the U.S. and in Europe. Clinical studies with sodium oxybate demonstrate dose-dependent pharmacokinetics, even at low, therapeutic doses [8,9]. Similar pharmacokinetic properties are reported in rats, in which nonlinearity has been attributed to several concentration-dependent processes including saturable metabolism, oral absorption, and renal reabsorption [10-12]. Saturable renal reabsorption in rats can be accounted for by saturable transport by monocarboxylate transporters (MCTs), of which GHB is a substrate [10,13]. In the kidney, MCTs act to conserve endogenous monocarboxylates, such as lactate, from being cleared into the urine, and serve a similar role in the conservation of GHB. Increasing GHB renal elimination by inhibition of these transporters represents a potential therapeutic strategy for the treatment of GHB overdose. This strategy has been validated using rat kidney membrane vesicles and in vivo rat studies to demonstrate that administration of MCT inhibitors inhibits GHB transport in the kidney and effectively increases GHB

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total and renal clearance at high GHB doses [10,13-15]. Using a human kidney cell line, MCT inhibitors similarly inhibited the transport of GHB in human tissue [16].

Although well-established in rodents, the role of MCTs in the renal elimination of GHB in humans has not been demonstrated in vivo. The purpose of this study was to provide proof-of-concept that administration of an MCT inhibitor, L-lactate, combined with osmotic diuresis increases the renal clearance of GHB in human subjects.

Materials and Methods

Study design and selection of participants

This was a prospective, open-label, crossover study performed at a university hospital from 2009-2011. This study was approved by the institutional review boards at the sponsoring institutions. Healthy male and female volunteers, ages 21-55, were recruited for the study. A screening visit was used to determine subjects in good health considering medical history, physical examination, and laboratory tests. Women of child-bearing age were administered a pregnancy test at the screening visit and were required to use an acceptable method of contraception throughout the study. Exclusion criteria included evidence of organ dysfunction as determined by physical examination and laboratory results, history of drug or alcohol abuse within 6 months prior to the study, allergy to study medications, known succinic semi-aldehyde dehydrogenase deficiency, women who were pregnant, breast-feeding or unwilling to use an acceptable method of contraception, and prescription or non-prescription drug use within 1 week prior to the study, excluding oral contraceptives or other medications approved by the investigator.

On study day 1, subjects were administered sodium oxybate 50 mg/kg (4.5 gm maximum dose) orally in water. Subjects were instructed to fast overnight until they were served breakfast 2 hours after drug administration and to withhold caffeine ingestion on study days. On study day 2, subjects were administered sodium oxybate 50 mg/kg (4.5 gm maximum dose) orally, and treatment was administered intravenously at 30 minutes after GHB ingestion. Treatment consisted of a 0.125 mmol/kg L-lactate bolus over 10 minutes and a 200 mg/kg bolus of mannitol over 3 – 5 minutes, followed by a 0.75 mmol/kg/hr L-lactate infusion for the duration of the study. The L-lactate bolus and infusion were administered as sodium lactate 1/6 M solution for infusion. Mannitol was administered as a 20% solution in normal saline. In some subjects the study days were conducted in opposite order. Regardless of order, a washout period of at least 1 week was required between study days.

Data collection

Blood samples were taken on both study days directly before and at 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 210, 240, and 360 minutes after GHB administration. A spot urine was collected predose and urine samples were collected at intervals of 0-30, 30-60, 60-120, 120-180, 180-240, and 240-360 minutes after GHB administration. GHB plasma and urine concentrations were determined using an LC/MS/MS assay [17,18]. Urine volume at urine collection intervals were recorded and multiplied by urine concentrations to determine the total amount of GHB recovered at each urine collection interval. On study day 2, plasma lactate concentrations were also taken at baseline and at 180 minutes after GHB administration. Plasma lactate concentrations were determined by a colorimetric lactate oxidase assay. Vital signs and adverse events were continuously monitored on both study days. On study day 2, an electrocardiogram was recorded via telemetry from time zero to 6 hours after study drug administration and a physical examination was performed prior to release.

Data and statistical analysis

Pharmacokinetic parameters were determined by noncompartmental analysis using WinNonlin 5.2 (Pharsight Corp., Palo Alto, CA). Primary outcome measurements included GHB renal clearance (Cl_R) and the percentage of GHB excreted unchanged in the urine. Other outcome measurements included total clearance (CL/F), area under the plasma-concentration time curve (AUC), and maximum plasma concentration (C_{max}). Individual AUCs were determined by the trapezoidal method, where the measured plasma concentrations represented >95% the total AUC. Cl/F was determined as Dose/AUC. Cl_R was determined by A_e/AUC where A_e represents the amount of GHB recovered unchanged in the urine. Paired t-tests were used to determine statistically significant differences in mean pharmacokinetic parameters between study days. Statistical analyses were performed using SigmaPlot 10.0 (Systat Software, Inc., San Jose, CA). In this study, a 20% change in Cl_R was considered significant. Considering previous reports of 25% coefficient of variation (CV) values in GHB clearance [19] using α=0.05 and power of 0.8, 12 subjects were determined necessary to detect significant differences in measured pharmacokinetic parameters.

Results

A total of 21 subjects voluntarily gave informed consent for the study. Of these, 15 subjects completed the screening visit and 10 subjects completed both study days and were included in the statistical analyses. Primary outcome measurements were met with this sample size; therefore recruitment was concluded. Demographics and baseline characteristics of the 10 subjects are given in Table 1. No significant differences in renal or liver function tests were detected between study days.

Pharmacokinetic parameters determined for both study days are given in Table 2. As shown, administration of L-lactate and mannitol significantly increased GHB renal clearance and the percentage of GHB dose excreted in the urine. Individual changes in renal clearance are displayed in Figure 1. GHB plasma AUC and total clearance were unchanged between study days. Administration of L-lactate on study day 2 increased the plasma lactate level from 0.9±0.2 mM at baseline to 2.6±0.8 mM at 180 minutes (mean±SD, P<0.001). Adverse effects were similar between study days and were limited to light sedation, headache, dizziness, and nausea/vomiting. No effects on heart rate, blood pressure, or ECG were observed.

Characteristic	Number or Mean (range)	
N	10	
Age, yr	24 (21-30)	
Gender, female	5	
Weight, kg	74 (53-120)	
Race, Caucasian	9	
Race, African American	1	
	Study Day 1 Mean (SD)	**Study Day 2 Mean (SD)**
Scr (mg/dL)	0.86 (0.1)	0.84 (0.1)
BUN (mg/dL)	12 (2)	12 (2)
AST (IU/L)	30 (12)	40 (29)
ALT (IU/L)	24 (16)	30 (22)

Study Day 1 represents day of administration of GHB alone. Study Day 2 represents day of administration of GHB + lactate/mannitol

Table 1: Baseline Characteristics of Study Population.

Pharmacokinetic Parameter	GHB	GHB + Lactate/mannitol	P-value
AUC (ug/mL·hr)	191 (71)	183 (52)	0.593
Cl/F (L/hr)	20.4 (7.7)	20.4 (6.6)	0.983
C_{max} (ug/mL)	102 (27)	111 (31)	0.533
% dose excreted in urine	2.2 % (1.2)	3.2 % (1.6)	0.021
Cl_R (mL/hr)	439 (222)	615 (263)	0.001

Results are from administration of GHB 50 mg/kg orally to 10 healthy volunteers alone and with L-lactate/mannitol. Data are presented as mean ± SD. Cl/F=total oral clearance, C_{max}=maximum plasma concentration, Cl_R=renal clearance. Paired t-tests were used to detect significant differences in pharmacokinetic parameters

Table 2: Effect of L-lactate/mannitol administration on GHB pharmacokinetics.

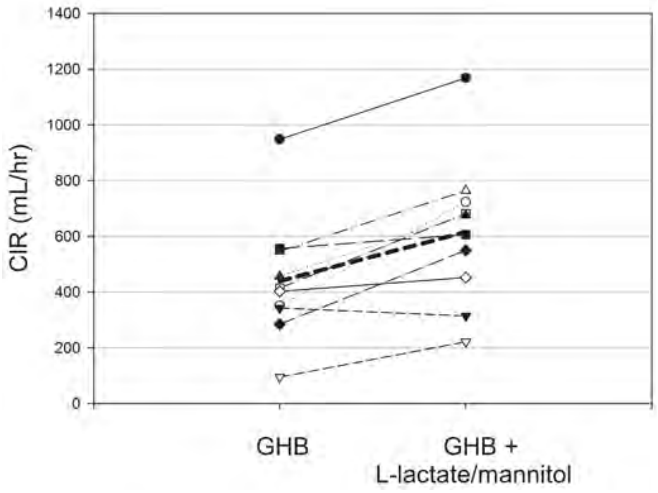

Figure 1: Individual renal clearances in 10 healthy volunteers administered GHB 50 mg/kg alone and with L-lactate/mannitol. Renal clearance (CIR) of each subject is represented by individual symbols. Heavy dashed line represents mean.

Discussion

Although GHB abuse has resulted in an increasing incidence of overdose case reports and fatalities, treatment for these cases remains limited to supportive measures. Treatment with antidotes including flumazenil, naloxone, and physostigmine has been attempted, with little effect on clinical course [6]. Administration of $GABA_B$ antagonists has been demonstrated effective for treating overdose in rodent studies [20,21]; however, these agents are not currently available for use in humans. This study was performed to assess the ability of a clinically available treatment strategy of L-lactate and mannitol to increase GHB renal elimination. The treatment strategy including MCT inhibition and osmotic diuresis was chosen based upon results of previous animal studies [10,14,15]. L-lactate was chosen as the MCT inhibitor based upon its ability to increase renal and total clearances of GHB in rats along with its demonstrated safety in humans at the effective doses [15,22,23]. The addition of mannitol as an osmotic diuretic was based upon animal experiments demonstrating an increased effect with the combination of L-lactate and mannitol compared to L-lactate alone [15]; however, the contribution of mannitol to overall effects on GHB toxicokinetics remains uncertain. L-lactate effectively increased GHB renal and total clearance in animal studies in the absence of mannitol [10,15], as has also been demonstrated with other MCT inhibitors [14]. Ongoing animal studies are being conducted to determine the benefit of mannitol co-administration on the improvement of GHB toxicokinetic and toxicodynamic endpoints with MCT inhibitors.

Although the renal clearance of GHB was increased in this study, there was no effect of treatment on total clearance. At low, therapeutic GHB doses, such as that studied currently, the primary route of elimination is metabolism by GHB dehydrogenase to form succinic semialdehyde, which is then metabolized by succinic semialdehyde dehydrogenase to succinic acid, which enters the Krebs cycle at is excreted as carbon dioxide [9]. Renal excretion represents a minor route of elimination at low doses, as illustrated in this study with only approximately 2% of the total dose excreted into the urine. In both humans and rats, GHB metabolism is saturable [8,10,11], and contributes less to overall clearance as doses are increased [10]. In rats, nonlinear GHB renal clearance has also been well-characterized, and, in contrast with metabolism, the contribution of renal elimination to total clearance increases with dose [10]. In clinical studies, the urinary recovery of GHB has also been demonstrated to increase with dose [9], suggesting similar nonlinear renal elimination in humans. Accordingly, extremely high urine concentrations have been reported in overdose cases [24-26]. At low GHB doses, when renal elimination is negligible, even a significant increase in renal clearance with treatment administration would not be expected to significantly affect total clearance, as was observed in this study. However, in clinical GHB overdose, due to both concentration-dependent renal reabsorption and saturation of metabolism, renal clearance may contribute significantly to total drug elimination, allowing the increase in renal clearance demonstrated in this study to translate to increased total clearance, a concept which has been demonstrated in our animal studies of GHB overdose. In animal studies, the increase in clearance and decrease in GHB plasma concentrations with MCT inhibition also resulted in improvement in the toxicodynamic endpoint of sedation [14,15]. In the current study, the low dose and lack of effect on total clearance limited the pharmacodynamic evaluation possible with this study, as changes in total AUC would be necessary to expect differences in pharmacodynamic endpoints. Animal studies are in progress to further assess the effect of a clinically relevant dose of L-lactate with and without mannitol on toxicodynamic endpoints of GHB overdose including sedation, respiratory depression, and fatality.

Although this study demonstrated a statistically significant increase in renal clearance with L-lactate/mannitol administration, this increase in renal clearance of approximately 40% is modest compared to that observed in animal studies [10,15]. Since L-lactate is a competitive inhibitor of MCTs, increasing the L-lactate dose may have greater effects on GHB renal clearance. The dose of L-lactate administered in this clinical study is moderate, and higher infusion rates have been administered safely to human subjects [23]. Animal studies are being conducted to compare a similar L-lactate/mannitol regimen as that administered in this study with high-dose L-lactate/mannitol regimens.

Limitations

The primary limitation of this study was the low GHB dose used, which reflects therapeutic dosing and not that in overdose cases. This excluded the evaluation of possible effects of treatment on total GHB clearance or pharmacodynamic endpoints. Due to the short half-life of GHB at the low dose used, treatment was administered 30 minutes after GHB administration in this study; in the clinic, treatment is not likely to be available this quickly after GHB ingestion. However, following overdoses, delayed peak plasma concentrations may occur, perhaps hours after ingestion as observed in animal studies [11], which may provide the opportunity for treatment at later time points. Finally, with the administration of L-lactate and mannitol concomitantly in this study, effects of MCT inhibition or osmotic diuresis alone on GHB renal clearance cannot be determined.

Conclusions

This pilot study demonstrates the proof-of-concept that MCT inhibition with L-lactate in combination with osmotic diuresis increases GHB renal elimination in humans. Administration of L-lactate and mannitol may represent a practical potential strategy for GHB overdose due to the clinical availability and low risk associated with these agents. Further research in GHB overdose cases is needed to determine the efficacy of this treatment strategy for increasing GHB total clearance and improving clinical course during GHB intoxication.

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References

1. Shannon M, Quang LS (2000) Gamma-hydroxybutyrate, gamma-butyrolactone, and 1,4-butanediol: a case report and review of the literature. Pediatr Emerg Care 16:435-440.
2. Zvosec DL, Smith SW, Porrata T, Strobl AQ, Dyer JE (2010) Case series of 226 gamma-hydroxybutyrate-associated deaths: lethal toxicity and trauma. Am J Emerg Med 29:319-332.
3. Caldicott DG, Chow FY, Burns BJ, Felgate PD, Byard RW (2004) Fatalities associated with the use of gamma-hydroxybutyrate and its analogues in Australasia. Med J Aust 181:310-313.
4. Chin RL, Sporer KA, Cullison B, Dyer JE, Wu TD (1998) Clinical course of gamma-hydroxybutyrate overdose. Ann Emerg Med 31:716-722.
5. Li J, Stokes SA, Woeckener A (1998) A tale of novel intoxication: seven cases of gamma-hydroxybutyric acid overdose. Ann Emerg Med 31:723-728.
6. Mason PE, Kerns WP (2002) Gamma hydroxybutyric acid (GHB) intoxication. Acad Emerg Med 9:730-739.
7. Okun MS, Boothby LA, Bartfield RB, Doering PL (2001) GHB: an important pharmacologic and clinical update. J Pharm Pharm Sci 4:167-175.
8. Palatini P, Tedeschi L, Frison G, Padrini R, Zordan R, et al.(1993) Dose-dependent absorption and elimination of gamma-hydroxybutyric acid in healthy volunteers. Eur J Clin Pharmacol 45:353-356.
9. Food and Drug Administration. Xyrem application, Clinical Pharmacology Biopharmaceutics Review. Orphan Medical Inc., 2002. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2002/21-196_Xyrem.cfm.
10. Morris ME, Hu K, Wang Q (2005) Renal clearance of gamma-hydroxybutyric acid in rats: increasing renal elimination as a detoxification strategy. J Pharmacol Exp Ther 313:1194-1202.
11. Lettieri JT, Fung HL(1979) Dose-dependent pharmacokinetics and hypnotic effects of sodium gamma-hydroxybutyrate in the rat. J Pharmacol Exp Ther 208:7-11.
12. Arena C, Fung HL (1980) Absorption of sodium gamma-hydroxybutyrate and its prodrug gamma-butyrolactone: relationship between in vitro transport and in vivo absorption. J Pharm Sci 69:356-358.
13. Wang Q, Darling IM, Morris ME (2006) Transport of gamma-hydroxybutyrate in rat kidney membrane vesicles: Role of monocarboxylate transporters. J Pharmacol Exp Ther 318: 751-761.
14. Wang X, Wang Q, Morris ME (2008) Pharmacokinetic interaction between the flavonoid luteolin and gamma-hydroxybutyrate in rats: potential involvement of monocarboxylate transporters. AAPS J 10: 47-55.
15. Wang Q, Wang X, Morris ME (2008) Effects of L-lactate and D-mannitol on gamma-hydroxybutyrate toxicokinetics and toxicodynamics in rats. Drug Metab Dispos 36:2244-2251.
16. Wang Q, Lu Y, Morris ME (2007) Monocarboxylate transporter (MCT) mediates the transport of gamma-hydroxybutyrate in human kidney HK-2 cells. Pharm Res 24:1067-1078.
17. Felmlee MA, Roiko SA, Morse BL, Morris ME (2010) Concentration-effect relationships for the drug of abuse gamma-hydroxybutyric acid. J Pharmacol Exp Ther 333:764-771.
18. Felmlee MA, Wang Q, Cui D, Roiko SA, Morris ME (2010) Mechanistic toxicokinetic model for gamma-hydroxybutyric acid: inhibition of active renal reabsorption as a potential therapeutic strategy. AAPS J 12:407-416.
19. Abanades S, Farre M, Segura M, Pichini S, Barral D, et al .(2006) Gamma-hydroxybutyrate (GHB) in humans: pharmacodynamics and pharmacokinetics. Ann N Y Acad Sci1074: 559-576.
20. Carai MA, Colombo G, Gessa GL (2005) Resuscitative effect of a gamma-aminobutyric acid B receptor antagonist on gamma-hydroxybutyric acid mortality in mice. Ann Emerg Med 45: 614-619.
21. Carai MA, Colombo G, Brunetti G, Melis S, Serra S, et al. (2001) Role of GABA(B) receptors in the sedative/hypnotic effect of gamma-hydroxybutyric acid. Eur J Pharmacol 428: 315-321.
22. Revelly JP, Tappy L, Martinez A, Bollmann M, Cayeux MC, et al. (2005) Lactate and glucose metabolism in severe sepsis and cardiogenic shock. Crit Care Med 33: 2235-2240.
23. Miller BF, Fattor JA, Jacobs KA, Horning MA, Navazio F, et al. (2002) Lactate and glucose interactions during rest and exercise in men: effect of exogenous lactate infusion. J Physiol 544: 963-975.
24. Kintz P, Villain M, Pelissier AL, Cirimele V, Leonetti G (2005) Unusually high concentrations in a fatal GHB case. J Anal Toxicol 29: 582-585.
25. Mazarr-Proo S, Kerrigan S (2005) Distribution of GHB in tissues and fluids following a fatal overdose. J Anal Toxicol 29: 398-400.
26. Kugelberg FC, Holmgren A, Eklund A, Jones AW (2010) Forensic toxicology findings in deaths involving gamma-hydroxybutyrate. Int J Legal Med 124:1-6.