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γ -Hydroxybutyric Acid (GHB) Pharmacokinetics and Pharmacodynamics: Semi-Mechanistic and Physiologically Relevant PK/PD Model

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Abstract

An overdose of γ -hydroxybutyric acid (GHB), a drug of abuse, results in fatality caused by severe respiratory depression. In this study, a semi-mechanistic pharmacokinetic/pharmacodynamic (PK/PD) model was developed to characterize monocarboxylate transporter 1 (MCT1)-mediated transport of GHB, as well as effects of GHB on respiration frequency, for IV doses of 200, 600, and 1500 mg/kg in rats. The proposed PK/PD model for GHB consists of nonlinear metabolism of GHB in the liver, MCT1-mediated renal reabsorption with physiologically relevant concurrent fluid reabsorption, MCT1-mediated uptake into the brain, and direct effects of binding of GHB to GABA_B receptors on the PD parameter, respiration frequency. Michaelis-Menten affinity constants for metabolism, renal reabsorption, and uptake into and efflux from the brain were fixed to the observed *in vitro* values. The *IC*₅₀ value for the effect of GHB on respiration frequency was fixed to a reported value for binding of GHB to GABA_B receptors. All physiological parameters were fixed to the reported values for a 300-g rat. The model successfully captured the GHB PK/PD data and was further validated using the data for a 600-mg/kg dose of GHB after IV bolus administration. Unbound GHB brain ECF/blood partition coefficient (*K*_{p,u,w}) values obtained from the model agreed well with values calculated using experimental ECF concentrations obtained with brain microdialysis, demonstrating the physiological relevance of this model. Sensitivity analysis indicated that the PK/PD model was stable. In conclusion, we developed a semi-mechanistic and physiologically relevant PK/PD model of GHB using *in vitro* drug-transporter kinetics and *in vivo* PK/PD data in rats.

Keywords

active renal reabsorption; active uptake into brain; monocarboxylate transporters (MCTs); nonlinear PK; PK/PD modeling and simulation; semi-mechanistic kidney model; γ -hydroxybutyric acid (GHB)

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INTRODUCTION

γ -Hydroxybutyric acid (GHB), a naturally occurring short-chain fatty acid and a metabolite of γ -aminobutyric acid (GABA), is found in the mammalian brain, heart, liver, and kidneys [1]. Apart from its therapeutic use for the treatment of narcolepsy (Xyrem®, USA) [2] and alcohol withdrawal (Europe) [3], GHB has been widely abused for recreational purposes [4] and sexual exploitation [5]. Overdose of GHB has been reported to cause many adverse events including loss of consciousness, coma, and death. Severe respiratory depression is most often the cause of GHB-induced fatality [6–8]. Currently, no treatment strategy exists to treat GHB overdose in emergency rooms except for the often-unsuccessful supportive care [8,9].

Nonlinear pharmacokinetics (PK) for GHB has been characterized in rats [10] and humans [11,12], where saturable absorption [11,13], metabolism [11], and renal clearance [14] have been shown to contribute to this observed nonlinearity in GHB PK. Renal clearance of GHB has been reported to contribute significantly to the total clearance at the high concentrations observed in overdose patients [15] and to increase with increasing doses in rats, in contrast to the decrease in the total clearance [14]. These findings indicate saturation of active reabsorption of GHB at very high concentrations. *In vitro* studies have characterized GHB as a substrate of proton-linked monocarboxylate transporters (MCTs) 1, 2, and 4 and sodium-dependent monocarboxylate transporter (SMCT) 1 [16–20]. Monocarboxylate transporter 1 (MCT1) is distributed ubiquitously in the mammalian brain, heart, intestine, kidneys, and red blood cells [21] and has been shown to play a predominant role in transport of GHB in kidneys and brain in rats and humans [17,18,22].

We have recently reported a semi-mechanistic kidney model incorporating physiologically relevant fluid reabsorption and transporter-mediated renal reabsorption to describe the pharmacokinetics of GHB and L-lactate in rats [23]. This model will be extended to incorporate MCT1-mediated uptake of GHB into the brain extracellular fluid (ECF) as well as GHB pharmacodynamics, namely, GHB-induced respiratory depression in rats. The overall objective of the present study was to develop and validate a semi-mechanistic and physiologically relevant PK/PD model for GHB using *in vitro* drug-transporter kinetics and *in vivo* PK/PD data in rats.

MATERIALS AND METHODS

Semi-Mechanistic PK/PD Modeling Framework

As illustrated in Fig. 1, the proposed model has five key components: (1) physiologically relevant fluid reabsorption in the kidney, (2) nonlinear renal and brain transport kinetics, (3) saturable hepatic metabolism, (4) mass balance disposition of GHB, and (5) GHB-induced respiratory depression. The description and values of all fixed parameters including the values of physiological flows (Q) and volumes (V) are provided in Table I.

PK/PD Model of GHB

The GHB-specific component followed our previously published semi-mechanistic PK model for GHB [23] with several modifications and was further extended to incorporate

uptake of GHB into the brain and incorporated the pharmacodynamics parameter of GHB, effect on respiratory frequency.

Pharmacokinetics of GHB

The semi-mechanistic PK model for GHB consists of physiologically relevant blood, liver, brain, and remainder compartments as well as a kidney component consisting of various nephron segments: proximal tubules (PT), loop of Henle (LOH), distal tubules (DT) and collecting ducts (CD), concurrent fluid reabsorption, and active renal reabsorption of GHB that is described briefly with equations below [23].

Blood Compartment

The blood compartment (Eq. 1) is the site for GHB input. From the blood (*BL*), GHB is distributed to the liver (*LI*), the kidneys (*KI*), the brain vasculature (*BR, VS*) and to the remainder of the body (*RM*). The model accounted for mass and flow balance. The initial condition for the blood compartment is the dose of GHB and is zero for all other compartments in the model.

$$\begin{aligned} \frac{dA_{BL}}{dt} = & -Q_{LI} \times \frac{A_{BL}}{V_{BL}} + Q_{LI} \times \frac{A_{LI}}{V_{LI} \times K_{P,LI}} - Q_{KI} \times \frac{A_{BL}}{V_{BL}} + (Q_{KI} - Q_U) \times \frac{A_{RBL}}{V_{RBL}} \\ & - Q_{BR} \times \frac{A_{BL}}{V_{BL}} + Q_{BR} \times \frac{A_{BR,VS}}{V_{BR,VS}} - Q_{RM} \times \frac{A_{BL}}{V_{BL}} + Q_{RM} \times \frac{A_{RM}}{V_{RM} \times K_{P,RM}} \end{aligned} \quad (1)$$

Liver and Remainder Compartments

At physiological pH, GHB is predominantly in the ionized form and its distribution into tissues requires MCT-mediated transport, predominantly by MCT1 [17,18,20]. GHB tissue distribution is dose-dependent and organ-specific (unpublished data). The two key assumptions are as follows: (1) GHB distribution into the liver and the remainder compartments can be described by the respective blood-to-tissue partition coefficients (K_P) and (2) the liver is the major site for capacity-limited metabolism of GHB [23]. Saturable tissue distribution of GHB was not observed for the doses of GHB studied (unpublished data). The capacity-limited metabolism of GHB was incorporated as a single Michaelis-Menten (MM) equation. Equations 2 and 3 describe the distribution of GHB into the liver and the remainder compartments as:

$$\begin{aligned} \frac{dA_{LI}}{dt} = & Q_{LI} \times \frac{A_{BL}}{V_{BL}} - Q_{LI} \\ & \times \frac{A_{LI}}{V_{LI} \times K_{P,LI}} - \frac{V_{MAX,MET} \times \frac{A_{LI}}{V_{LI} \times K_{P,LI}}}{K_{M,MET} + \frac{A_{LI}}{V_{LI} \times K_{P,LI}}} \end{aligned} \quad (2)$$

$$\frac{dA_{RM}}{dt} = Q_{RM} \times \frac{A_{BL}}{V_{BL}} - Q_{RM} \times \frac{A_{RM}}{V_{RM} \times K_{P, RM}} \quad (3)$$

Brain Compartments

Brain ECF is the key site of GHB pharmacodynamics [26,35,36]. MCT1 is the sole MCT isoform expressed at the BBB [37], and concentration-dependent uptake of GHB into immortalized rat (RBE4) and human (hCMEC/D3) brain capillary endothelial cells is best characterized by single transporter kinetics [22]. Uptake of GHB into the brain is given by Eqs. 4 and 5, where MCT1 mediates bidirectional transport of GHB [26] from the brain vasculature (BR, VS), across the BBB, and into the brain ECF (BR, ECF).

$$\frac{dA_{BR, VS}}{dt} = Q_{BR} \times \frac{A_{BL}}{V_{BL}} - Q_{BR} \quad (4)$$

$$\begin{aligned} & \times \frac{A_{BR, VS}}{V_{BR, VS}} - \frac{V_{MAX, UP} \times \frac{A_{BR, VS}}{V_{BR, VS}}}{K_{M, UP} + \frac{A_{BR, VS}}{V_{BR, VS}}} \\ & + \frac{V_{MAX, EF} \times \frac{A_{BR, ECF}}{V_{BR, ECF}}}{K_{M, EF} + \frac{A_{BR, ECF}}{V_{BR, ECF}}} \end{aligned}$$

$$\frac{dA_{BR, ECF}}{dt} = \frac{V_{MAX, UP} \times \frac{A_{BR, VS}}{V_{BR, VS}}}{K_{M, UP} + \frac{A_{BR, VS}}{V_{BR, VS}}} - \frac{V_{MAX, EF} \times \frac{A_{BR, ECF}}{V_{BR, ECF}}}{K_{M, EF} + \frac{A_{BR, ECF}}{V_{BR, ECF}}} \quad (5)$$

Physiologically Relevant Kidney Component

The semi-mechanistic kidney component was independently developed in an adaptive manner as described previously [23]. It consists of several compartments representing various lumen segments of the nephron containing the ultra-filtrate. These compartments do not represent physiological lumen space of nephron segments but ultra-filtrate within a specific nephron segment. Since fluid filtration and reabsorption across the nephrons are continuous processes, decreases in the volumes and flows of the filtrate across the nephron segments are described as constant, proportional in degree, and equal in magnitude, both, spatially and temporally. In other words, the numerical values of the flows and the volumes of the filtrate at given nephron segments would be the same and can be described as fractions of GFR, reflecting the volume of the filtrate just prior to its respective fractional

reabsorption in that segment. The fractional fluid reabsorption from each segment is characterized by a decrease in the flow exiting that segment. The physiologically relevant assumptions for the kidney component are the following: (1) fractions of fluid reabsorption and urine pH across the nephrons are identical in all animals, (2) fractions of fluid reabsorption are identical between rats and humans, (3) and the numerical value of flow exiting one segment is the numerical value of the volume of the next segment accounting for the volume remaining in the latter segment after fractional fluid reabsorption in the former [23].

The blood flow to the kidneys (Q_{KI}) carries GHB to the glomerulus (GLM), where a fraction of Q_{KI} becomes the GFR and the remaining fraction drains into the peritubular capillaries as:

$$\frac{dA_{GLM}}{dt} = Q_{KI} \times \frac{A_{BL}}{V_{BL}} - GFR \times \frac{A_{GLM}}{V_{GLM}} - (Q_{KI} - GFR) \times \frac{A_{GLM}}{V_{GLM}} \quad (6)$$

The model assumes that 67% of the total filtrate is reabsorbed from the proximal tubule (PT), where 66.7% gets reabsorbed from the S1 segment (divided into three subsegments, $S1_1$, $S1_2$, and $S1_3$) and 33.3% from S2+S3 segment as given by Eqs. 7–10 [23,29–31].

$$\frac{dA_{S1_1}}{dt} = GFR \times \frac{A_{GLM}}{V_{GLM}} - Q_{S1_2} \times \frac{A_{S1_1}}{V_{S1_1}} \quad (7)$$

$$\frac{dA_{S1_2}}{dt} = Q_{S1_2} \times \frac{A_{S1_1}}{V_{S1_1}} - Q_{S1_3} \times \frac{A_{S1_2}}{V_{S1_2}} \quad (8)$$

$$\frac{dA_{S1_3}}{dt} = Q_{S1_3} \times \frac{A_{S1_2}}{V_{S1_2}} - Q_{S2+S3} \times \frac{A_{S1_3}}{V_{S1_3}} \quad (9)$$

Uptake of GHB is pH- and sodium-dependent in brush border membrane (BBM) vesicles and pH-dependent in basolateral membrane (BLM) vesicles in rats [17], suggesting the involvement of sodium-dependent monocarboxylate transporter (SMCT1)-, MCT1-, and MCT2-mediated transport from PT lumen into renal blood [17]. MCT1 mediates uptake of GHB in rat kidney BLM membrane vesicles [17] and in human kidney (HK-2) cells [18]. MCT1 expression is observed at the BBM and BLM in rat kidney vesicles [17] and in HK-2 cells [18]. The model assumes predominantly MCT1-mediated reabsorption of GHB from

the S2+S3 segment into the peritubular capillaries containing the renal blood (RBL) as single active reabsorption process in Eqs. 10 and 11 as:

$$\frac{dA_{S2+S3}}{dt} = Q_{S2+S3} \times \frac{A_{S1-3}}{V_{S1-3}} - Q_{LOH} \quad (10)$$

$$\times \frac{A_{S2+S3}}{V_{S2+S3}} - \frac{V_{MAX,R} \times \frac{A_{S2+S3}}{V_{S2+S3}}}{K_{M,R} + \frac{A_{S2+S3}}{V_{S2+S3}}}$$

$$\frac{dA_{RBL}}{dt} = \frac{V_{MAX,R} \times \frac{A_{S2+S3}}{V_{S2+S3}}}{K_{M,R} + \frac{A_{S2+S3}}{V_{S2+S3}}} + (Q_{KI} - GFR) \quad (11)$$

$$\times \frac{A_{GLM}}{V_{GLM}} - (Q_{KI} - Q_U) \times \frac{A_{RBL}}{V_{RBL}}$$

The renal blood also receives GHB that is carried within the unfiltered fraction ($Q_{KI}-GFR$) exiting the glomerulus and returns to the systemic circulation along with the reabsorbed GHB. Moreover, the term $Q_{KI}-Q_U$ accounts for the flow balance in the system, where 98% of fluid is reabsorbed every minute, where urine flow (Q_U) is ~1–2% of GFR [23,29–31].

The model assumes that 33% of remaining fluid filtrate enters the LOH, where after 15% of fluid reabsorption (Eq. 12), about 18% of the total filtrate enters the lumen of the distal nephrons consisting mainly of the DT and the CD [23,29–31].

$$\frac{dA_{LOH}}{dt} = Q_{LOH} \times \frac{A_{S2+S3}}{V_{S2+S3}} - Q_{DT+CD} \times \frac{A_{LOH}}{V_{LOH}} \quad (12)$$

Effects of antidiuretic hormone (ADH)-mediated fluid reabsorption from late distal tubules and early collecting ducts were not considered in this model with the assumption that the hydration status of all animals was normal for the duration of this study [23,29,31,38]. The model assumes that about 16% of fluid reabsorption occurs from the CD+DT compartment (Eq. 13), which results in 2% of the filtrate as the urine flow (Q_U) and urine volume (V_U) per minute as described in Eq. 14 [23,29–31].

$$\frac{dA_{DT+CD}}{dt} = Q_{DT+CD} \times \frac{A_{LOH}}{V_{LOH}} - Q_U \times \frac{A_{DT+CD}}{V_{DT+CD}} \quad (13)$$

$$\frac{dA_U}{dt} = Q_U \times \frac{A_{DT+CD}}{V_{DT+CD}} - Q_U \times \frac{A_U}{V_U} \quad (14)$$

The cumulative amount of GHB excreted unchanged into the urine (A_E) was fitted to capture the saturable renal reabsorption process and facilitate the estimation of associated parameters (described in Eqs. 10 and 11), which is given by summation form:

$$\frac{dA_E}{dt} = Q_U \times \frac{A_U}{V_U} \quad (15)$$

The model outputs for the two PK endpoints of GHB are:

$$Y(C_{BL}) = \frac{A_{BL}}{V_{BL}} \quad (16)$$

$$Y(A_E) = A_E \quad (17)$$

Pharmacodynamics of GHB

The PD endpoint for GHB is decline in respiration frequency ($FREQ$) (breaths/min), also known as respiratory depression, which is mediated by direct effects of binding of GHB in brain ECF to GABA_b receptors [26]:

$$Y(FREQ) = E_0 \times \left\{ 1 - \frac{\left(I_{MAX} \times \frac{A_{BR,ECF}}{V_{BR,ECF}} \right)}{IC_{50} + \frac{A_{BR,ECF}}{V_{BR,ECF}}} \right\} \quad (18)$$

Data for PK/PD of GHB

Data for the two PK endpoints of GHB-plasma concentrations (C_{PL}) and cumulative amount excreted unchanged into the urine (A_E), and a PD endpoint, respiration frequency, in rats was obtained from Morse *et al.* [26]. In brief, all the studies were performed in male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 270–330 g ($N=3-4$ per dose group). Animals were allowed to acclimatize to the chambers before drug

administration, and respiration frequency was measured using whole-body plethysmography as described previously [26]. GHB was administered IV as 200, 600, or 1500 mg/kg bolus doses. Blood and urine samples were collected at regular intervals up to 8 h following GHB administration ($n = 3\text{--}4$ Sprague-Dawley rats per time point). The respiration frequency was also continuously recorded until 8 h ($n = 3\text{--}4$ Sprague-Dawley rats per time point). GHB plasma and urine concentrations were measured using a validated LC/MS/MS method published previously [26,39,40].

Model development and validation

Data from the following doses were used for model development: (1) GHB doses of 200, 600, and 1500 mg/kg for model development and (2) an independent dataset of GHB PK/PD after a 600-mg/kg IV bolus dose for model validation ($n = 3$ male Sprague-Dawley rats per time point). PK/PD modeling was performed using the maximum likelihood estimation algorithm in ADAPT V software (BMSR, Los Angeles, CA) [41]. Since blood concentrations of GHB are more relevant in a clinical setting, blood concentrations were calculated from plasma concentrations using the GHB B/P partition ratio of 0.75 [33]. Since GHB has negligible (1.2%) and concentration-independent protein binding, total and not unbound GHB concentrations are considered for the model development [14]. Mean values of GHB concentrations in blood, amount of GHB excreted unchanged into urine, and breathing frequency data were simultaneously utilized for PK/PD model fitting.

For GHB PK/PD (Table I), (1) metabolic MM affinity constant ($K_{M,MET}$) was fixed to 63 $\mu\text{g/mL}$ [14,23,32]; (2) MM affinity constant for renal reabsorption ($K_{M,R}$) was fixed to a value of 228 $\mu\text{g/mL}$, which is the K_M of MCT1-mediated uptake of GHB into rat erythrocytes at a pH of 6.5 [33]; (3) MM affinity constant for uptake into the brain ($K_{M,UP}$) was fixed to 1882 $\mu\text{g/mL}$, which is the K_M of GHB uptake into hCMEC/D3 cells [22]; (4) MM affinity constant for efflux out of the brain ($K_{M,EF}$) was fixed to a previously estimated value of 63 $\mu\text{g/mL}$ [26]; (5) Maximum inhibitory effect of GHB on respiration frequency (I_{MAX}) was fixed to 1, as complete cessation of respiration is reported at very high concentrations of GHB [26]; (6) The inhibitory constant for GHB on respiration frequency (IC_{50}) was fixed to 82.8 $\mu\text{g/mL}$, which represents the reported value (K_M) for binding of GHB to GABA_b receptors [26,34]; and (7) baseline respiration frequency (E_0) was fixed to the observed dose-specific values.

Maximal metabolic capacity ($V_{MAX,MET}$) was estimated since GHB is metabolized by several enzymes, namely, aldo-keto reductases 1A1 and 7A2, succinic semialdehyde reductase, and hydroxyacid-oxoacid transhydrogenase [42], for which the protein expression and relative contribution of these enzymes to the metabolism of GHB are not well-understood. Maximal renal reabsorption capacity ($V_{MAX,R}$), maximal capacity of uptake into brain ($V_{MAX,UP}$), and maximal capacity of efflux out of brain ($V_{MAX,EF}$) were estimated. Moreover, GHB blood-to-tissue partition coefficients for liver and remainder compartment were also estimated to better capture the PK/PD of GHB. GHB blood concentrations below the lower limit of quantification (LLQ = 1 $\mu\text{g/mL}$) were estimated using the M3 method in ADAPT V [41]. Constant, proportional, and the combined constant and proportional error models were tested to capture the residual variability. Model selection

and performance were evaluated using visual inspection of observed vs. model predicted data and standard goodness-of-fit criteria including Akaike Information Criterion, Schwarz Criterion, model diagnostic plots, and coefficient of variation ($CV\% = SE/Mean \times 100\%$, where SE is standard error of the mean estimate) as performed previously [23,26]. The residual variability for all PK/PD endpoints was best described by the combined, constant and proportional, error model, where i represents the predicted value:

$$Var(i) = (\sigma_I + \sigma_S \times Y_i)^2 \quad (19)$$

Several model modifications were implemented and the model performance was evaluated (using the abovementioned criterion) in order to select the final model: (1) altering number of proximal tubule S1 sub-compartments from 1 to 5; three sub-compartments were deemed optimal as less than three compartments resulted in rapid accumulation of A_E (or severe overprediction), whereas greater than three compartments resulted in slower accumulation of A_E (or severe underprediction). (2) Separate S2 and S3 compartments (resulted in slow accumulation of A_E or severe underprediction and overprediction of blood concentrations; similar observation with separate DT and CD compartments). (3) K_P value for GHB partition in to the brain vasculature (the estimated K_P value was close to one; hence, this parameter was not included).

The PK/PD model was validated using an independent dataset for 600 mg/kg GHB that was not used for model development. PK/PD profiles were simulated in ADAPT V software (BMSR, Los Angeles, CA) using the model-estimated parameter values. Due to the complex dose-dependent kinetics of GHB including nonlinear renal and brain transport kinetics, and saturable hepatic metabolism, concentration predictions for different doses are not straightforward (as expected for a drug displaying linear and doseindependent PK over a wide dose range), making independent datasets at doses outside of the dose range evaluated in this study (200–1500 mg/kg) not suitable as validation sets [23,26]. Moreover, LD₅₀ of GHB is 1750 mg/kg in rats [9]; therefore, the dose range evaluated in this study is in fact optimal for characterizing toxicokinetics and toxicodynamics of GHB.

Evaluation of Physiological Relevance of Mechanistic Brain Model

ECF profiles for the doses 200–1500 mg/kg were simulated by fixing all parameters to their literature or estimated values. These profiles were utilized to obtain unbound GHB brain ECF/blood partition coefficients ($K_{p,u,u}$) given negligible plasma protein binding of GHB, as demonstrated previously [14]. $AUC_{0-\infty}$ values were obtained using the NCA feature in PKSolver add-on package in MS Excel [43].

Sensitivity Analysis

A sensitivity analysis was performed to assess the overall stability of the model and to identify the most sensitive parameters that contribute to the PK/PD of GHB. Physiologically relevant parameters were not included in the sensitivity analysis. The parameters describing metabolism and transport affinity/capacity, K_M, MET , K_M, R , K_M, UP , K_M, EF , $V_{MAX, MET}$, $V_{MAX, R}$, $V_{MAX, UP}$ and $V_{MAX, EF}$ and tissue partitioning (K_{PLB} , K_{PRM}) of GHB were

included in the sensitivity analysis. Simulations were performed perturbing the parameters individually ± 1 and ± 3 SE using ADAPT V software (BMSR, Los Angeles, CA). SEs for K_M parameters were obtained from literature, with the exception of $K_{M,R}$, which did not have a reported SE or CV% [33]. For this parameter, a CV% of 20% was utilized for the analysis as this is a typical CV% indicating an acceptable fit of a parameter. For the remaining parameters (V_{MAX} , K_P), SE was calculated using the CV% obtained from fitting; these are reported in Table II. The impact of these perturbations on blood $AUC_{0-\infty}$, CL_R , and maximum decrease in the breathing frequency relative to baseline (E_{MAX}) was evaluated. $AUC_{0-\infty}$ values were obtained using the NCA feature in PKSolver add-on package in MS Excel [43]. CL_R was calculated as the ratio of amount of GHB excreted unchanged into urine at time infinity ($A_{e,\infty}$) and $AUC_{0-\infty}$. E_{MAX} values were determined by subtracting the lowest value of breathing frequency from the dose-specific baseline breathing frequency (84, 88, and 85 breaths/min for the 200-, 600-, and 1500-mg/kg doses, respectively). Parameters were deemed sensitive for an endpoint, if the endpoint was altered $\pm 20\%$ or greater.

RESULTS

PK/PD of GHB

PK/PD of GHB was best characterized using a semi-mechanistic and physiologically relevant modeling framework incorporating (1) MCT1-mediated renal reabsorption and uptake into the brain ECF and (2) respiratory depression induced by direct effects of binding of GHB in brain ECF to GABA_b receptors, as illustrated in Fig. 1. All model parameters are estimated with reasonable CV% as detailed in Table II.

GHB C_{BL} vs. time profiles in Fig. 2a show that the model reasonably captured the GHB C_{BL} data for all doses. There is a very strong agreement between the observed and the predicted values of C_{BL} ($R^2 > 0.90$). The cumulative A_E of GHB is shown in Fig. 2b, with good agreement between the observed and the predicted data ($R^2 > 0.8$). A_E at 60 min for the 200-mg/kg dose was significantly overpredicted, whereas A_E at 60 min for 600- and 1500-mg/kg doses was moderately underpredicted. However, A_E at later time points was well captured at all doses, which resulted in the estimate of the $V_{MAX,R}$ parameter with reasonable CV%. Introducing nonlinear MM kinetics for uptake and efflux of GHB into/out of brain ECF facilitated the prediction of GHB-induced and GHB dose-dependent decline in respiration frequency (respiratory depression), which was reasonably captured ($R^2 > 0.7$) by the model as illustrated in Fig. 2c. Moderate overestimation of PD until 120 min for 200-mg/kg dose was observed. The estimated value of $V_{MAX,MET}$ (730 $\mu\text{g}/\text{min}$) is comparable to previously reported values of 581 $\mu\text{g}/\text{min}$ [39], 680 $\mu\text{g}/\text{min}$ [14], and 670 $\mu\text{g}/\text{min}$ [23]. The estimated value of $V_{MAX,R}$ (2237 $\mu\text{g}/\text{min}$) is similar to 2808 $\mu\text{g}/\text{min}$ [26] and 2340 $\mu\text{g}/\text{min}$ [40]. Values of $V_{MAX,UP}$ and $V_{MAX,EF}$ are also estimated with low CV% and are in agreement with our previous evaluation [26]. Additional model diagnostic plots including the observed vs. predicted data plots and standardized residuals vs. predicted data plots are illustrated in supplementary Figs. S1 and S2, respectively. Observed vs. predicted data for (a) blood concentrations, (b) cumulative amount excreted unchanged into urine (A_E), and (c) breathing frequency (respiratory depression) are distributed evenly about the line of unity as

illustrated in Fig. S1a–c, respectively. The model (Fig. S1c) generally overpredicted the highly dynamic return-to-baseline phase of the breathing frequency. Standardized residuals vs. predicted data for (a) blood concentrations, (b) cumulative amount excreted unchanged into urine (A_E), and (c) breathing frequency (respiratory depression) are distributed evenly about the line of unity as illustrated in Fig. S2a–c, respectively. The absence of any outstanding trends indicates that the residual error model was appropriate. The majority of data points fell within ± 2 standard deviation of the standardized residuals for all three outputs as illustrated in Fig. S2a–c. Overall, our proposed modeling framework adequately described GHB PK/PD.

Model Validation

Results of the model validation using a separate data set for the administration of GHB 600 mg/kg are shown in Fig. 3. Simulated profiles of C_{BL} (Fig. 3a), A_E (Fig. 3b), and $FREQ$ (Fig. 3c) are in strong agreement with the observed data indicating that the model has a strong quantitative power and it was successfully validated.

Evaluation of Physiological Relevance of Mechanistic Brain Model

Table III shows the obtained $K_{p,u,u}$ values which agree well with values that have been calculated using experimental ECF values obtained with brain microdialysis, with these values ranging from 0.08 to 0.13 [22,44]. While the simulated ECF/blood AUC ratios demonstrated a trend towards an increase with dose, the change was small and would be determined by the nonlinear CL_R and by uptake and efflux at the BBB.

Sensitivity Analysis

Overall, blood $AUC_{0-\infty}$, CL_R , and E_{MAX} were not altered by greater than $\pm 20\%$ with perturbations in parameters evaluated (± 1 and ± 3 SE), indicating the model is stable (Table IV and Figs. 4 and 5 and S3–S5). The parameters describing GHB transport across BBB (uptake and efflux) were only found to be sensitive with respect to E_{MAX} when changes in the parameters were ± 3 SE (Table IV and Figs. S3 and S4). In general, the impact of perturbations in these parameters ($K_{M,EF}$, $K_{M,UP}$, $V_{MAX,EF}$, $V_{MAX,UP}$) increased as the dose was decreased (Table IV and Figs. S3 and S4).

GHB hepatic metabolism was also found to be stable within the model. Perturbations in the capacity parameter for metabolism ($V_{MAX,MET}$) exhibited no sensitivity, while the affinity parameter ($K_{M,MET}$) showed some sensitivity with respect to $AUC_{0-\infty}$ or when $K_{M,MET}$ was altered ± 3 SE (Table IV and Fig. S5). Moreover, the impact of these alterations on the endpoints increased as the dose decreased (Table IV and Fig. S5). The parameters describing the tissue partitioning of GHB produced converse results; no sensitivity in endpoints was observed when K_{PRM} was perturbed, while each endpoint was moderately sensitive to changes in K_{PLI} parameter (Table IV and Fig. 4). Endpoints were greatly sensitive only when K_{PLI} was decreased by 3 SE and was the most pronounced for the 600-mg/kg dose for CL_R and E_{MAX} (Table IV and Fig. 4). $AUC_{0-\infty}$ increased when K_{PLI} was decreased by 3 SE (Table IV and Fig. 4). The affinity parameter for GHB renal reabsorption showed some sensitivity with respect to CL_R (Table IV and Fig. 5). The change in CL_R was greater than 20% only when K_{MR} was perturbed ± 3 -fold, and this change was greater at lower doses

(Table IV and Fig. 5). Changes in CL_R were most sensitive to perturbation in $V_{MAX,R}$ parameter (Table IV and Fig. 5). CL_R was altered greater than 20% for all doses when $V_{MAX,R}$ was set to ± 3 SE.

DISCUSSION

To our best knowledge, this is the first report of a semimechanistic and physiologically relevant model on the pharmacokinetics and pharmacodynamics of the drug of abuse, γ -hydroxybutyric acid. GHB has a high abuse potential, which results in severe respiratory depression and cardiac arrest in overdose patients [7–9]. Postmortem concentrations of GHB in blood are reported to be as high as 4400 mg/L [8].

The proposed modeling framework consists of a PK/PD model for GHB consisting of nonlinear metabolism, MCT1-mediated renal reabsorption with physiologically relevant concurrent fluid reabsorption and uptake into the brain, and direct effects of binding of GHB (in brain ECF) to GABA_B receptors on respiration frequency in rats. The model utilized *in vitro* GHB-MCT1 transport kinetics as well as the *in vivo* PK/PD data in rats, which significantly minimized the number of estimated parameters, thereby ensuring that the model was not overparameterized. For the evaluation of PK/PD of GHB, all MM affinity constants (K_M) were fixed to previously reported values to ensure model stability and to circumvent any bias that could arise from potential covariance and correlation between MM capacity (V_{MAX}) and K_M terms during parameter estimation [23]. The fixed value of $K_{M,R}$ (228 $\mu\text{g/mL}$), which represents the K_M value for MCT1-mediated uptake of GHB into rat erythrocytes at a pH of 6.5 [33], is similar to the K_M (480 $\mu\text{g/mL}$) of GHB uptake into rMCT1-MDA-MB231 cells at a pH of 6.0 [17] and the K_M value (215 $\mu\text{g/mL}$) of GHB uptake into HK-2 cells at a pH of 6.0 [18]. These similarities are in agreement with our assumption that MCT1 plays a predominant role in the renal reabsorption of GHB. The fixed value of $K_{M,UP}$ (1882 $\mu\text{g/mL}$), the K_M of GHB uptake into hCMEC/D3 cells [22], is comparable to the K_M (1768 $\mu\text{g/mL}$) of MCT1-mediated uptake of GHB into rat erythrocytes at a pH of 7.4 [33] and the K_M (2423 $\mu\text{g/mL}$) of GHB uptake into RBE4 cells at a pH of 7.4 [22].

Differences in the values of $K_{M,UP}$ and $K_{M,EF}$ indicate potential differences in intra- and extracellular pH and/or involvement of other transporters, which is currently not well-understood [26]. Fixing the I_{MAX} and IC_{50} values (for effects of GHB on respiration frequency) facilitated the establishment of direct relationships between brain ECF concentrations of GHB and its PD effects, as brain ECF concentrations of GHB are functions of its nonlinear uptake and efflux kinetics. This also facilitated the modeling of PD data in the absence of GHB concentrations in brain ECF [26]. The estimated value of GHB K_{PLI} is 10.5, which suggests significant distribution of GHB in the liver, which is consistent with liver being the major site of GHB metabolism.

Overall, the model exhibited stability for the majority of the parameters and endpoints. Sensitivity was minimally observed for most parameters, but significant alterations in relevant endpoints were observed when parameters were altered ± 3 SE. For instance, the parameters describing GHB brain transport ($K_{M,EF}$, $K_{M,UP}$, $V_{MAX,EF}$, $V_{MAX,UP}$) were only

sensitive with respect to the PD endpoint E_{MAX} . This is expected, as these parameters govern the ECF concentrations of GHB. The impact of altering the metabolism-related parameters $V_{MAX,MET}$ and $K_{M,MET}$ on $AUC_{0-\infty}$ and K_{PLI} on all three endpoints is consistent with the nonlinear PK properties of GHB. GHB has been shown to exhibit saturable metabolism; therefore, changes in the parameters describing the metabolism of GHB and its partitioning into the metabolic organ (liver) are expected to impact the PK and therefore PD of GHB [40]. The nonlinearity of GHB PK also explains the observed sensitivity for the parameters describing active renal reabsorption of GHB ($V_{MAX,R}$; $K_{M,R}$). GHB exhibits saturable renal reabsorption and therefore when the capacity for transport is decreased, CL_R increases; the converse is also true, i.e., CL_R decreases, when transport capacity is increased.

The contribution of CL_R to total CL increases as the dose of GHB is increased due to the presence of high concentrations of GHB, which saturate the active renal reabsorption of the GHB in the kidney. Therefore, the impact of changes in CL_R only affects $AUC_{0-\infty}$ when CL_R significantly contributes to the total CL , which was observed in the sensitivity analysis. The sensitivity of CL_R with respect to reabsorption parameters is consistent with our previous studies [45]. In summary, the sensitivity analysis demonstrated that the model was stable and any changes in the endpoints evaluated are in accordance with the established PK/PD profile of GHB.

While the simulated ECF/blood AUC ratios showed a trend towards an increase with dose, the change was small and might be explained by the nonlinear transport across BBB. GHB uptake into and efflux out of the brain is characterized by low affinity/high capacity and high affinity/low capacity transport processes, respectively. This would lead to increasing GHB exposure into brain ECF with increasing doses of GHB.

While the model was able to capture the PK for all doses, there was slight overestimation for the lowest dose of GHB up to 120 min (Fig. 2a). This is likely due to an initial overprediction of CL_R , which is supported by the initial overprediction of A_e , but $A_{e,\infty}$ was adequately predicted for the lowest dose (Fig. 2b). This may be due to the contribution of additional transporters in GHB renal reabsorption. Currently, the model includes reabsorption via MCT1, as this is the dominant transporter for GHB transport at this site. However, the contribution of sodium-dependent transport, likely mediated by the SMCT1, has also been demonstrated in rat brush border membrane vesicles [17]. SMCT1 is a high affinity/low capacity transporter for GHB with a reported K_M of 0.68 mM (70.8 μ M, over threefold lower than MCT1) [16] and may contribute to active renal reabsorption of GHB at lower doses. SMCT-mediated reabsorption was not included in the model as its quantitative contribution to overall active renal reabsorption of GHB is not known; it is known that SMCT1 has a much lower capacity and therefore its contribution to GHB reabsorption is likely to be minor after high GHB doses [16,33]. MCT1-mediated transport, being the predominant transport process, is sufficient to capture the overall PK of GHB and thus only MCT1-mediated transport was considered in this model.

The main limitation of the current model is that the limited *in vivo* experimental data on GHB ECF concentrations (obtained from the frontal cortex) could not be utilized in the

model development, since we have no PD data for these experiments. PD data is needed since, in the microdialysis experiments, GHB clearance was significantly decreased compared to animals without microdialysis probes, resulting in higher plasma concentrations for the doses examined, compared with the animals used in the PD studies [22]. Determination of the model simulated GHB concentrations in ECF found that they were initially overpredicted, followed by systematically lower concentrations (underpredicted), consistent with the lower clearance of GHB in the microdialysis experiments (Fig. S6) [22]. The systemic underprediction is at least partially explained by the impact of the microdialysis surgery utilized to obtain ECF concentrations [22], although it may also reflect the site of ECF collection which may differ from the relevant ECF concentrations associated with GABA_b binding and toxicity. The differences between predicted ECF concentrations at the relevant physiological sites and our limited ECF data obtained in the frontal cortex demonstrate the complexity of GHB PK/PD and highlight the need for continued investigations to incorporate physiological relevant GHB distribution. Additional limitations of this model include the following: (1) the impact of urine pH and hydration status on active renal reabsorption of GHB; (2) impact of GABA_b receptor expression, turnover, and micro binding kinetics (i.e., K_{ON} , K_{OFF} , etc.) on PD of GHB; and (3) uptake and efflux of GHB in and out of the brain cells on GHB ECF concentrations and GHB PD.

Taken together, model fittings, parameter estimates, and validation study results indicate that the model has a strong quantitative power and PK/PD of GHB was reasonably captured. In our future studies, we will expand the current model to quantitatively evaluate the inhibition of MCT1-mediated renal reabsorption and uptake into brain of GHB as a potential treatment strategy in GHB overdose. In conclusion, we developed and validated a semi-mechanistic and physiologically relevant PK/PD model of GHB using *in vitro* drug-transporter kinetics and *in vivo* PK/PD data in rats.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

A semi-mechanistic PK/PD model elucidating MCT1-mediated active renal reabsorption and brain uptake of GHB. **a** Overview of PK/PD model for GHB. **b** Mechanistic kidney component for GHB. **c** Mechanistic brain component for GHB. Symbols are defined in Tables I and II

Fig. 2. GHB PK/PD model fittings. **a** Blood concentrations. **b** Cumulative amount excreted unchanged into urine. **c** Breathing frequency (respiratory depression). *Symbols and lines* represent observed data (mean $\pm$ SD, $n = 3-4$) and model fittings

Fig. 3. GHB PK/PD model validation. **a** Blood concentrations. **b** Cumulative amount excreted unchanged into urine. **c** Breathing frequency (respiratory depression). *Symbols* and *lines* represent observed data (mean $\pm$ SD, $n = 3-4$) and model fittings

Fig. 4. Sensitivity analysis for the parameters describing GHB tissue partitioning in the kidney and remainder compartments. Simulations were performed holding all parameters constant, while modifying the parameter of interest ± 1 and 3 standard error (SE)

Fig. 5.
Sensitivity analysis for the affinity ($K_{M,R}$) and capacity ($V_{MAX,R}$) for GHB reabsorption in the proximal tubules. Simulations were performed holding all parameters constant, while modifying the parameter of interest ± 1 and 3 standard error (SE)

Table I.

List of Fixed Physiological and GHB-Specific Parameters Utilized for the PK/PD Model

Parameter	Definition	Value	Ref.
V_{BL} (mL)	Volume of blood	20.3	[24,25]
V_{RM} (mL)	Volume of remainder compartment	265	[24,25]
V_{LI} (mL)	Volume of liver	12.4	[24,25]
V_{BK} v_s (mL)	Volume of brain vascular space	0.08	[24,25]
V_{BK} ECF (mL)	Volume of brain extracellular fluid	0.35	[26]
Q_{LI} (mL/min)	Blood flow to liver	11.6	[24,25]
Q_{KI} (mL/min)	Blood flow to kidneys	10.5	[24,25]
Q_{RM} (mL/min)	Blood flow to remainder compartment	27.1	[24,25]
Q_{BK} (mL/min)	Blood flow to brain	1.53	[24,25]
V_{GLM} (mL)	Volume of glomerulus	0.08	[27]
V_{RBL} (mL)	Volume of renal blood	0.375	[27]
GFR (mL/min)	Glomerular filtration rate	2.2	[28]
$V_{SL,1}$ (mL), $Q_{SL,1}$ (mL/min)	Volume and flow of filtrate in lumen of 1st sub-segment of S1 segment of proximal tubule	GFR	
$V_{SL,2}$ (mL), $Q_{SL,2}$ (mL/min)	Volume and flow of filtrate in lumen of 2nd sub-segment of S1 segment of proximal tubule	$0.85 \times GFR$	[29–31]
$V_{SL,3}$ (mL), $Q_{SL,3}$ (mL/min)	Volume and flow of filtrate in lumen of 3rd sub-segment of S1 segment of proximal tubule	$0.70 \times GFR$	[29–31]
$V_{S2,S3}$ (mL), $Q_{S2,S3}$ (mL/min)	Volume and flow of filtrate in lumen of S2 and S3 segments of proximal tubule	$0.55 \times GFR$	[29–31]
V_{LOH} (mL), Q_{LOH} (mL/min)	Volume and flow of filtrate in lumen of loop of Henle	$0.33 \times GFR$	[29–31]
$V_{DT,CD}$ (mL), $Q_{DT,CD}$ (mL/min)	Volume and flow of filtrate in lumen of distal tubules and collecting ducts	$0.18 \times GFR$	[29–31]
V_U (mL), Q_U (mL/min)	Volume and flow of urine	$0.02 \times GFR$	[29–31]
Parameters for GHB			
$K_{M, MET}$ (μ g/mL)	Metabolic Michaelis-Menten affinity constant	63	[14,23,32]
$K_{M, R}$ (μ g/mL)	Renal reabsorption Michaelis-Menten affinity constant	228	[33]
$K_{M, UP}$ (μ g/mL)	Michaelis-Menten affinity constant for uptake into brain	1882	[22]
$K_{M, EF}$ (μ g/mL)	Michaelis-Menten affinity constant for efflux out of brain	63	[26]
I_{MAX}	Maximum inhibitory effect of GHB on breathing frequency	1	[26]
IC_{50} (μ g/mL)	Affinity constant for inhibitory effect of GHB on breathing frequency	82.8	[26,34]

Parameter	Definition	Value	Ref.
E_T (breaths/min)	Baseline breathing frequency (fixed to observed values)		
200 mg/kg GHB			84
600 mg/kg GHB			88
1500 mg/kg GHB			85

Physiological flow and volume parameters were obtained and optimized for 300-g rats

Ref. references, *GHB* γ -hydroxybutyric acid

Estimated PK/PD Parameters for GHB

Table II.

Parameter	Definition	Estimate	CV%
$V_{MAX,MET}$ (µg/min)	Maximal metabolic capacity	730	7.2
$V_{MAX,R}$ (µg/min)	Maximal renal reabsorption capacity	2237	12
$V_{MAX,UP}$ (µg/min)	Maximal capacity of uptake into brain	12.8	11
$V_{MAX,EF}$ (µg/min)	Maximal capacity of efflux out of brain	6.34	10
$K_{P,LI}$	GHB blood-to-tissue partition coefficient for liver	10.5	18
$K_{P,RM}$	GHB blood-to-tissue partition coefficient for remainder compartment	0.12	11
$\sigma_S(C_{BL})$	Slope of the residual variability of blood concentrations	0.11	9.2
$\sigma_S(A_E)$	Slope of the residual variability of cumulative amount excreted into urine	0.33	14
$\sigma_S(FREQ)$	Slope of the residual variability of breathing frequency	0.05	7.8
$\sigma_I(C_{BL})$ (µg/mL)	Intercept of the residual variability of blood concentrations	2.71	35
$\sigma_I(A_E)$ (µg)	Intercept of the residual variability of cumulative amount excreted into urine	0.93	26
$\sigma_I(FREQ)$ (breaths/min)	Intercept of the residual variability of breathing frequency	0.01	Fixed

Table III.Calculated ECF/Blood Partition Coefficients ($Kp_{u,u}$)

Dose (mg/kg)	$AUC_{0-\infty}$ blood ($\mu\text{g/mL} \times \text{min} \times 10^4$)	$AUC_{0-\infty}$ ECF ($\mu\text{g/mL} \times \text{min} \times 10^4$)	$Kp_{u,u}$
200	2.05	0.160	0.078
400	5.80	0.501	0.086
600	10.4	1.01	0.097
800	15.0	1.62	0.107
1000	19.6	2.33	0.119
1500	30.0	4.53	0.151

Table IV.

Parameters and Conditions with Exhibited Sensitivity with Respect to One or More Endpoints

Parameter	Dose	±SE	$AUC_{0-\infty}$	CL_R	E_{MAX}
% change					
K_{PLI}	200	-3	28.7	7.68	19.6
K_{PLI}	600	-3	15.8	38.3	21.0
K_{PLI}	1500	-3	-2.98	34.6	6.77
K_{MR}	200	-3	4.55	-56.5	0.876
K_{MR}	600	-3	11.3	-41.0	1.57
K_{MR}	200	3	-3.58	50.0	-0.705
K_{MR}	600	3	-6.88	32.3	-0.767
$V_{MAX,R}$	200	-3	-5.26	71.0	-1.36
$V_{MAX,R}$	600	-3	-16.9	81.2	-3.05
$V_{MAX,R}$	1500	-3	-20.6	50.0	-2.59
$V_{MAX,R}$	600	-1	-5.01	20.9	-0.98
$V_{MAX,R}$	200	3	2.40	-29.5	0.539
$V_{MAX,R}$	600	3	11.4	-37.4	2.66
$V_{MAX,R}$	1500	3	23.8	-32.1	1.97
K_{MEF}	200	-3	0.006	0.470	-45.5
K_{MEF}	600	-3	0.213	0.605	-36.7
K_{MEF}	200	3	-0.006	0.466	28.1
$V_{MAX,EF}$	200	-3	-0.005	0.467	26.2
$V_{MAX,EF}$	600	-3	-0.135	0.653	29.0
$V_{MAX,EF}$	200	3	0.002	0.469	-20.9
$K_{M,UP}$	200	-3	-0.011	0.464	59.9
$K_{M,UP}$	600	-3	0.003	0.626	43.0
$K_{M,UP}$	200	3	0.003	0.469	-28.7
$K_{M,UP}$	600	3	0.023	0.650	-22.7
$V_{MAX,UP}$	200	-3	0.004	0.469	-33.0
$V_{MAX,UP}$	600	-3	0.024	0.652	-31.6
$V_{MAX,UP}$	1500	-3	1.04	-0.155	-27.9
$V_{MAX,UP}$	200	3	-0.004	0.466	31.1
$V_{MAX,UP}$	600	3	0.013	0.634	27.1
$K_{M,MET}$	200	-3	-21.0	-0.794	-0.971
$K_{M,MET}$	200	3	48.7	-2.36	0.888
$K_{M,MET}$	600	3	23.0	-5.93	0.681

Values are in bold to highlight the instances of sensitivity that the endpoint changed greater than or equal to 20%