Published in final edited form as:

Anesthesiology. 2018 June ; 128(6): 1107–1116. doi:10.1097/ALN.0000000000002157.

Pharmacokinetic/Pharmacodynamic Model of CW002, an Investigational Intermediate Neuromuscular Blocking Agent, in Healthy Volunteers

Josh D. Kaullen, Pharm.D.¹, Joel S. Owen, Ph.D.², Kim L.R. Brouwer, Pharm.D., Ph.D.¹, Paul M. Heerdt, M.D., Ph.D.³, Cynthia A. Lien, M.D.⁴, John J. Savarese, M.D.⁴, and Virginia D. Schmith, Ph.D.⁵

¹UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill

²Union University

³Yale Medical School

⁴Weill Cornell Medical College

⁵Nuventra Pharma Sciences

Abstract

Background—CW002 is an investigational non-depolarizing, neuromuscular blocking agent with a rapid onset and intermediate duration of action in animals. This is a single ascending dose, healthy subject study exploring tolerability, pharmacokinetics, and potency.

Methods—Population PK (PopPK) and PK/pharmacodynamic (PK-PD) models were developed using plasma drug concentration data from a previously published dose-response study in 28 healthy subjects receiving single doses of CW002 during sevoflurane anesthesia. Subjects included in the models were from five different dose cohorts (Cohorts 3, 4, 5, 6, and 8 receiving 0.04, 0.06, 0.08, 0.10, and 0.14 mg/kg, respectively). Serial arterial plasma concentrations and muscle twitch heights were monitored.

Results—A 4-compartment model was fit to the concentration-time data, while a transit compartment with a sigmoid Emax model was fit to the PK-PD data. The PopPK of CW002 was linear with very low inter-individual variability in clearance (10.8%). Simulations were conducted to predict the onset and offset of effect at 2×, 3×, and 4× ED₉₅. The time to 80% block was predicted to be 1.5, 0.8, and 0.7 min for 2×, 3×, and 4× ED₉₅ doses, respectively. The simulated 25–75% recovery index was independent of dose.

Correspondence. Name: Virginia D Schmith, Ph.D., FCP, Address: Nuventra Pharma Sciences, Inc., 2525 Meridian Parkway, Suite 280, Durham, NC 27713, Telephone number: 919-260-7870, gschmith@nuventra.com.

Clinical Trial Number and registry URL: NCT01338935 <https://clinicaltrials.gov/ct2/show/NCT01338935?term=NCT01338935&rank=1>

Prior Presentations: ASCPT, March 17, 2017, Washington D.C.

Conflict of interests: Dr. Savarese is the inventor of CW002 and Drs. Heerdt and Savarese are the inventors of cysteine formulations for reversal of CW002. The patents for the molecules are held by Weill Cornell Medical College, New York, NY. Dr. Owen served as a paid consultant for the population PK analysis.

Conclusions—CW002 has predictable PK and is likely to have a rapid onset with an intermediate duration of action at 3×ED95. This model provides information to inform critical decisions (e.g., dose, study design) for continued development of CW002.

Introduction

Neuromuscular blockade is imperative for patients who require complete muscular relaxation during surgery, prior to rapid or routine endotracheal intubation, or prior to and during mechanical ventilation. CW002 is an investigational non-depolarizing neuromuscular blocking (NMB) agent with a rapid onset and intermediate duration of action. CW002 exhibits rapid reversal upon administration of L-cysteine HCl in animals^{1,2}, in addition to inactivation by endogenous cysteine.

The primary focus of this study was to collect safety, PK, and dose-response data in healthy subjects (not undergoing surgery) receiving escalating single doses of CW002. While the safety (including hemodynamic stability) and neuromuscular blockade data (including the estimated effective dose producing 95% neuromuscular blockade, ED95 of 0.077 mg/kg) were reported previously³, the present manuscript describes the noncompartmental PK of CW002 in humans from this study, the development of a PopPK model to predict plasma concentrations over time after various doses, and the development of a PK-PD model to describe the relationship between concentration and neuromuscular blockade. This PK-PD model, with the collection of serial blood samples and intensive measurement of neuromuscular blockade over a wide range of doses, allowed a better understanding of the delay between plasma concentrations and neuromuscular blockade as well as inter-individual variability (IIV) and unexplained variability in the PK-PD of CW002. Simulations were conducted to predict efficacy results under a variety of dosing regimens not studied, to answer clinical drug development questions, and to help in the design of future clinical studies. Therefore, the purpose of the analyses detailed below was to describe the PK and PopPK of CW002, develop a PK-PD model for CW002, and simulate the time to onset and offset of neuromuscular blockade at higher doses that may be administered to patients who require complete neuromuscular blockade for their surgical procedures.

Materials and Methods

Study Recruitment

This single ascending dose study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01338935) NCT01338935) was approved by the Institutional Review Board of Weill Cornell Medical Center³. Thirty-four healthy male and female subjects ranging from 18–55 years old were recruited for the study between October 2012 and October 2013. Exclusion criteria included significant chronic disease, tobacco use, weight 30% above or below ideal body weight, a history of drug use, allergies, or a history of difficult tracheal intubation. Female subjects were using an accepted form of birth control if they were of child bearing potential. Female subjects were not pregnant during the conduct of this study. All subjects underwent a screening physical assessment within 14 days of the study to confirm the absence of cardiopulmonary abnormalities (based on history, physical exam and ECG), unremarkable airway anatomy, and normal laboratory data (chemistry panel, urine analysis and hematology). All laboratory tests were repeated the

morning prior to dosing, and for female participants, a negative urine pregnancy test was confirmed. All subjects gave written, informed consent before participation in the study.

Subjects were assigned sequentially into seven cohorts of six subjects, each receiving a single CW002 dose of 0.02 (cohort 1), 0.04 (cohort 2), 0.04 (cohort 3, repeated), 0.06 (cohort 4), 0.08 (cohort 5), 0.1 (cohort 6), and 0.02 mg/kg (cohort 7, repeated), as described previously³. Data from the first two cohorts were lost due a bioanalytical issue (see Bioanalytical procedure below). Men and women were included in each study group. After definition of potency (quantified as the effective dose producing 95% neuromuscular blockade, or ED95), an additional cohort (cohort 8) of four subjects received 0.14 mg/kg CW002 (~ 2×ED95). The response to 0.02 mg/kg CW002 (cohort 7) was negligible and plasma concentrations were not collected from this cohort because of the expected low concentrations; therefore, this cohort was omitted from the PK-PD model. In addition, data from one volunteer who received 0.08 mg/kg were omitted from the efficacy and safety analysis due to a transcription error that led to the weight-normalized dose being incorrect³; however, these data were included in the PK and PopPK analyses. Neuromuscular blocking data were unavailable from this subject, so this subject was not included in the PK-PD analysis.

Study protocol

Prior to dosing, subjects had been fasting since midnight, and following morning admission to the Weill Cornell Clinical Research Center, they were hydrated with intravenous Lactated Ringers solution to ensure that pre-study hydration was similar in all subjects³. For the initial two cohorts (CW002 0.02 mg/kg and 0.04 mg/kg), anesthesia was induced and maintained with propofol, and fentanyl. However, because there was an interference with the bioanalytical assay (see Bioanalytical procedure below), the protocol was amended so that anesthesia was induced with propofol (3–5 mg/kg) and maintained using sevoflurane and these cohorts were repeated (cohorts 3 for 0.04 mg/kg and 7 for 0.02 mg/kg). The trachea was intubated without neuromuscular blockade. Following induction and tracheal intubation, subjects were mechanically ventilated with a tidal volume of 6–8 mL/kg and 5 cm H₂O positive end-expiratory pressure (PEEP). Respiratory rate was adjusted to maintain end-tidal CO₂ at 32–40 mmHg. Anesthesia was maintained with nitrous oxide (70%) and sevoflurane (0.8–1.2 % end-tidal).

A radial arterial catheter was placed for sample collection and continuous blood pressure recording (data reported previously³). Arterial blood samples were collected at predose and at 0.5, 1, 3, 5, 10, 20, 30, 45, 75, and 90 min post-dose for PK analysis. Depth of neuromuscular blockade was assessed at the adductor pollicis using stimulation of the ulnar nerve and mechanomyography³. Supramaximal stimuli were delivered via surface electrodes placed over the ulnar nerve at a frequency of 0.10 Hz from a Grass (Quincy, MA) Model S88 stimulator in conjunction with a Grass Stimulation Isolation unit. The strength of contraction of the adductor pollicis in response to stimulation was measured with a Grass Model FT-10 force displacement transducer applied to the thumb.

At least 30 min after achieving a steady end-tidal concentration of sevoflurane and 20 min after beginning single twitch (referred to as T1 or ST) neuromuscular stimulation, CW002

was administered as an intravenous (IV) bolus dose over 5 sec. The adductor pollicis response to ulnar nerve stimulation was monitored continuously for maximal suppression of T1. Once initial recovery of neuromuscular function was marked, the stimulation mode was changed to train-of-four (TOF) (0.2 msec square waves at 2 Hz for 2 sec) applied every 20 sec and continued until the ratio of T4 to T1 was greater than or equal to 0.9. When recovery of neuromuscular function was complete, subjects were allowed to emerge from anesthesia, the trachea was extubated, and monitoring was continued in the post-anesthesia care unit for a minimum of two hours.

Bioanalytical Method

CW002 concentrations in plasma (collected in tubes with potassium EDTA) were determined using a high performance liquid chromatography with tandem mass spectrometry assay following protein precipitation with solid phase extraction for concentrations between 10 and 5000 ng/mL. Initially, the analytical method was validated using an analogue (CV005) of CW002 as the internal standard. When samples from Cohort 2 (where subjects received propofol and fentanyl) were analyzed, the internal standard response was variable (45–90%), which was due to interference with the extraction and ionization suppression of the internal standard. This interference was suspected to be related to the nonmedicinal components of the propofol and fentanyl formulations. Therefore, two changes were made to the method, which was successfully validated for CW002 when CW002 was present along with low concentrations of propofol (0.933 µg/mL) and standard concentrations of sevoflurane (320 µg/mL): (1) formic acid was added to acetonitrile and methanol during extraction; and (2) the internal standard was changed to a stable isotope of CW002. Concentration data from the first two cohorts, analyzed prior to this updated method, were lost and the anesthesia regimen was changed to propofol for induction followed by sevoflurane for all remaining cohorts (see Study protocol). The final bioanalytical method used in the analysis of samples from this study was conducted using a high performance liquid chromatography with tandem mass spectrometry. Plasma samples were stabilized with 5 µL of 1N HCl and frozen at –80°C until analysis. Thawed samples were extracted using acetonitrile:methanol:formic acid (50:50:0.1) and 5 µL was injected onto an HPLC column (Phenomenex Jupiter C18 300 A, 5µm) with a mobile phase consisting of 10mM ammonium acetate (aq): acetic acid (100:1 v/v) (Mobile Phase A) and 10mM ammonium acetate (methanol):water (90:10 v/v) (Mobile Phase B) using a gradient flow rate. The validated standard curve included concentrations ranging from 10 to 5000 ng/mL. Accuracy (%CV) and % bias were reported as 2.2 to 2.9% and 0 to 0.5%, respectively.

Data Acquisition

Continuous mechanomyography data were recorded to a disc (LabChart, ADInstruments, New South Wales, Australia), and a peak detection algorithm was used to discriminate the individual components of the TOF response.

Data Analysis

Plasma concentration-time data from Cohorts 3, 4, 5, 6, and 8 were included in the PK population and the PopPK population. Doses in these cohorts were 0.04, 0.06, 0.08, 0.10,

and 0.14 mg/kg, respectively. Cohorts 1 and 2 (0.02 and 0.04 mg/kg dose) were not included in the PK population because the study initially included fentanyl and propofol for induction and maintenance of anesthesia, which interfered with the bioanalytical method for CW002 (see Bioanalytical method). The protocol was then amended to use propofol (for induction) and sevoflurane (for maintenance) for all future cohorts (Cohorts 3–8). Cohort 7 (0.02 mg/kg) did not have any PK sample analysis performed because a lower dose of CW002 was administered, which would have had a minimal contribution to the PK analysis. This cohort was added to the protocol to determine the low end of the dose response curve to allow for adequate data for an accurate estimation of the ED95 dose under sevoflurane anesthesia. The total number of cohorts included in the PK, PopPK and PK-PD analyses was 5, after excluding Cohorts 1, 2 and 7, resulting in the inclusion of evaluable data from 28 healthy subjects.

Noncompartmental Analysis

Noncompartmental analysis using Phoenix® WinNonlin® version 6.4 (Certara USA, Inc., Princeton, NJ) was conducted to estimate the maximal concentration (C_{max}), half-life, clearance (CL), area under the concentration time curve from time 0 to infinity (AUC_{0-inf}), and volume of distribution at steady state (V_{ss}).

PopPK-PD Modeling

A 2-step approach was taken to model CW002 PopPK and PD data using NONMEM v7.1.2 (ICON, PLC, Dublin, Ireland) and first-order conditional estimation with interaction. A PopPK model was developed first to describe the concentration-time profile of CW002 (including the early concentrations during the onset of neuromuscular blockade). The PopPK parameters were then fixed and a PK-PD model was developed. Each model was developed using standard criteria, where the best model was chosen based on a significant drop in the objective function value (OFV) (where a decrease in the OFV of >3.84 (χ^2 -distribution, $\alpha = 0.05$ and 1 degree of freedom) was considered statistically significant) and improved goodness-of-fit plots. Model performance was evaluated using a nonparametric bootstrap ($n=1000$ for PopPK and 200 for PK-PD) with sampling stratified by cohort and individuals and a visual predictive check ($n=1000$).

PopPK Model

Three- and 4-compartment open mammillary models were attempted, with and without elimination from the peripheral compartments. The dose was modeled as a 5-sec IV infusion. All PopPK parameters were weight normalized and allometrically scaled, where $CL = \theta_i \times \left(\frac{Weight}{70}\right)^{0.75}$, for clearance parameters, and $V = \theta_i \times \left(\frac{Weight}{70}\right)$, for volume parameters. Allometric scaling was chosen to provide the ability to predict plasma concentration-time data in pediatrics through simulation. Plasma concentrations were log-transformed for parameter estimation. Inter-individual variability was estimated as an exponential error on CL, central volume, and distributional clearances from the central to the 1st (Q1) and 2nd (Q2) peripheral compartments (but not the 3rd peripheral compartment (Q3)), using an omega block structure. Residual error was modeled as additive error with log-transformed concentrations.

PK-PD Model

The PD effect was calculated as the maximal T1 percent change relative to baseline, where baseline is defined as the average T1 amplitude over the 2 minutes prior to drug injection. The amplitude of T1 was expressed as a % of baseline, with this value then used to quantify the response, i.e., 25% of baseline equals 75% blockade. During the time when the twitch response was abolished, 100% blockade was input into the dataset at the same frequency they were collected throughout the study. Once T1 amplitude had returned to baseline, the twitch data were only included once every minute to avoid repetitive data at the quantification limit.

The PK-PD model was attempted using an effect compartment model⁴ to describe the delay observed between plasma concentrations and PD effect using a first-order rate constant (K_{eo}) along with the sigmoid Emax model (Equation 1):

$$Effect = \frac{Emax * CE^{Hill}}{(CE^{Hill} + EC50^{Hill})} \quad (1)$$

where CE is the concentration in the effect compartment, Emax is the maximum effect (set to 1 to indicate 100% blockade), EC_{50} is the concentration in the effect compartment producing 50% blockade (a measure of sensitivity), and Hill is the Hill coefficient for a sigmoid Emax model, which describes the steepness of the concentration-effect curve. The EC_{50} and Hill coefficient were estimated, along with first-order rate constants to describe the time delay between plasma concentrations and the appearance of neuromuscular blockade. Models evaluated also included variants of a transit model with an effect compartment (with first-order rate constants of $1/\tau$ and K_{out}) (Figure 1). Interindividual variability was added to K_{out} and EC_{50} as an omega block matrix. An additive residual error was used for the PK-PD model.

Simulations

Simulations were conducted using the final PopPK and PK-PD models using the final NONMEM estimates for parameters, inter-individual variability and residual error. Plasma concentrations and % blockade were simulated following single 2×, 3×, or 4× the ED95 doses in 1000 subjects each. Weights were randomly selected from a normal distribution from the study population³. During the simulations, plasma concentration and % blockade observations were made every 15 sec for the first 4 min, and every min thereafter for a total of 150 min. All simulated PD measurements were used to identify the times to reach clinical thresholds describing the onset (to 80% and 95% blockade) and the recovery (to 5%, 25%, 75%, and 95% recovery from full blockade, if reached, and 25%–75% recovery index). The times to reach various degrees of blockade and recovery were defined as the 3rd consecutive simulated value that exceeded the clinical cutoff. A time of 10 min post-dose was chosen empirically based on the observed data of the latest time for maximal blockade to begin looking for the various levels of recovery. This criterion was chosen because residual variability from the PopPK and PK-PD models were included in the simulations and led to some fluctuations in the predicted values.

Results

A total of 28 subjects were included in the PK and PopPK analyses and 27 subjects were included in the PK-PD analysis. There were 14 males and 14 females who were an average of 35 years of age (19 to 50 years of age), weighing an average of 75 kg (range 52 to 95.7 kg) with an average BMI of 25.5 kg/m² (range: 19 to 30 kg/m²). There were 9 Caucasians, 15 African Americans, and 4 subjects with race classified as other. The dose-response, safety and tolerability data from these subjects are described in detail by Heerdt³.

The noncompartmental PK parameters of CW002 are summarized in Table 1. The CL was intermediate, while the V_{ss} was low. The half-life was 23 to 27 minutes across doses.

PopPK Model

The 4-compartment model with elimination from the central compartment was selected as the final PopPK model because it resulted in improvements in the goodness-of-fit plots and a statistically significant drop in the OFV when compared to a 3-compartmental model and any of the following competing 3- and 4-compartmental models: a) models where central volume was fixed to the plasma volume reported in the literature⁵, (0.03575*Weight {kg}), assuming hematocrit is 45% of blood volume, b) models that excluded the initial two data points (0.5 and 1 minute) to evaluate whether the lack of homogeneity of mixing was influencing the parameter estimates, and c) models with elimination from the central and peripheral compartments (where rate constant is assumed to be equivalent in the central and peripheral compartments).

The 4-compartment model with elimination from the central compartment had significantly improved goodness-of-fit plots compared to the 3-compartment model with elimination from the central compartment, especially for the conditional weighted residuals-vs-time and individual-weighted residuals versus individual predictions (Supplemental Figure 1) due in part to its ability to accurately predict the initial time points. In addition, there was a much lower OFV with the addition of a fourth compartment, decreasing from -642.704 to -906.165 for the 3-compartment and 4-compartment models, respectively.

The final PopPK model parameters are listed in Table 2 with goodness-of-fit plots given in Supplemental Figure 2. All PopPK parameters were estimated with good precision. All random effects were estimated with low shrinkage (7.4%). The inter-individual variability was very low for weight-normalized CL (10.8%), low to moderate for distributional clearances from the central to the 1st and 2nd peripheral compartments (23.3% and 15.2%, respectively), and moderate for the central volume (34.6%). An omega block matrix was estimated to account for correlation between the CL, distributional clearances from the central to the 1st and 2nd peripheral compartments, and central volume in individual subjects. There was no appearance of nonlinearity of PK parameters as a function of dose. The estimates from the nonparametric bootstrap showed good agreement with the NONMEM estimates for all PK parameters (Table 2). The visual predictive checks showed good agreement overall between observed and predicted plasma concentrations over time, as shown for the 1×ED95 cohort (Figure 2) and additional cohorts (Supplemental Figure 3). All

simulations of concentrations at matched observation times required for the PK-PD model were computed using the NONMEM final parameter estimates presented in Table 2.

When compared to the noncompartmental parameters (CL and V_{ss}), the predicted parameters from the PopPK model were lower than the observed parameters, yet the predicted half-life was only minimally longer (27 min) than the observed half-life (24.8 min).

PK-PD Model

Final parameters for the PopPK model were fixed and the PD parameters were estimated for the PK-PD model. During PK-PD model development, the basic effect compartment model or a single transit model (i.e., either of which only had 1 parameter to describe the time delay between plasma concentrations and neuromuscular blockade) were not able to describe the rapid onset of neuromuscular blockade adequately. Therefore, a combined model (a single transit compartment with an effect compartment) was selected as the final model (Figure 1). Equations 2 and 3 describe the movement of drug from the central compartment into the transit compartment and effect compartment.

$$dC5/dt = 1/\text{Tau} * (C1 - C5) \quad (2)$$

$$dC6/dt = 1/\text{Tau} * C5 - K_{out} * C6 \quad (3)$$

Where C1, C5 and C6 represent drug concentrations in the central, transit, and effect compartments, respectively, and Tau represents the transit time. The final PK-PD model parameters for the transit model are listed in Table 3. There was moderate inter-individual variability observed for EC_{50} (27.6%) and K_{out} (24.8%).

The NONMEM estimates were nearly identical to the nonparametric bootstrap estimates (Table 3). The visual predictive check plots showed good agreement between observed and predicted values (Supplemental Figure 4). Model performance was assessed visually by plotting the observed clinically important times to onset and recovery for the 2×ED₉₅ dose (0.14 mg/kg)³ versus the simulated times for each clinical endpoint using the PK-PD model. As shown in Figure 3, the predicted and observed times to various levels of blockade and recovery for the 2×ED₉₅ dose are in good agreement. Therefore, simulations to predict the onset and recovery at higher doses are summarized in Table 4. These simulations showed that the times to 80% blockade were predicted to be 1.5, 0.8 and 0.7 min for a 2×, 3×, and 4× ED₉₅ dose, respectively. The 25–75% recovery indices were consistent across all simulated doses with predicted times of 12, 11, and 12 min for 2×, 3×, and 4× ED₉₅ doses, respectively. The simulated times to 95% recovery for the 2×, 3×, and 4× ED₉₅ doses were 57, 66, and 75 min, respectively.

Discussion

In the present study, the PK, PopPK, and PK-PD of CW002, an investigational intermediate duration NMB agent, are presented for this healthy subject study. These results confirm that, despite the novel process of cysteine inactivation, CW002 has very similar PK properties to other intermediate acting NMBs in humans^{6,7,8}. PopPK and PK-PD models were developed to predict the neuromuscular blocking profile of CW002 at higher doses, where patients require complete blockade to carry out the surgical procedures. This information will help optimize the drug development program (e.g., selecting doses for future studies, understanding drug supply issues). The PopPK and PK-PD analyses also contributed to information about the general properties of CW002.

The half-life of CW002 (20–30 min) is consistent with intermediate duration NMB agents. CW002 has a very low inter-individual variability (10.8%) in CL suggesting that variability in endogenous cysteine is low. CW002 also appears to exhibit linear kinetics across a dose range of 0.04–0.14 mg/kg suggesting that this NMB agent would have very predictable exposures. The mean noncompartmental CL estimate for CW002 was 5.36 mL/min/kg. This CL is similar to or faster than CL estimates for other drugs in this class, e.g., vecuronium (5.2 mL/min/kg)⁹, rocuronium (2.67 mL/min/kg)¹⁰, and cisatracurium (4.57 mL/min/kg)¹¹.

The final PopPK model selected was an empirical 4-compartment open mammillary model. The CL and V_{ss} estimates for this model were much lower than that derived from the noncompartmental analysis, and the model parameter estimates generally lacked physiologic plausibility. Numerous models (e.g., eliminating early concentrations, fixing central volume to plasma volume, addition of elimination from the peripheral compartment) were evaluated in an effort to develop a PopPK model with parameter estimates that were similar to the noncompartmental analysis, and that were more physiologically realistic. These models led to significant bias between observed and predicted concentrations early and late in the concentration-time profile after CW002 administration, eliminating the potential for accurate simulations of PK-PD after administration of higher CW002 doses, which was the primary objective of this analysis.

The first challenge in the development of the PopPK model using data from the present study was that the first arterial sample was collected at 0.5 min after the bolus dose (treated as a 5 sec infusion), as part of the safety monitoring protocol. Clearly, homogenous mixing has not occurred within 0.5 min. Previous physiologic modeling efforts have attempted to evaluate this non-homogeneity of mixing unsuccessfully (unpublished observations). For the current model, models were tested that excluded the early time-points (0.5 min and 1 min), from fitting with 3- and 4-compartment models, either as the 0.5 min sample alone or both together. The 4-compartment model OFV was 72 points lower than the 3-compartment model when the 1 min data were excluded from fitting. In both models, the average CL values were still 70 to 100 mL/min lower than those estimated from noncompartmental analysis. The V_{ss} of the 4-compartment model began to approach that of the noncompartmental analysis, but was still somewhat lower. Thus, excluding these two early time-points only partially improved the correspondence of the CL and V_{ss} estimates from the PopPK model with results from noncompartmental analysis. Still, V_1 was only ~0.6 L

for both models (i.e., lower than plasma volume). With the early time-points excluded, the 4-compartment model still outperformed the 3-compartment model in OFV and goodness-of-fit plots. Since fixing V_1 to an estimate of the plasma volume did not resolve the issue of parameter interpretation, the initial unconstrained model was retained.

The second challenge is that the central volume (0.0822 L or ~1 mL/kg) estimated from the final model, values that are much lower than plasma volume, are likely underestimated (with the resulting C_{max} overestimated). Administering CW002 as a 15-min infusion, which results in homogenous mixing, would allow for a better estimation of central volume in a future study. As part of the present model development, fixing the volume of the central compartment to the plasma volume was attempted for a 3- and 4-compartment model. When the central volume of a 3-compartment model was fixed to the estimate of plasma volume, the CL and V_{ss} were closer to the estimates derived from noncompartmental analysis, but the early concentrations were very biased (Supplemental Figure 5), and, as seen in the resulting visual predictive check plots, the terminal slope was much too fast to accurately describe the later data points (Supplemental Figure 6). While the misfit in the early time points may be acceptable because the system is not well-stirred, fixing the volume to plasma volume led to large deviations between predicted and observed concentrations later in the profile (e.g., model suggests much faster elimination than observed). In a 4-compartment model with central volume fixed to the estimate of the plasma volume, inter-individual variabilities on CL and distributional clearances from the central to the 1st and 2nd peripheral compartments, each approached zero; reducing the number of parameters with inter-individual variability led to a very large volume of the fourth compartment (4×10^{10} L), and multiple numerical problems. Thus, fixing the central volume to plasma volume did not result in an adequate model.

The third challenge is that CW002 is thought to be eliminated throughout the body by cysteine adduction and the low central volume may also be a function of failure to account for peripheral elimination¹². The modeling approach for estimating peripheral elimination rates would require *in vitro* data that were not available at the time of modeling for successful implementation. Instead, 3- and 4-compartment models were tested that allowed first-order elimination from the central compartment and each of the peripheral compartments (assuming that the elimination rate was the same in the central and peripheral compartments), but did not improve the fit over comparable models without peripheral elimination, and thus the unconstrained 4-compartment model (with elimination from the central compartment only) was retained as the final model.

Each of the models tested had competing strengths and weaknesses. The underestimation of CL and central volume in the current model (relative to the noncompartmental estimates) is likely due to the lack of homogenous mixing early after a bolus dose, the lack of plasma concentration-time data after an infusion, and/or insufficient information to separate the sources of CL from multiple routes. Since the primary goal of this project was to predict neuromuscular blockade at higher doses, we chose the PopPK model that best described the observed concentration-time profile in order to predict the neuromuscular blockade over time. Of the PK-PD models evaluated, the current transit model with the 4-compartment PopPK model did the best job of describing the plasma concentrations over time and the

onset and offset of neuromuscular blockade after CW002 administration. The model describes the current data well for the purpose of predicting concentrations for the PK-PD model development. Evaluation of a more physiologically relevant model will occur when data from a larger number of patients receiving CW002 at higher doses or as infusions are available with alternative sampling protocols. In the meantime, it is best to use noncompartmental analysis for determination of CL and V_{ss} , while the PopPK model is most suitable for predictions of concentrations over time, which can serve as input into the PK-PD model to predict the resulting neuromuscular blocking profile.

The PK-PD model revealed that CW002 is an intermediate duration NMB with a rapid onset. Simulations of higher CW002 doses predicted that the onset of complete neuromuscular blockade may be achieved in 60 sec with a $3\times ED_{95}$ or $4\times ED_{95}$ dose, which can be critical for patients that need rapid intubation. The $3\times ED_{95}$ dose is predicted to keep an intermediate duration of action for CW002, while a $4\times ED_{95}$ dose is predicted to result in a duration consistent with long-acting NMB agents. Either way, L-cysteine reverses the effect of CW002 in animals with complete blockade^{1,2} (i.e., not just after 5% recovery has occurred), suggesting a similar potential in humans. Prior to understanding this potential, the appropriate doses of L-cysteine will need to be studied in humans, and they must be well characterized and well tolerated (data still unknown). Typically, PK-PD studies are conducted using balanced anesthesia. The present study could not use balanced anesthesia because fentanyl and propofol interfered with the bioanalytical method for CW002. Since sevoflurane was used instead of balanced anesthesia, and inhalation agents are known to potentiate neuromuscular blockade, one would expect the neuromuscular blocking profile results presented in this manuscript to differ (i.e., shorter duration, slightly slower onset) when CW002 is administered with balanced anesthesia.

It is important to note that the PK-PD model was developed based on ~175 measurements of neuromuscular blockade per subject and ~10 concentrations per subject. A transit compartment model best described the onset of action for CW002. A similar approach with a transit compartment has been used to describe prothrombin time¹³ and propofol¹⁴. The effect compartment model did not allow for an adequate description of the onset of action as well as the recovery data with only one parameter (Supplemental Figure 7), regardless of whether a 3- or 4-compartment PopPK model was used. This was surprising given that the effect compartment model was specifically developed for NMB agents⁴ and may be related to the low availability of data at higher doses (e.g., only 4 subjects at $2\times ED_{95}$) with complete blockade. The mean transit time of 1.40 min indicates that rapid onset is observed with CW002. CW002 has an EC_{50} of 4730 ng/mL, which is much higher than that reported for cisatracurium¹⁵ or rocuronium¹⁶. The inter-individual variability for EC_{50} was moderate, as expected due to differences in sensitivity. The agreement between the simulated and observed onset and offset profile at the $2\times ED_{95}$ dose provides confidence in the predictions for higher doses ($3\times$ and $4\times ED_{95}$). The percent blockade observations and predictions vs time graphs for the individuals in Cohorts 4, 5, 6 and 8 are provided in Supplemental Figure 8.

Predicting the time to 5% recovery was limited by the fact that once recovery from the blockade began, a few subjects began rapid recovery beyond 5% recovery within seconds

after being at 100% blockade, yet neuromuscular blockade was only predicted once per minute during recovery. This phenomenon can be seen with a few subjects in Cohort 8 in Supplemental Figure 8. This led to disruption in the PD effect curve for these subjects and some difficulty in determining exactly when 5% recovery was achieved. Another difficulty in the simulated times to 5% recovery was the error included in the predicted % blockade and concentration values. This led to intervals where the simulated subjects were demonstrating full blockade but their predictions fell between 93–100% blockade. Importantly, this is consistent with other intermediate NMB agents. Once recovery begins, recovery is relatively rapid. For instance, the simulations showed that the 25–75% recovery index is predicted to be independent of dose (12, 11, and 12 min for 2×, 3×, and 4× ED₉₅ doses, respectively), with values similar to other NMB blocking agents such as cisatracurium¹¹, atracurium¹⁷, vecuronium¹⁷ and rocuronium¹⁰ (with 25–75% recovery index values of 13, 13, 10 and 13 min, respectively).

In summary, the current manuscript presents the PK, PopPK, and PK-PD of CW002, an investigational NMB agent, with a rapid onset and an intermediate duration of action. The PK-PD model provides information to inform critical decisions (e.g., dose, study design) for the clinical development of CW002. Information gained from these simulations can be tested in the next clinical study in patients receiving higher doses of CW002.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Disclosure of Funding: Research supported by Dept. of Anesthesiology, Weill Cornell Medical College, New York, NY.

Phoenix WinNonlin software was generously provided to the Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, by Certara as a member of the Pharsight Academic Center of Excellence Program.

J.D.K. was supported by a UNC-QuintilesIMS Pharmacokinetics/Pharmacodynamics Fellowship.

K.L.R.B is supported by the National Institute of General Medical Sciences of the National Institutes of Health (NIH) under Award Numbers R01 GM041935 and R35 GM122576.

Funding Statement: Support was provided solely from institutional and/or departmental sources.

References

1. Sunaga H, Malhotra JK, Yoon E, Savarese JJ, Heerdt PM. Cysteine reversal of the novel neuromuscular blocking drug CW002 in dogs: pharmacodynamics, acute cardiovascular effects, and preliminary toxicology. *Anesthesiology*. 2010; 112:900–9. [PubMed: 20234310]
2. Sunaga H, Savarese JJ, McGilvra JD, Heerdt PM, Belmont MR, Van Ornum SG, Murrell MT, Malhotra JK, Savard PM, Jeannotte E, Petty BJ, Allen E, Carnathan GW. Preclinical Pharmacology of CW002: A Nondepolarizing Neuromuscular Blocking Drug of Intermediate Duration, Degraded and Antagonized by L-cysteine: Additional Studies of Safety and Efficacy in the Anesthetized Rhesus Monkey and Cat. *Anesthesiology*. 2016; 125:732–43. [PubMed: 27466033]
3. Heerdt PM, Sunaga H, Owen JS, Murrell MT, Malhotra JK, Godfrey D, Steinkamp M, Savard P, Savarese JJ, Lien CA. Dose-response and Cardiopulmonary Side Effects of the Novel

- Neuromuscular-blocking Drug CW002 in Man. *Anesthesiology*. 2016; 125:1136–43. [PubMed: 27749289]
4. Sheiner LB, Stankisi DR, Vozeh S, Miller RD, Ham J. Simultaneous modeling of pharmacokinetics and pharmacodynamics: Application to d-tubocurarine. *Clin Pharmacol Ther*. 1979; 25:358–71. [PubMed: 761446]
 5. Kaplan AA. A simple and accurate method for prescribing plasma exchange. *ASAIO Trans*. 1990; 36:M597–9. [PubMed: 2252761]
 6. Vega-Villa KR, Kaneda K, Yamashita S, Woo S, Han TH. Vecuronium pharmacokinetics in patients with major burns. *Br J Anaesth*. 2014; 112:304–10. [PubMed: 24067332]
 7. Magorian T, Wood P, Caldwell J, Fisher D, Segredo V, Szenohradszky J, Sharma M, Gruenke L, Miller R. The Pharmacokinetics and Neuromuscular Effects of Rocuronium Bromide in Patients with Liver Disease. *Anesth Analg*. 1995; 80:754–9. [PubMed: 7893030]
 8. Szenohradszky J, Fisher DM, Segredo V, Caldwell JE, Bragg P, Sharma ML, Gruenke LD, Miller RD. Pharmacokinetics of Rocuronium Bromide (ORG 9426) in Patients with Normal Renal Function or Patients Undergoing Cadaver Renal Transplantation. *Anesthesiology*. 1992; 77:899–904. [PubMed: 1443743]
 9. Cronnelly R, Fisher DM, Miller RD, Gencarelli P, Nguyen-Gruenke L, Castagnoli N Jr. Pharmacokinetics and pharmacodynamics of vecuronium (ORG NC45) and pancuronium in anesthetized humans. *Anesthesiology*. 1983; 58:405–8. [PubMed: 6132566]
 10. Rocuronium [package insert]. Teva Parenteral Medicines, Inc; Irvine, CA: 2008.
 11. Nimbex® [package insert]. GlaxoSmithKline Australia Pty. Ltd; Boronia, Victoria: 2002.
 12. Kisor DF, Schmith VD. Clinical pharmacokinetics of cisatracurium besilate. *Clin Pharmacokin*. 1999; 36:27–40.
 13. Hirt D, Richardet JP, Urien S, Poupon J, Sogni P, Batteux F, Laurent A, Pavlovic S, Debray M, Treluyer JM, Weill B. Pharmacokinetic-Pharmacodynamic Modeling of Manganese After a Single Intravenous Infusion of Mangafodipir in Patients With Acute Alcoholic Hepatitis. *Ther Drug Monit*. 2009; 31:557–65. [PubMed: 19834427]
 14. Bjornsson MA, Norberg A, Kalman S, Karlsson MO, Simonsson US. A two-compartment effect site model describes the bispectral index after different rates of propofol infusion. *J Pharmacokinet Pharmacodyn*. 2010; 37:243–55. [PubMed: 20414709]
 15. Schmith VD, Fiedler-Kelly J, Phillips L, Grasela TH Jr. Prospective use of population pharmacokinetics/pharmacodynamics in the development of cisatracurium. *Pharm Res*. 1997; 14:91–7. [PubMed: 9034227]
 16. Vega EA, Ibacache ME, Anderson BJ, Holford NH, Nazar CE, Solari S, Allende FA, Cortínez LI. Rocuronium pharmacokinetics and pharmacodynamics in the adductor pollicis and masseter muscles. *Acta Anaesthesiol Scand*. 2016; 60:734–46. [PubMed: 26899676]
 17. Erkola O, Karhunen U, Sandelin-Hellqvist E. Spontaneous recovery of residual neuromuscular blockade after atracurium or vecuronium during isoflurane anaesthesia. *Acta Anaesthesiol Scand*. 1989; 33:290–4. [PubMed: 2566251]

Figure 1.

Illustration of the Final PKPD Model

V1-4: Volume for central and peripheral compartments, IV: Intravenous, $Q1-3$:

Distributional clearance from the central compartment to peripheral compartments, CL :

Clearance from the central compartment, Tau : First-order rate constant for drug into transit

and effect compartments, $Kout$: First-order rate constant for drug leaving the effect compartment.

Figure 2.
Visual Predictive Check for the PK Model for 1×ED95 Dose Cohort

Figure 3.
Predicted vs Observed Time to Onset (A) and Offset (B)

Table 1Noncompartmental PK Parameters^a for CW002

Dose	Half-life (min)	C _{max} (ng/mL)	AUC _{0-inf} (min*ng/mL)	CL (mL/min)	V _{ss} (mL)
0.04 mg/kg	27.4 (3.15)	2360 (995)	7900 (684)	397 (55.9)	9330 (2110)
0.06 mg/kg	26.3 (2.13)	2660 (1500)	9610 (1560)	399 (109)	8800 (1540)
0.08 mg/kg	27.0 (2.14)	3610 (1950)	14900 (2250)	387 (51.5)	9580 (1310)
0.10 mg/kg	23.9 (2.44)	5020 (1630)	18200 (1940)	438 (66.9)	9020 (1470)
0.14 mg/kg	24.8 (1.06)	9120 (5060)	30400 (4880)	366 (41.9)	7840 (1610)

C_{max} = Maximum concentration; AUC_{0-inf} = Area under the concentration-time curve from zero to infinity; CL = Clearance; V_{ss} = Volume of distribution at steady state.^a presented as geometric mean (SD).

Table 2

NONMEM and Bootstrap Estimates for the Population Pharmacokinetic Model (Assuming a 70 kg Subject)

Parameter	Final Parameter Estimate		Interindividual Variability		Bootstrap Parameter Estimate		Interindividual Variability	
	Typical Value	%RSE	Magnitude	%RSE	Typical Value	95% CI	Magnitude	95% CI
CL: (L/min)	0.243	4.78	10.8% CV	25.7	0.242	0.220–0.266	10.6% CV	7.72–13.1% CV
Q1: (L/min)	0.0882	16.4	23.3% CV	33.6	0.087	0.0600–0.118	23.9% CV	14.8–32.3% CV
Q2: (L/min)	0.130	11.0	15.2% CV	34.8	0.127	0.101–0.161	15.0% CV	9.12–20.1% CV
V1: (L)	0.0821	13.5	34.6% CV	26.0	0.081	0.061–0.107	35.0% CV	25.3–43.9% CV
V2: (L)	0.157	23.3	0% CV	FIXED	0.157	0.0890–0.237	0% CV	FIXED
V3: (L)	1.22	16.8	0% CV	FIXED	1.19	0.868–1.60	0% CV	FIXED
V4: (L)	2.35	8.31	0% CV	FIXED	2.36	2.04–2.78	0% CV	FIXED
Q3: (L/min)	0.0664	20.2	0% CV	FIXED	0.0670	0.0450–0.0910	0% CV	FIXED
cov(IIV in V1, IIV in CL)	0.0152	53.8	NA	NA	0.0146	–0.000820 –0.0313	NA	NA
cov(IIV in Q1, IIV in CL)	0.0113	57.4	NA	NA	0.0113	0.000250 –0.0238	NA	NA
cov(IIV in Q1, IIV in V1)	–0.0147	141	NA	NA	–0.0140	–0.0569 –0.0213	NA	NA
cov(IIV in Q2, IIV in CL)	0.00715	63.0	NA	NA	0.00694	–0.000400 –0.0172	NA	NA
cov(IIV in Q2, IIV in V1)	0.0261	48.1	NA	NA	0.0246	0.00283 –0.0502	NA	NA
cov(IIV in Q2, IIV in Q1)	0.0173	51.1	NA	NA	0.0169	0.00144 –0.0348	NA	NA
Log Residual Variability	0.00462	16.5	NA	NA	0.00435	0.00296 – 0.00587	NA	NA
Minimum value of the objective function = –906		Minimum value of the objective function = –933						

CL = Clearance; Q = Distributional clearance; V = Volume; RSE = Relative standard error; IIV = inter-individual variability; CV = Coefficient of variation; CI = confidence interval; NA = Not Applicable.

The following parameter estimates were found to be highly correlated ($r^2 = 0.854$): (V2: L), Q1: (L/min)).

The calculated correlation coefficients (r^2) of the off-diagonal omegas were as follows: 0.168 for cov (IIV in V1, IIV in CL), 0.203 for cov(IIV in Q1, IIV in CL), 0.0332 for cov(IIV in Q1, IIV in V1), 0.190 for cov(IIV in Q2, IIV in CL), 0.246 for cov(IIV in Q2, IIV in V1), 0.236 for cov(IIV in Q2, IIV in Q1).

Parameters in this model are allometrically scaled to a weight of 70-kg. CL, Q1, Q2, and Q3 have a power of 0.75 and V1, V2, V3, and V4 have a power of 1; e.g., CL = $0.243 \times (\text{WT}/70)^{0.75}$.

Table 3

NONMEM and Bootstrap Estimates for the Population PK-PD Model

Parameter	Final Model Estimates			Interindividual Variability			Bootstrap Estimates			Interindividual Variability		
	Typical Value	%RSE	Magnitude	%RSE	Magnitude	%CI	Median	95% CI	Magnitude	95% CI	Magnitude	95% CI
EC₅₀ (ng/mL)	4730	17.2	27.6%CV	16.6	4770	3370–6870	0.0562	0.0485–0.0658	27.6 %CV	19.3–35.0 %CV	24.5 %CV	15.8–33.5 %CV
K_{out} (1/min)	0.0566	7.50	24.8%CV	17.3	0.0562	0.0485–0.0658	5.90	4.90–7.20	0%CV	Fixed	0%CV	Fixed
Hill Coefficient	5.80	9.00	0%CV	Fixed	0.717	0.566–0.932	0.717	0.566–0.932	0%CV	Fixed	0%CV	Fixed
1/Tau (1/min)^a	0.712	12.6	0%CV	Fixed	NA	NA	NA	NA	NA	NA	NA	NA
Cov(IIV in Kout, IIV in EC50)	–0.0398	–50.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Residual Variability	3.60%	8.30	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

CI = confidence interval; IIV = inter-individual variability; CV = Coefficient of variation; RSE = Relative standard error; NA = Not applicable.

^aTau was estimated at 1.40 min for delivery of drug from the central compartment to the effect site.

Table 4

Predicted Times to Onset and Offset for 2×, 3×, and 4×ED₉₅ Doses

Time to Onset	Dose		
	2×ED ₉₅ 0.14 mg/kg (min)	3×ED ₉₅ 0.21 mg/kg (min)	4×ED ₉₅ 0.28 mg/kg (min)
80% Blockade	1.5 (1.0, 2.3)	0.8 (0.5, 1.2)	0.7 (0.5, 1.0)
95% Blockade	1.8 (1.3, 3.5)	1.0 (0.7, 1.7)	0.8 (0.5, 1.3)
Time to Offset			
5% Recovery	23 (15, 35)	17 (11, 33)	18 (11, 38)
25% Recovery	36 (24, 53)	46 (32, 65)	54 (39, 76)
75% Recovery	48 (34, 67)	57 (41, 80)	66 (48, 92)
95% Recovery	57 (41, 79)	66 (46, 91)	75 (55, 100)
25–75% Recovery Index	12 (10, 14)	11 (9.0, 15)	12 (9.0, 16)

Values are presented as the median (5th and 95th percentile).

Recovery was determined by the third observation at or below the threshold 10 min post-dose for each simulation.