Effects of Monocarboxylate Transporter Inhibition on the Oral Toxicokinetics/Toxicodynamics of \(\gamma\)-hydroxybutyrate and \(\gamma\)-butyrolactone

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Running Title: Treatment of oral GHB/GBL overdose

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Text Pages: 33
Tables: 4
Figures: 6
References: 35
Abstract: 250 words
Introduction: 745 words
Discussion: 1484 words

Abbreviations: ABEC, area below the effect curve; AUC, area under the plasma concentration-time curve; Cl, clearance; Cl_m, metabolic clearance; Cl_R, renal clearance; E_max, maximum pharmacodynamic effect; GBL, γ-butyrolactone; GHB, γ-hydroxybutyrate; MCT, monocarboxylate transporter; T_d, duration of effect

Recommended Section Assignment: Metabolism, Transport, and Pharmacogenomics
Abstract

Respiratory depression and death secondary to respiratory arrest have occurred following oral overdoses of γ-hydroxybutyrate (GHB) and its precursor, γ-butyrolactone (GBL). GHB is a substrate for monocarboxylate transporters (MCTs), and increasing GHB renal clearance or decreasing GHB absorption via MCT inhibition represent potential treatment strategies for GHB/GBL overdose. In these studies, GHB and GBL were administered in doses of 1.92, 5.77 and 14.4 mmol/kg orally, with and without MCT inhibition, to determine effects of this treatment strategy on oral toxicokinetics and toxicodynamics of GHB and GBL. The competitive MCT inhibitor L-lactate was administered by intravenous infusion, starting 1 hr after GHB and GBL. Oral administration of L-lactate and the MCT inhibitor luteolin was also evaluated. Respiratory depression was measured using plethysmography. IV L-lactate, but not oral treatments, significantly increased GHB renal and/or oral clearances. At the low dose of GHB and GBL, IV L-lactate increased GHB renal clearance. Due to the increased contribution of renal clearance to total clearance at the moderate dose, increased renal clearance translated to an increase in oral clearance. At the highest GHB dose, oral clearance was increased without a significant change in renal clearance. A lack of effect of IV L-lactate on renal clearance following a high oral GHB dose suggests possible effects of IV L-lactate on MCT-mediated absorption. The resulting increases in oral clearance improved respiratory depression. IV L-lactate also reduced mortality with the high GBL dose. These data indicate IV L-lactate represents a potential treatment strategy in oral overdose of GHB and GBL.
Introduction

Since recognition of the abuse of γ-hydroxybutyrate (GHB) over a decade ago, overdose of GHB and its precursors remains an important issue in public health. While some reports indicate a drop in GHB abuse in certain geographical areas (Anderson et al., 2006), the Drug Abuse Warning Network still consistently reports 1,000-2,000 emergency department visits due to GHB intoxication annually in the United States (Substance Abuse and Mental Health Services Administration, 2011). Additionally, reports indicate that abuse of the GHB precursor, γ-butyrolactone (GBL), may be increasing in the US and other countries, due to the classification of GHB as a controlled substance (Wood et al., 2011). GBL is rapidly converted to GHB by lactonases present in blood (Giarman and Roth, 1964), and studies suggest that, compared to GHB, GBL is more rapidly absorbed following oral administration (Root, 1965; Lettieri and Fung, 1976; Goodwin et al., 2009), potentially making GBL abuse particularly dangerous. While overdose of GHB and GBL remains a significant concern, resulting in coma, respiratory depression, and fatalities, there exists no specific treatment for GHB intoxication.

Preclinical studies have evaluated a number of potential therapeutic strategies for treating GHB overdose, including those affecting GHB toxicodynamics directly through receptor inhibition and indirectly through alteration of GHB toxicokinetics (Carai et al., 2001; Carai et al., 2005; Morris et al., 2005; Wang et al., 2008a; Morse et al., 2012). The pharmacokinetics of GHB are complex, and dose-dependent oral absorption and metabolism are observed in humans even at therapeutic dosages (Palatini et al., 1993). At therapeutic dosages, metabolism is the primary route of GHB elimination and renal excretion is negligible (Brenneisen et al., 2004). Toxicokinetic evaluation of GHB in rats indicates similar dose-dependent properties as observed in humans at low doses, including saturable oral absorption and saturable metabolism (Lettieri and Fung, 1979), and has also revealed nonlinear renal elimination of GHB (Morris et al., 2005). Nonlinear
renal clearance has been demonstrated in our laboratory to involve concentration-dependent transport by a group of proton-dependent transporters known as the monocarboxylate transporters (MCTs) (Morris et al., 2005; Wang et al., 2006). While renal elimination of GHB is negligible at therapeutic dosages, we previously demonstrated that GHB undergoes saturable renal reabsorption, and renal clearance increases with dose (Morris et al., 2005). Our studies further demonstrated that administration of MCT inhibitors increases the renal clearance of GHB administered intravenously in rats, resulting in increased total clearance and improvement in toxicodynamics (Wang et al., 2008a; Wang et al., 2008b; Morse et al., 2012). Additionally, in our pilot clinical study, L-lactate administered with the osmotic diuretic D-mannitol increased GHB renal clearance following oral GHB administration in humans (Morris et al., 2011). Effects on total oral clearance were limited in our clinical study, as renal clearance negligibly contributes to GHB clearance at low GHB doses safe for evaluation in humans. In combination, these clinical and preclinical data advocate increasing the renal clearance of GHB through MCT inhibition as a potential therapeutic strategy in GHB overdose.

GHB and GBL are abused orally and the oral absorption of high doses of GHB is dose-dependent in rats, with maximum plasma concentrations increasing less than proportionally with dose, indicating a saturable absorption process (Lettieri and Fung, 1979). Our in vitro studies in CaCo-2 cells also indicate saturable transport of GHB, which was pH-dependent and could be inhibited by MCT inhibitors (Lam et al., 2010). These studies suggest that, along with its concentration-dependent renal reabsorption, the dose-dependent oral absorption of GHB is also due to saturation of MCTs, which are present throughout the intestine in both rats and humans (Kirat et al., 2009; Merezhinskaya and Fishbein, 2009). As a lactone, GBL does not represent an MCT
substrate, and as such does not exhibit a similar dose-dependent oral absorption process, but is rapidly absorbed in rats (Lettieri and Fung, 1978).

While we have assessed the effects of MCT inhibition on GHB renal clearance when administered intravenously in rats, and at low oral doses in humans, the effect of MCT inhibition on GHB toxicokinetics and toxicodynamics in oral GHB overdose has yet to be evaluated, nor have effects on GHB toxicokinetics following overdose of GBL been examined. Considering the proposed role of MCTs in the oral absorption and renal reabsorption of GHB, both processes may be affected by MCT inhibitor administration in oral GHB overdose. In these studies, we aim to assess the effect of MCT inhibition on the oral and renal clearances of GHB in rats following oral overdoses of GHB and GBL.
Materials and Methods

Chemicals and Reagents. Sodium GHB used in these studies was provided by the National Institute on Drug Abuse (NIDA). Sodium L-lactate, calcium L-lactate, GBL, and activated charcoal (DARCO®), were purchased from Sigma-Aldrich (St. Louis, MO). Luteolin was obtained from Indofine Chemical Company (Hillsborough, NJ). Deuterated GHB (GHB-d6) was purchased from Cerilliant Corporation (Round Rock, TX).

Animals and Animal Surgery. Male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 250-350 g were used for all experiments. Animals were housed under controlled temperature and humidity with an artificial 12 hour light/dark cycle and food was available ad libitum. All animal protocols were approved by the Institutional Animal Care and Use Committee at the University at Buffalo. Animals were allowed to acclimate to their environment for a minimum of one week prior to surgical implantation of jugular and femoral vein cannulae under anesthesia with ketamine/xylazine. Cannulae were flushed daily with 40 IU/mL heparinized saline to maintain patency. Animals were allowed a minimum of 72 hours for recovery from surgery before drug administration.

Toxicokinetic/Toxicodynamic Experiments. Rats were administered GHB and GBL in doses of 1.92, 5.77, and 14.4 mmol/kg by oral gavage, with and without treatment strategies. All animals were fasted for 12-15 hours prior to drug administration. Blood and urine samples were collected for up to 15 hours following drug administration, and sampling times optimized for each dose. Feces were also collected for 48 hours after drug administration to determine the percentage of GHB dose absorbed. In treatment groups, treatment strategies were administered at 30 minutes after GHB/GBL in the 1.92 mmol/kg dose group and at 60 minutes after GHB/GBL administration in the 5.77 and
14.4 mmol/kg dose groups. Intravenous L-lactate (0.75 mmol/kg + 6.75 mmol/kg/hr) was administered after both GHB and GBL to target a 2 mM increase in steady-state plasma lactate concentrations, similar to our previous studies (Morris et al., 2011; Morse et al., 2012). L-lactate infusion continued up to 6, 8, and 12 hours for the 1.92, 5.77, and 14.4 mmol/kg doses, respectively. Plasma L-lactate concentrations were measured hourly for the duration of each infusion. With GHB administration, oral MCT inhibitor treatment strategies were also evaluated including administration of L-lactate (7.2 mmol/kg) and luteolin (0.175 mmol/kg), administered by oral gavage 60 minutes following 14.4 mmol/kg GHB. As a conventional treatment for oral drug overdoses, activated charcoal (1 g/kg) was also administered 60 minutes after GHB 14.4 mmol/kg. In these studies, GHB and GBL were administered as 300 mg/mL solutions in water. IV L-lactate was administered as a sodium L-lactate solution in sterile water (40 mg/mL L-lactate). Oral L-lactate was administered as a sodium/calcium L-lactate solution in water (150 mg/mL L-lactate, equimolar doses of sodium and calcium salt), luteolin as a 5 mg/mL suspension in water, and activated charcoal as a 200 mg/ml solution in water.

Respiratory depression was used a toxicodynamic endpoint in these studies, measured using a whole-body plethysmograph (Model PLY4213, Buxco Research Systems, Wilmington, NC), as previously described (Morse et al., 2012). Briefly, rats were placed in plethysmography chambers 1 hour prior to drug administration and allowed a 45 minute acclimation, after which 5 baseline measurements of 1 minute each were recorded over 15 minutes. Following the collection of baseline measurements, rats were removed from chambers and administered GHB/GBL by oral gavage. Rats were then placed back in chambers and allowed a 15 minute re-acclimation prior to the collection of respiratory measurements, which were recorded every 15 minutes for up to 12 hours following GHB/GBL administration. GHB/GBL administration was considered time 0 in all experiments. Respiratory rate was the primary respiratory parameter used
for evaluation of treatment strategies on respiratory depression, as we have previously identified change in this parameter as the primary effect of GHB on respiration (Morse et al., 2012).

The 14.4 mmol/kg GBL dose group was used to assess the effect of IV L-lactate on mortality rate (n=10 in control and treatment groups). The treatment group was administered IV L-lactate 60 minutes following GBL at the same dose as above (0.75 mmol/kg + 6.75 mmol/kg/hr). Animals were pronounced dead following cessation of respiration for 15 minutes. In the treatment group, L-lactate infusion was continued until animals resumed normal behavior (baseline respiratory rate, regained righting reflex, and normal motility). Animals were monitored for 24 hours following GBL administration.

**Sample analysis.** GHB plasma and urine concentrations were determined using previously validated LC/MS/MS methods (Felmlee et al., 2010; Morse et al., 2012). As GBL is rapidly converted to GHB in the blood, only GHB is detectable following GBL administration (Giarman and Roth, 1964); therefore plasma concentrations of GBL were not quantified. An LC/MS/MS method for the measurement of GHB in feces was developed and validated for use in these studies. Dry feces samples were ground to a fine powder. For standards, 5 μL of GHB standard solution and 5 μL of GHB-d6 were added to 50 μg of powdered blank feces. For samples 5 μL double-distilled water and 5 μL GHB-d6 were added to 50 μg of powdered sample. A water extraction was performed by adding 750 μL of double-distilled water to each sample, followed by sonication for 5 minutes and centrifugation at 10,000 rpm for 15 minutes. Protein precipitation of 400 μL of the resulting supernatant was performed by adding 1.2 mL of 0.1% formic acid in acetonitrile, followed by centrifugation at 10,000 rpm for 15 minutes. The supernatant (1.4 mL) was then dried under nitrogen gas and reconstituted in 250 μL of aqueous
mobile phase. A final centrifugation was performed at 10,000 rpm for 15 minutes and 175 μL of the supernatant used for analysis. LC/MS/MS conditions were similar to those used in the previously published assay for plasma GHB quantification (Morse et al., 2012). Interday and intraday precision and accuracy were determined using quality controls (10, 25, and 250 μg/mL), run in triplicate over 3 days. Recovery of GHB in feces was determined at all 3 quality control levels. Stability of GHB in feces was determined at each quality control level by spiking GHB into blank feces followed by incubation at 37°C for 24 and 48 hours.

Plasma lactate concentrations were measured using a YSI 1500 Sport Lactate Analyzer (Yellow Springs Instruments, Yellow Springs, CO).

Data and Statistical Analysis. Toxicokinetic parameters were determined via noncompartmental analysis using WinNonLin 5.2 (Pharsight Corp., Palo Alto, CA). Area under the plasma concentration-time curve (AUC) was determined using the trapezoidal method, and oral clearance (Cl/F) was determined as Dose/AUC. Renal clearance (ClR) was determined as Ae,urine/AUC, where Ae,urine represents the total amount of GHB excreted in the urine. Nonrenal clearance (ClNR) was determined as Cl-ClR. Fraction excreted in the feces (Fe) was determined as Ae,feces/dose, and the fraction absorbed (Fa) determined as 1-Fe. Steady-state lactate concentrations were determined as the mean value from 60 minutes until the end of L-lactate infusion (or similar time frame for control groups). Toxicodynamic descriptors of area below the effect curve (ABEC), maximum effect (E_max) and duration of respiratory depression (T_d) were used to determine effects of treatment strategies on GHB-induced respiratory depression. ABEC was determined using WinNonLin. T_d was determined at the time required for each animal to return to its individual baseline respiratory rate. Student’s t-tests or one-way
ANOVA were used to compare toxicokinetic/toxicodynamic parameters between dose groups of GHB and GBL, between equimolar doses of GHB and GBL, and between treatment and control groups. Paired t-tests were used to determine significant changes in respiratory rate compared to baseline. Statistical analyses were performed using SigmaPlot 10.0 (Systat Software, Inc., San Jose, CA). Using a power level of 0.8, to detect a 2-fold increase in oral and renal clearance, 4-5 animals were included in each GBL group, and 7-10 animals in each GHB group. Differences resulting in p<0.05 were considered significant.
Results

**LC/MS/MS assay for detection of GHB in feces.** The standard curve for GHB in feces ranged from 5 to 500 µg/g with an excellent correlation coefficient ($r^2 > 0.999$). Intraday accuracy values were 98.4%, 101%, and 101% for the 10, 25 and 250 µg/mL quality control concentrations, respectively; interday values were 99.4%, 100%, and 100%. Precision was acceptable at all quality control concentrations, with both intraday and interday CV% values of <5%. Recovery of GHB with this method ranged from 96-101% at quality control levels. At 37°C, after 24 hours, >95% of GHB remained in feces samples at all quality control levels, and >90% remained after 48 hours at each level, indicating GHB to be stable in feces over the collection period. Endogenous amounts of GHB in blank feces were determined to be lower than our lower limit of quantitation of 5 µg/g.

**GHB toxicokinetics/toxicodynamics following oral administration of GHB and GBL.** As shown in Table 1 and Figure 1A, saturation of GHB absorption was evident at high oral doses, as a less than proportional increase in the maximum plasma concentration ($C_{max}$) and significant increase in time to maximum plasma concentration ($T_{max}$) was observed between the 5.77 and 14.4 mmol/kg doses. Three distinct individual plasma profiles following the highest dose of GHB are shown in Figure 1B, demonstrating the variable and complex oral absorption patterns at this dose. Oral administration of GBL resulted in significantly higher GHB $C_{max}$ values compared to equimolar doses of GHB. Compared to the 1.92 mmol/kg doses of each compound, oral clearance of GHB was significantly lower at the 5.77 and 14.4 mmol/kg doses, consistent with saturable GHB metabolism, while renal clearance of GHB was significantly higher. Negligible renal elimination was observed at low GHB plasma concentrations, with ~1-2% of the dose excreted in the urine following oral administration of 1.92 mmol/kg GHB and GBL. However, with the
higher doses of both compounds, renal elimination represented 10-30% of GHB total oral clearance. Negligible amounts of GHB were detected in the feces at all GHB doses.

No decrease in respiratory rate was seen at the 1.92 mmol/kg doses of GHB or GBL. While the 5.77 mmol/kg dose of GHB resulted in no significant change in respiratory rate, significant respiratory depression was observed at this dose of GBL, (Figure 2 and Table 2). Administration of 14.4 mmol/kg GHB resulted in a significant, but moderate decrease in respiratory rate (Figure 2 and Table 2), while administration of 14.4 mmol/kg GBL resulted in fatality in the majority (7 out of 10) animals due to respiratory arrest (Figure 3). No fatalities were observed at 14.4 mmol/kg GHB.

**Effect of IV L-lactate administration on the oral toxicokinetics/toxicodynamics of GHB/GBL.** IV L-lactate administration following 1.92 mmol/kg GHB and GBL significantly increased GHB renal clearance, while having no effect on GHB plasma concentrations or total oral clearance of GHB, as shown in Figures 4 and 5. GHB renal, nonrenal, and total oral clearance were significantly increased in the 5.77 mmol/kg groups with IV L-lactate administration; however, renal clearance of GHB was unchanged with the 14.4 mmol/kg dose of GHB, although nonrenal and total oral clearance of GHB were significantly increased in this group. Negligible amounts of GHB were detected in the feces of animals treated with IV L-lactate, at each GHB dose. Administration of IV L-lactate in the 5.77 mmol/kg GBL and 14.4 mmol/kg GHB groups resulted in significant improvements in the ABEC, E\text{max}, and T\text{d} for respiratory rate compared to 5.77 mmol/kg GBL alone and completely prevented any significant decrease in respiratory rate following GHB 14.4 mmol/kg (Figure 2 and Table 2). When administered following a fatal dose of 14.4 mmol/kg GBL, IV L-lactate administration completely prevented lethality (Figure 3).
In this study, higher than normal baseline plasma lactate concentrations were observed in all groups, presumably due to prolonged fasting, while this level returned to normal quickly after oral drug administration in control groups. Therefore, steady-state plasma lactate concentrations were compared to control groups instead of baseline since lactate concentrations changed over time even in control groups not administered L-lactate. Steady-state plasma lactate concentrations in groups administered IV L-lactate were maintained at approximately 2 mM higher than control groups over the same time period, as shown in Table 3. Higher steady-state concentrations in the 1.92 mmol/kg GBL groups are due to higher baseline levels in this control and treatment group. However, baseline levels were similar between control and treatment groups at this and all other doses (p>0.05 compared by student’s t-test for all dose groups).

Effect of oral treatment strategies on GHB oral toxicokinetics. The administration of L-lactate or luteolin orally 1 hour after GHB 14.4 mmol/kg resulted in no significant effect on GHB oral or renal clearances (Figure 6 and Table 4). The administration of oral L-lactate appeared to only delay the absorption of GHB, as the time to peak plasma concentration (T_{max}) was significantly longer in the L-lactate treated group. Luteolin had no significant effect on any toxicokinetic parameters, nor did the administration of activated charcoal. The administration of activated charcoal actually appeared to cause respiratory distress, likely due to aspiration, in 50% of the animals in this treatment group.
Discussion

GHB overdose remains a concern in several countries, primarily due to the lack of pharmacological intervention for GHB intoxication. Current treatment for GHB overdose includes supportive care and intubation in cases of significant respiratory depression. In rodent studies, administration of GABA<sub>B</sub> receptor antagonists has improved many toxicodynamic measures of GHB intoxication, including sedation, respiratory depression, and fatality (Carai et al., 2001; Carai et al., 2005; Morse et al., 2012); however, GABA<sub>B</sub> receptor antagonists are not currently available for human use. Here we investigate the utility of a clinically available potential treatment option, MCT inhibition with L-lactate, for improving GHB toxicokinetics and toxicodynamics following oral overdose of GHB and GBL.

Our data concur with previous data demonstrating a saturable absorption process for GHB at high oral doses (Lettieri and Fung, 1979). Despite saturable absorption, the previous study estimated GHB bioavailability to be high, ranging from 65-100% at doses of 200-1600 mg/kg, although bioavailability of GHB is difficult to determine experimentally due to many concentration-dependent processes. However, our data also indicate that GHB is well-absorbed even at high oral doses, with negligible excretion in the feces. The current studies show that, similar to humans, renal elimination of GHB is negligible in rats at low plasma concentrations, however, significantly contributes to total GHB clearance in rats at high oral doses. Case reports of GHB intoxication have reported very high urine concentrations, also suggesting significant renal elimination of GHB in clinical overdose (Kintz et al., 2005; Mazarr-Proo and Kerrigan, 2005; Kugelberg et al., 2010). Our data are also consistent with previous studies in rats demonstrating much higher plasma concentrations of GHB following GBL administration compared to administration of equimolar doses of GHB itself (Lettieri and Fung, 1978). Although toxicokinetic data are not available for comparison in humans,
similar data have been reported in non-human primates (Goodwin et al., 2009). We additionally report that, similar to GHB administration, renal clearance significantly contributes to total GHB elimination following a high GBL dose. The toxicodynamic data from these experiments indicate that in rats respiratory depression and death secondary to respiratory arrest are more likely with GBL than GHB overdose, due to higher GHB plasma concentrations.

The administration of IV L-lactate with 1.92 mmol/kg GHB and GBL lead to similar results as in our clinical study (Morris et al., 2011); GHB renal clearance was increased, but due to the negligible role of renal elimination, this did not lead to increased GHB total oral clearance. However, with the 5.77 mmol/kg dose of each agent, contribution of GHB renal clearance to GHB oral clearance was much greater, and increases in renal and total oral clearance of GHB were observed. An interesting effect of L-lactate in this dose group was a substantial increase in GHB nonrenal clearance, an effect previously observed in IV studies (Morris et al., 2005), suggesting effects of L-lactate on GHB metabolism. GHB is metabolized primarily by GHB dehydrogenase as well as a mitochondrial transhydrogenase (Kaufman and Nelson, 1991). Based on the current data, direct effect of L-lactate on either pathway is unlikely, as this should have also increased GHB metabolism at the low dose of GHB and GBL, and nonrenal clearance was unchanged in these groups. Alternatively, the increase in nonrenal clearance with L-lactate administration is more likely an indirect effect due to lower plasma concentrations caused by increased renal clearance, which would increase GHB metabolism due to its nonlinearity. Surprisingly, at GHB 14.4 mmol/kg, the administration of IV L-lactate increased nonrenal and total oral clearance of GHB without a significant change in renal clearance. Our previous IV studies indicate a 2-fold change in renal clearance is expected with L-lactate administration at GHB plasma concentrations observed at this high dose (Morris et al., 2005; Wang et al., 2008a), but
this was not observed in the current study with oral GHB administration. As the increased nonrenal and total clearance cannot be attributed to lower plasma concentrations caused by increased renal clearance, these results suggest another mechanism for the effects of IV L-lactate at this GHB dose, possibly through an interaction of MCT-mediated GHB absorption. No significant amount of GHB was detected in the feces of L-lactate-treated animals at this or any dose, suggesting no effect of L-lactate on the extent of GHB absorption. However, while the extent may not be affected, the rate of GHB absorption may be decreased by inhibition of intestinal transport, leading to lower plasma concentrations and potentially increased nonrenal clearance via nonlinear metabolism, thereby increasing total oral clearance without requiring an effect on the percentage of GHB absorbed. This potential effect of L-lactate on GHB absorption is supported by the secondary peak in GHB plasma concentration after L-lactate is discontinued. While the MCT-mediated absorption of GHB allows the potential for this interaction at the intestine with L-lactate administration, further studies are needed to confirm the mechanisms behind the effect of IV L-lactate on high oral doses of GHB. Additionally, although we have demonstrated GHB to be a substrate of MCT1, 2, and 4, and demonstrated pH-dependent transport of GHB in kidney and intestinal cells (Wang et al., 2006; Wang and Morris, 2007a; Lam et al., 2010), GHB and L-lactate are also substrates for the sodium-coupled SMCT1(SLC5A8) (Cui and Morris, 2009), also present in rat intestine and kidney (Ganapathy et al., 2008). Further experiments may also be warranted to distinguish the contribution of MCTs and SMCTs to GHB transport, and the effects of L-lactate, at both physiologic sites.

IV L-lactate administration following GHB and GBL improved respiratory depression with both agents, in agreement with our previous IV data indicating that increasing GHB clearance with L-lactate administration improves respiratory rate (Morse et al., 2012). Along with effects on renal clearance, our recent data indicate L-lactate can
also decrease the brain uptake of GHB (Roiko et al., 2013), as GHB blood-brain barrier transport is also MCT-mediated (Bhattacharya and Boje, 2004). Along with decreased plasma concentrations, decreased brain:plasma partitioning may also play a role in the improvement of GHB toxicodynamics with IV L-lactate administration in the current study. Perhaps of greatest significance, when administered following a fatal dose of 14.4 mmol/kg GBL, IV L-lactate prevented death in all animals, suggesting that L-lactate can not only improve respiratory rate but also prevent death due to respiratory arrest. To our knowledge this is the first study demonstrating improvement in mortality associated with GHB intoxication using a clinically available treatment option.

Due to the suggested role of MCTs in the absorption of GHB, oral administration of MCT inhibitors was also assessed as a potential treatment strategy. Even though IV L-lactate increased GHB oral clearance, oral L-lactate had no significant effect on this parameter, nor did the oral administration of a more potent MCT inhibitor, luteolin, despite a very low IC_{50} for MCT1 of less than 1 µM for this flavonoid (Wang and Morris, 2007b). The lack of effect of was likely due to rapid absorption and elimination of both agents following oral administration. Following oral L-lactate administration, plasma lactate concentrations peaked just after administration at 60 minutes, then quickly declined to that of control animals (Table 3). Luteolin is also rapidly absorbed and metabolized following oral administration in rats (Chen et al., 2010). From these experiments, it can be concluded that for oral MCT inhibition to be effective, the inhibitor must be present throughout the prolonged duration of GHB absorption. While a lack of effect of activated charcoal on GHB toxicokinetics is somewhat surprising given the relatively rapid timing of treatment, it is in agreement with recent in vitro data indicating low binding of GHB to activated charcoal at intestinal pH (Neijzen et al., 2012). The risk of aspiration with this compound may outweigh the potential benefit in GHB overdose.
In summary, the renal elimination of GHB is negligible at low oral doses in rats, as in humans, while at high oral doses of GHB and GBL, renal elimination contributes significantly to GHB total oral clearance. Similarly, an increase in GHB renal clearance with IV L-lactate administration at a low dose of GHB mimics clinical results, while at higher plasma concentrations of GHB, increases in GHB renal and nonrenal clearances with L-lactate translates to an increase in total oral clearance following overdose of GHB and GBL. An increase in total oral clearance at a very high GHB dose without a significant effect on renal clearance suggests other mechanisms are responsible for the increase in total clearance at high oral GHB doses. In both cases of GHB and GBL overdose, an increase in GHB total oral clearance translates to improvement in respiratory rate, as well as decreased GBL-induced fatality, suggesting L-lactate as a potential therapeutic strategy for improving respiratory depression/arrest following overdose of both agents. Further mechanistic studies evaluating the effect of IV L-lactate on GHB absorption and metabolism would be useful for determining effects on high oral doses of GHB as well as translating the potential treatment strategy of MCT inhibition to clinical GHB overdose.
Acknowledgements

The authors would like to thank Donna Ruszaj for assistance in developing the current LC/MS/MS method.
Authorship Contributions

Participated in research design: Morris and Morse

Conducted experiments: Morse

Contributed new reagents or analytic tools: Morris and Morse

Performed data analysis: Morse

Wrote or contributed to the writing of the manuscript: Morris and Morse
References


Goodwin AK, Brown PR, Jansen EE, Jakobs C, Gibson KM, and Weerts EM (2009) Behavioral effects and pharmacokinetics of gamma-hydroxybutyrate (GHB) precursors gamma-butyrolactone (GBL) and 1,4-butanediol (1,4-BD) in baboons. Psychopharmacology (Berl) 204:465-476.


Wood DM, Brailsford AD, and Dargan PI (2011) Acute toxicity and withdrawal syndromes related to gamma-hydroxybutyrate (GHB) and its analogues gamma-butyrolactone (GBL) and 1,4-butanediol (1,4-BD). Drug Test Anal 3:417-425.
Footnotes

a) This work was supported by the National Institutes of Health National Institute on Drug Abuse [grant DA023223] and by a fellowship from Pfizer Global Research and Development.

b) A portion of this work was previously presented as an abstract: Effect of Monocarboxylate Transporter Inhibition on the Oral Toxicokinetics/Toxicodynamics of γ-hydroxybutyrate (GHB) and γ-butyrolactone (GBL), American Association of Pharmaceutical Sciences Annual Meeting 2012, Chicago, IL.

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Figure 1. Plasma concentrations of GHB following oral administration of GHB and GBL.  A) Mean plasma concentrations B) Plasma concentrations for 3 individual rats following oral administration of GHB 14.4 mmol/kg. Data presented as mean ± SD, n=4-10.

Figure 2. Effect of oral GHB and GBL administration on respiratory rate with and without IV L-lactate administration.  A) GBL 5.77 mmol/kg B) GHB 14.4 mmol/kg. (Respiratory data is shown only for non-fatal doses of GHB/GBL which alone resulted in significant respiratory depression.) GHB/GBL were administered by oral gavage at time 0 and intravenous L-lactate treatment (0.75 mmol/kg + 6.75 mmol/kg/hr) initiated 60 minutes after GHB/GBL. L-lactate infusion was continued for up to 8 and 12 hours for the 5.77 and 14.4 mmol/kg doses, respectively. Data presented as mean ± SD, n=4-5.

Figure 3. Fatality rates following oral administration of GBL 14.4 mmol/kg with and without IV L-lactate.  GBL was administered at time 0 by oral gavage. Intravenous L-lactate (0.75 mmol/kg + 6.75 mmol/kg/hr) was initiated 60 minutes after GBL. L-lactate infusion was continued until normal behavior of animals resumed.

Figure 4. Effect of IV L-lactate on GHB clearances following oral administration of GHB (A) and GBL (B).  Cl/F=total oral GHB clearance(clearance/bioavailability)

\[ Clr = \text{GHB renal clearance} \]
\[ Clnr = \text{GHB nonrenal clearance} \]
\[ Fa = \text{fraction of GHB absorbed.} \]

GHB and GBL were administered by oral gavage and intravenous L-lactate (0.75 mmol/kg + 6.75 mmol/kg/hr) was initiated 30 and 60 minutes later, for the 1.92 and 5.77/14.4 mmol/kg doses, respectively. L-lactate infusion was continued for up to 6, 8,
and 12 hours for the 1.92, 5.77, and 14.4 mmol/kg doses, respectively. Data presented as mean ± SD, n=7-10 (GHB) and 4-5 (GBL).

* P<0.05  ** P<0.01  *** P<0.001, compared to control group of same dose.

**Figure 5. Effect of IV L-lactate on GHB plasma concentrations following oral administration of GHB and GBL.** A) GHB/GBL 1.92 mmol/kg B) GHB/GBL 5.77 mmol/kg C) GHB 14.4 mmol/kg. Closed symbols represent GHB/GBL alone, open symbols represent GHB/GBL + L-lactate. Circles= GHB administration, Squares= GBL administration. GHB and GBL were administered by oral gavage and intravenous L-lactate (0.75 mmol/kg + 6.75 mmol/kg/hr) was initiated 30 and 60 minutes later, for the 1.92 and 5.77/14.4 mmol/kg doses, respectively. L-lactate infusion was continued for up to 6, 8, and 12 hours for the 1.92, 5.77, and 14.4 mmol/kg doses, respectively. Data presented as mean ± SD, n=7-10 (GHB) and 4-5 (GBL).

**Figure 6. Plasma concentrations of GHB following oral administration of GHB (14.4 mmol/kg) with and without oral treatment options.** A) Effect of MCT inhibitors B) Effect of activated charcoal. GHB was administered by oral gavage and L-lactate (7.2 mmol/kg), luteolin (0.175 mmol/kg), or activated charcoal (1 g/kg) were administered by oral gavage 1 hour later. Data presented as mean ± SD, n=4-7.
Table 1. GHB toxicokinetics following oral administration of GHB and GBL. GHB and GBL were administered by oral gavage. Student’s t-tests or one-way ANOVA were used to compare mean toxicokinetic parameters between dose groups of GHB or GBL and between equimolar doses of GHB and GBL. Data presented as mean (SD), n=4-9.

<table>
<thead>
<tr>
<th>Toxicokinetic Parameter</th>
<th>GHB 1.92 mmol/kg</th>
<th>GHB 5.77 mmol/kg</th>
<th>GHB 14.4 mmol/kg</th>
<th>GBL 1.92 mmol/kg</th>
<th>GBL 5.77 mmol/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl/F (mL/kg/min)</td>
<td>18.5 (6.3)</td>
<td>7.64&lt;sup&gt;a&lt;/sup&gt; (2.8)</td>
<td>6.36&lt;sup&gt;a&lt;/sup&gt; (1.1)</td>
<td>14.3 (4.9)</td>
<td>3.11&lt;sup&gt;a,c&lt;/sup&gt; (0.26)</td>
</tr>
<tr>
<td>Cl&lt;sub&gt;R&lt;/sub&gt; (mL/kg/min)</td>
<td>0.194 (0.098)</td>
<td>0.867&lt;sup&gt;a&lt;/sup&gt; (0.51)</td>
<td>1.82&lt;sup&gt;a&lt;/sup&gt; (0.63)</td>
<td>0.197 (0.07)</td>
<td>0.545&lt;sup&gt;a&lt;/sup&gt; (0.28)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ug/mL)</td>
<td>122 (35)</td>
<td>401&lt;sup&gt;a&lt;/sup&gt; (150)</td>
<td>651&lt;sup&gt;b&lt;/sup&gt; (268)</td>
<td>230&lt;sup&gt;c&lt;/sup&gt; (5.7)</td>
<td>972&lt;sup&gt;a,c&lt;/sup&gt; (93)</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (hr)</td>
<td>0.85 (0.14)</td>
<td>1.72 (0.57)</td>
<td>6.0&lt;sup&gt;b&lt;/sup&gt; (2.1)</td>
<td>0.25&lt;sup&gt;c&lt;/sup&gt; (0.12)</td>
<td>1.13&lt;sup&gt;a&lt;/sup&gt; (0.63)</td>
</tr>
<tr>
<td>Fa (%)</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
<td>------</td>
<td>------</td>
</tr>
</tbody>
</table>

Cl/F = GHB oral clearance (total clearance/bioavailability)  
Cl<sub>R</sub> = GHB renal clearance  
C<sub>max</sub> = maximum GHB plasma concentration  
T<sub>max</sub> = time of maximum plasma concentration  
Fa = fraction of GHB absorbed  
<sup>a</sup> significantly different from 1.92 mmol/kg of the same compound (P<0.05)  
<sup>b</sup> significantly different from 1.92 and 5.77 mmol/kg of the same compound (P<0.05)  
<sup>c</sup> significantly different from an equimolar dose of GHB (P<0.05)
Table 2. Respiratory rate following oral administration of GHB and GBL with and without IV L-lactate. GHB and GBL were administered by oral gavage and intravenous L-lactate treatment (0.75 mmol/kg + 6.75 mmol/kg/hr) was initiated 1 hour later. L-lactate infusion was continued for up to 8 and 12 hours for the 5.77 and 14.4 mmol/kg doses, respectively. Data presented as mean (SD), n=4-5. Student’s t-tests were used to compare GHB/GBL + L-lactate to the same dose of GHB/GBL alone.

<table>
<thead>
<tr>
<th>Toxicodynamic Parameter</th>
<th>GHB 14.4 mmol/kg</th>
<th>GHB 14.4 mmol/kg + L-lactate</th>
<th>GBL 5.77 mmol/kg</th>
<th>GBL 5.77 mmol/kg + L-lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABEC (breaths)</td>
<td>167 (96)</td>
<td>-----a</td>
<td>131 (38)</td>
<td>56 (20)**</td>
</tr>
<tr>
<td>E_{max} (breaths/min)</td>
<td>33 (9)</td>
<td>-----a</td>
<td>18 (3)</td>
<td>36 (8)**</td>
</tr>
<tr>
<td>T_{d} (hr)</td>
<td>5.94 (2.7)</td>
<td>-----a</td>
<td>4.13 (0.43)</td>
<td>2.20 (0.21)***</td>
</tr>
</tbody>
</table>

ABEC=area below the effect curve  E_{max}=maximum effect  T_{d}=duration of effect

a----- no significant decrease in respiratory rate was observed at GHB 14.4 mmol/kg + L-lactate compared to baseline

** P<0.01 compared to control group of same dose

*** P<0.001 compared to control group of same dose
Table 3. Plasma lactate concentrations following oral administration of GHB and GBL with and without L-lactate. L-lactate was administered IV (0.75 mmol/kg + 6.75 mmol/kg/hr) and PO (7.2 mmol/kg) at 60 minutes after GHB/GBL (30 minutes after 1.92 mmol/kg doses). IV L-lactate infusion was continued for up to 6, 8, and 12 hours for the 1.92, 5.77, and 14.4 mmol/kg doses, respectively. Steady-state lactate concentrations represent the average plasma lactate concentrations from 1 hr until the end of infusion (or representative time period for control groups). Data presented as mean (SD).

<table>
<thead>
<tr>
<th>Regimen</th>
<th>GHB 1.92 mmol/kg</th>
<th>GHB 5.77 mmol/kg</th>
<th>GHB 14.4 mmol/kg</th>
<th>GHB 14.4 mmol/kg + L-lactate (IV)</th>
<th>GBL 1.92 mmol/kg</th>
<th>GBL 5.77 mmol/kg + L-lactate (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS plasma lactate (mM)</td>
<td>0.92 (0.10)</td>
<td>2.92 (0.24)</td>
<td>1.32 (0.28)</td>
<td>4.01 (1.2)</td>
<td>1.53 (0.36)</td>
<td>3.54 (0.30)</td>
</tr>
<tr>
<td>Difference from control (mean)</td>
<td>----- 2.00</td>
<td>----- 2.69</td>
<td>----- 2.01</td>
<td>-0.06</td>
<td>----- 2.23</td>
<td>----- 2.11</td>
</tr>
</tbody>
</table>

SS=steady-state
Table 4. Effect of oral treatment strategies on the oral toxicokinetics of GHB 14.4 mmol/kg. GHB was administered by oral gavage. L-lactate (7.2 mmol/kg), luteolin (0.175 mmol/kg), and activated charcoal (1 g/kg) were administered by oral gavage 1 hour later. Data presented as mean (SD), n=4-7. One-way ANOVA followed by a Dunnett’s post-hoc test was used to compare toxicokinetic parameters between treatment groups and GHB alone.

<table>
<thead>
<tr>
<th>Toxicokinetic Parameter</th>
<th>GHB 14.4 mmol/kg</th>
<th>GHB 14.4 mmol/kg + L-lactate</th>
<th>GHB 14.4 mmol/kg + luteolin</th>
<th>GHB 14.4 mmol/kg + charcoal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl/F (mL/kg/min)</td>
<td>6.36 (1.1)</td>
<td>6.85 (0.52)</td>
<td>6.45 (2.0)</td>
<td>5.72 (0.92)</td>
</tr>
<tr>
<td>ClR (mL/kg/min)</td>
<td>1.89 (0.58)</td>
<td>1.55 (0.63)</td>
<td>1.11 (0.71)</td>
<td>1.90 (0.24)</td>
</tr>
<tr>
<td>Cmax (ug/mL)</td>
<td>651 (268)</td>
<td>454 (61)</td>
<td>634 (424)</td>
<td>691 (130)</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>6.0 (2.1)</td>
<td>9.5 (2.4)*</td>
<td>4.0 (2.4)</td>
<td>6.75 (0.96)</td>
</tr>
<tr>
<td>Fa (% dose)</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
<td>------</td>
</tr>
</tbody>
</table>

Cl/F=GHB oral clearance (total clearance/bioavailability)  
ClR=GHB renal clearance  
Cmax=maximum GHB plasma concentration  
Tmax=time of maximum plasma concentration  
Fa= fraction of GHB absorbed (------ , due to apparent aspiration and respiratory distress, animals administered charcoal were not kept for 48 hr feces collection)  
* P<0.05 compared to GHB alone
Fig. 2

A

Frequency (breaths/min)

- GBL 5.77 mmol/kg
- GBL 5.77 mmol/kg + L-lactate

Time (hr)

B

Frequency (breaths/min)

- GHB 14.4 mmol/kg
- GHB 14.4 mmol/kg + L-lactate

Time (hr)
Fig. 3

![Graph showing the number of animals alive over time for two conditions: GBL 14.4 mmol/kg and GBL 14.4 mmol/kg + L-lactate. The graph indicates a rapid decline in the number of animals alive for the GBL 14.4 mmol/kg condition, while the GBL 14.4 mmol/kg + L-lactate condition shows a more sustained decline.]