

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

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ABSTRACT

Background: CW002 is an investigational nondepolarizing, 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 pharmacokinetic and pharmacokinetic/pharmacodynamic 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 four-compartment model was fit to the concentration–time data, whereas a transit compartment with a sigmoid E_{\max} model was fit to the pharmacokinetic/pharmacodynamic data. The population pharmacokinetics of CW002 was linear with very low interindividual variability in clearance (10.8%). Simulations were conducted to predict the onset and offset of effect at 2 \times , 3 \times , and 4 \times ED_{95} . The time to 80% block was predicted to be 1.5, 0.8, and 0.7 min for 2 \times , 3 \times , and 4 \times ED_{95} doses, respectively. The simulated 25 to 75% recovery index was independent of dose.

Conclusions: CW002 has predictable pharmacokinetics and is likely to have a rapid onset with an intermediate duration of action at 3 \times ED_{95} . This model provides information to inform critical decisions (*e.g.*, dose, study design) for continued development of CW002. (ANESTHESIOLOGY 2018; 128:1107-16)

NEUROMUSCULAR blockade is imperative for patients who require complete muscular relaxation during surgery, before rapid or routine endotracheal intubation, or before and during mechanical ventilation. CW002 is an investigational nondepolarizing neuromuscular blocking 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, pharmacokinetic, and dose–response data in healthy subjects (not undergoing surgery) receiving escalating single doses of CW002. Although the safety (including hemodynamic stability) and neuromuscular blockade data (including the estimated effective dose producing 95% neuromuscular blockade, ED_{95} of 0.077 mg/kg) were reported previously,³ the present article describes the noncompartmental pharmacokinetics of CW002 in humans from this study, the development of a population pharmacokinetic model to predict plasma concentrations over time after various doses, and the development of a pharmacokinetic/

What We Already Know about This Topic

- CW002 is an investigational nondepolarizing neuromuscular blocking agent with a rapid onset of effect and intermediate duration of action
- CW002 is inactivated by endogenous cysteine, and its effects in animals are reversed rapidly by administration of L-cysteine

What This Article Tells Us That Is New

- The pharmacokinetic properties of CW002 in humans are very similar to those of other neuromuscular blocking agents with intermediate durations of action

pharmacodynamic model to describe the relationship between concentration and neuromuscular blockade. This pharmacokinetic/pharmacodynamic 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 interindividual variability and unexplained variability in the pharmacokinetics/pharmacodynamics of CW002.

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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 pharmacokinetics and population pharmacokinetics of CW002, develop a pharmacokinetic/pharmacodynamic 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, NCT01338935) was approved by the Institutional Review Board of Weill Cornell Medical Center (New York, New York).³ Thirty-four healthy male and female subjects ranging from 18 to 55 yr 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 electrocardiogram), unremarkable airway anatomy, and normal laboratory data (chemistry panel, urine analysis, and hematology). All laboratory tests were repeated the morning before 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 previously.³ Data from the first two cohorts were lost due to a bioanalytical issue (see “Bioanalytical Methods,” 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 pharmacokinetic/pharmacodynamic 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 pharmacokinetic and population pharmacokinetic analyses. Neuromuscular blocking data were unavailable from this subject, so this subject was not included in the pharmacokinetic/pharmacodynamic analysis.

Study Protocol

Before dosing, subjects had been fasting since midnight, and after morning admission to the Weill Cornell Clinical Research Center (New York, New York), they were hydrated with intravenous lactated Ringer's solution to ensure that prestudy hydration was similar in all subjects.³ For the initial two cohorts (CW002 0.02 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 Methods,” below), the protocol was amended so that anesthesia was induced with propofol (3 to 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. After induction and tracheal intubation, subjects were mechanically ventilated with a tidal volume of 6 to 8 ml/kg and 5 cm H₂O positive end-expiratory pressure. The respiratory rate was adjusted to maintain end-tidal carbon dioxide at 32 to 40 mmHg. Anesthesia was maintained with nitrous oxide (70%) and sevoflurane (0.8 to 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 pharmacokinetic analysis. Depth of neuromuscular blockade was assessed at the adductor pollicis using stimulation of the ulnar nerve and mechanomyography.³ Supramaximal stimuli were delivered *via* surface electrodes placed over the ulnar nerve at a frequency of 0.10 Hz from a Grass (USA) 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) neuromuscular stimulation, CW002 was administered as an IV bolus dose over 5 s. 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 (0.2-ms square waves at 2 Hz for 2 s) applied every 20 s 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 postanesthesia care unit for a minimum of 2 h.

Bioanalytical Methods

CW002 concentrations in plasma (collected in tubes with potassium EDTA) were determined using a high-performance liquid chromatography with tandem mass spectrometry assay after protein precipitation with solid-phase extraction for concentrations between 10 and 5,000 ng/ml. Initially, the analytical method was validated using an analog (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 to 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 before 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 1 N 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 a high-performance liquid chromatography column (Phenomenex Jupiter C18 300 A, 5 µm) with a mobile phase consisting of 10 mM ammonium acetate:acetic acid (100:1 v/v) (mobile phase A) and 10 mM 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 5,000 ng/ml. Accuracy (percentage of coefficient of variation) and percentage of 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, Australia), and a peak detection algorithm was used to discriminate the individual components of the train-of-four response.

Data Analysis

Plasma concentration–time data from cohorts 3, 4, 5, 6, and 8 were included in the pharmacokinetic population and the population pharmacokinetic 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 pharmacokinetic 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 Methods”). The protocol was then amended to use propofol (for induction) and sevoflurane (for maintenance) for all future cohorts (cohorts 3 to 8). Cohort 7 (0.02 mg/kg) did not have any pharmacokinetic sample analysis performed because a lower dose of CW002 was administered, which would have had a minimal contribution to the pharmacokinetic 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 pharmacokinetic, population pharmacokinetic, and pharmacokinetic/pharmacodynamic 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., USA) was conducted to estimate the maximal concentration (C_{max}), half-life, clearance, area under the concentration time curve from time 0 to infinity (AUC_{0-inf}), and volume of distribution at steady state (V_{ss}).

Population Pharmacokinetic/Pharmacodynamic Modeling.

A two-step approach was taken to model CW002 population pharmacokinetic and pharmacodynamic data using NONMEM v7.1.2 (ICON plc, Dublin, Ireland) and first-order conditional estimation with interaction. A population pharmacokinetic model was developed first to describe the concentration–time profile of CW002 (including the early concentrations during the onset of neuromuscular blockade). The population pharmacokinetic parameters were then fixed, and a pharmacokinetic/pharmacodynamic 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 (where a decrease in the objective function value of greater than 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 ($n = 1,000$ for population pharmacokinetics and 200 for pharmacokinetics/pharmacodynamics) with sampling stratified by cohort and individuals and a visual predictive check ($n = 1,000$).

Population Pharmacokinetic Model. Three- and four-compartment open mammillary models were attempted, with and without elimination from the peripheral compartments. The dose was modeled as a 5-s IV infusion. All population pharmacokinetic 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. Interindividual variability was estimated as an exponential error on clearance, central volume, and distributional clearances from the central to the first and second peripheral compartments (but not the third peripheral compartment), using an ω block structure. Residual error was modeled as additive error with log-transformed concentrations.

Pharmacokinetic/Pharmacodynamic Model. The pharmacodynamic effect was calculated as the maximal T1 percentage change relative to baseline, where baseline is defined as the average T1 amplitude over the 2 min before drug injection. The amplitude of T1 was expressed as a percentage 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 data set 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 pharmacokinetic/pharmacodynamic model was attempted using an effect compartment model⁴ to describe the delay observed between plasma concentrations and pharmacodynamic effect using a first-order rate constant (K_{eo}) along with the sigmoid E_{max} model (equation 1):

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

where CE is the concentration in the effect compartment, E_{max} 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 E_{max} 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} ; fig. 1). Interindividual variability was added to K_{out} and EC_{50} as an ω block matrix. An additive residual error was used for the pharmacokinetic/pharmacodynamic model.

Simulations. Simulations were conducted using the final population pharmacokinetic and pharmacokinetic/pharmacodynamic models using the final NONMEM estimates for parameters, interindividual variability, and residual error. Plasma concentrations and percentage of blockade were simulated after single 2 \times , 3 \times , or 4 \times the ED_{95} doses in 1,000 subjects each. Weights were randomly selected from a normal distribution from the study population.³ During the simulations, plasma concentration and percentage of blockade observations were made every 15 s for the first

4 min, and every min thereafter for a total of 150 min. All simulated pharmacodynamic 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 to 75% recovery index). The times to reach various degrees of blockade and recovery were defined as the third consecutive simulated value that exceeded the clinical cutoff. A time of 10 min postdose 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 population pharmacokinetic and pharmacokinetic/pharmacodynamic 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 pharmacokinetic and population pharmacokinetic analyses and 27 subjects were included in the pharmacokinetic/pharmacodynamic analysis. There were 14 males and 14 females who were an average of 35 yr of age (19 to 50 yr of age), weighing an average of 75 kg (range, 52 to 95.7 kg) with an average body mass index 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 Heerd *et al.*³ The non-compartmental pharmacokinetic parameters of CW002 are summarized in table 1. The clearance was intermediate, whereas the V_{ss} was low. The half-life was 23 to 27 min across doses.

Population Pharmacokinetic Model

The four-compartment model with elimination from the central compartment was selected as the final population pharmacokinetic model because it resulted in improvements in the goodness-of-fit plots and a statistically significant drop in the objective function value when compared to a three-compartmental model and any of the following competing three- and four-compartmental models: (1) models where central volume was fixed to the plasma volume reported in the literature,⁵ ($0.03575 \times \text{weight [kg]}$), assuming hematocrit is 45% of blood volume; (2) models that excluded the initial two data points (0.5 and 1 min) to evaluate whether the lack of homogeneity of mixing was influencing the parameter estimates; and (3) models with elimination from the central and peripheral compartments (where rate constant is assumed to be equivalent in the central and peripheral compartments).

The four-compartment model with elimination from the central compartment had significantly improved goodness-of-fit plots compared to the three-compartment model with elimination from the central compartment, especially for the conditional weighted residuals *versus* time and individual-weighted residuals *versus* individual predictions (Supplemental Digital Content 1, <http://links.lww.com/ALN/B660>)

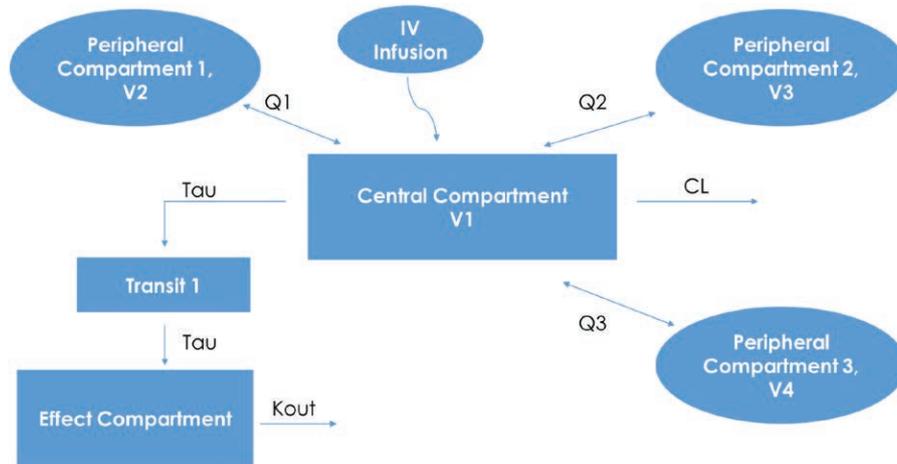


Fig. 1. Schematic of the pharmacokinetic/pharmacodynamic model. CL = clearance from the central compartment; IV = intravenous; Kout = first-order rate constant for drug leaving the effect compartment; Q1–3 = distributional clearance from the central compartment to peripheral compartments; Tau = first-order rate constant for drug into transit and effect compartments; V1–4 = volume for central and peripheral compartments.

Table 1. Noncompartmental Pharmacokinetic Parameters 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)	2,360 (995)	7,900 (684)	397 (55.9)	9,330 (2,110)
0.06 mg/kg	26.3 (2.13)	2,660 (1,500)	9,610 (1,560)	399 (109)	8,800 (1,540)
0.08 mg/kg	27.0 (2.14)	3,610 (1,950)	14,900 (2,250)	387 (51.5)	9,580 (1,310)
0.10 mg/kg	23.9 (2.44)	5,020 (1,630)	18,200 (1,940)	438 (66.9)	9,020 (1,470)
0.14 mg/kg	24.8 (1.06)	9,120 (5,060)	30,400 (4,880)	366 (41.9)	7,840 (1,610)

The pharmacokinetics parameters are presented as geometric means (SD).

AUC_{0-inf} = area under the concentration-time curve from zero to infinity; CL = clearance; C_{max} = maximum concentration; V_{ss} = volume of distribution at steady state.

due in part to its ability to accurately predict the initial time points. In addition, there was a much lower objective function value with the addition of a fourth compartment, decreasing from -642.704 to -906.165 for the three- and four-compartment models, respectively.

The final population pharmacokinetic model parameters are listed in table 2 with goodness-of-fit plots given in Supplemental Digital Content 2 (<http://links.lww.com/ALN/B661>). All population pharmacokinetic parameters were estimated with good precision. All random effects were estimated with low shrinkage (less than or equal to 7.4%). The interindividual variability was very low for weight-normalized clearance (10.8%), low to moderate for distributional clearances from the central to the first and second peripheral compartments (23.3 and 15.2%, respectively), and moderate for the central volume (34.6%). An ω block matrix was estimated to account for correlation between the clearance, distributional clearances from the central to the first and second peripheral compartments, and central volume in individual subjects. There was no appearance of nonlinearity of pharmacokinetic parameters as a function of dose. The estimates from the nonparametric bootstrap showed good agreement with the NONMEM estimates for all pharmacokinetic 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 (fig. 2) and additional cohorts (Supplemental Digital Content 3, <http://links.lww.com/ALN/B662>). All simulations of concentrations at matched observation times required for the pharmacokinetic/pharmacodynamic model were computed using the NONMEM final parameter estimates presented in table 2. When compared to the noncompartmental parameters (clearance and V_{ss}), the predicted parameters from the population pharmacokinetic 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).

Pharmacokinetic/Pharmacodynamic Model

Final parameters for the population pharmacokinetic model were fixed, and the pharmacodynamic parameters were estimated for the pharmacokinetic/pharmacodynamic model. During pharmacokinetic/pharmacodynamic model development, the basic effect compartment model or a single transit model (*i.e.*, either of which only had one 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

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 Digital Content 4, <http://links.lww.com/ALN/B665>). Model performance was assessed visually by plotting the observed clinically important times to onset and recovery for the $2 \times \text{ED}_{95}$ dose (0.14 mg/kg)³ versus the simulated times for each clinical endpoint using the pharmacokinetic/pharmacodynamic model. As shown in figure 3, the predicted and observed times to various levels of blockade and recovery for the $2 \times \text{ED}_{95}$ 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 $2 \times$, $3 \times$, and $4 \times \text{ED}_{95}$ dose, respectively. The 25 to 75% recovery indices were consistent across all simulated doses with predicted times of 12, 11, and 12 min for $2 \times$, $3 \times$, and $4 \times \text{ED}_{95}$ doses, respectively. The simulated times to 95% recovery for the $2 \times$, $3 \times$, and $4 \times \text{ED}_{95}$ doses were 57, 66, and 75 min, respectively.

Discussion

In the present study, the pharmacokinetics, population pharmacokinetics, and pharmacokinetics/pharmacodynamics of CW002, an investigational intermediate duration neuromuscular blocking agent, are presented for this healthy subject study. These results confirm that, despite the novel process of cysteine inactivation, CW002 has very similar pharmacokinetic properties to other intermediate acting neuromuscular blockings in humans.^{6–8} Population pharmacokinetic and pharmacokinetic/pharmacodynamic 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 population pharmacokinetic and pharmacokinetic/pharmacodynamic analyses also contributed to information about the general properties of CW002.

The half-life of CW002 (20 to 30 min) is consistent with intermediate duration neuromuscular blocking agents. CW002 has a very low interindividual variability (10.8%) in clearance, suggesting that variability in endogenous cysteine is low. CW002 also appears to exhibit linear kinetics across a dose range of 0.04 to 0.14 mg/kg, suggesting that this neuromuscular blocking agent would have very predictable exposures. The mean noncompartmental clearance estimate for CW002 was $5.36 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$. This clearance is similar to or faster than clearance estimates for other drugs in this class, e.g., vecuronium ($5.2 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$),⁹ rocuronium ($2.67 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$),¹⁰ and cisatracurium ($4.57 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$).¹¹

The final population pharmacokinetic model selected was an empirical four-compartment open mammillary model. The clearance 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 population pharmacokinetic 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 pharmacokinetics/pharmacodynamics after administration of higher CW002 doses, which was the primary objective of this analysis.

The first challenge in the development of the population pharmacokinetic 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-s 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 nonhomogeneity of mixing unsuccessfully (unpublished observations). For the current model, models were tested that excluded the early time points (less than or equal to 0.5 and less than or equal to 1 min), from fitting with three- and four-compartment

Table 3. NONMEM and Bootstrap Estimates for the Population Pharmacokinetic/Pharmacodynamic Model

Parameter	Final Model Estimates		Interindividual Variability		Bootstrap Estimates		Interindividual Variability	
	Typical Value	RSE, %	Magnitude	RSE, %	Median	95% CI	Magnitude	95% CI
EC_{50} , ng/ml	4,730	17.2	27.6% CV	16.6	4,770	3,370–6,870	27.6% CV	19.3–35.0% CV
K_{out} , 1/min	0.0566	7.50	24.8% CV	17.3	0.0562	0.0485–0.0658	24.5% CV	15.8–33.5% CV
Hill coefficient	5.80	9.00	0% CV	Fixed	5.90	4.90–7.20	0% CV	Fixed
$1/\text{Tau}$, 1/min*	0.712	12.6	0% CV	Fixed	0.717	0.566–0.932	0% CV	Fixed
cov(IIV in K_{out} , IIV in EC_{50})	–0.0398	–50.0	NA	NA	NA	NA	NA	NA
Residual variability	3.60%	8.30	NA	NA	NA	NA	NA	NA

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

CV = coefficient of variation; IIV = interindividual variability; NA = not applicable; RSE = relative standard error.

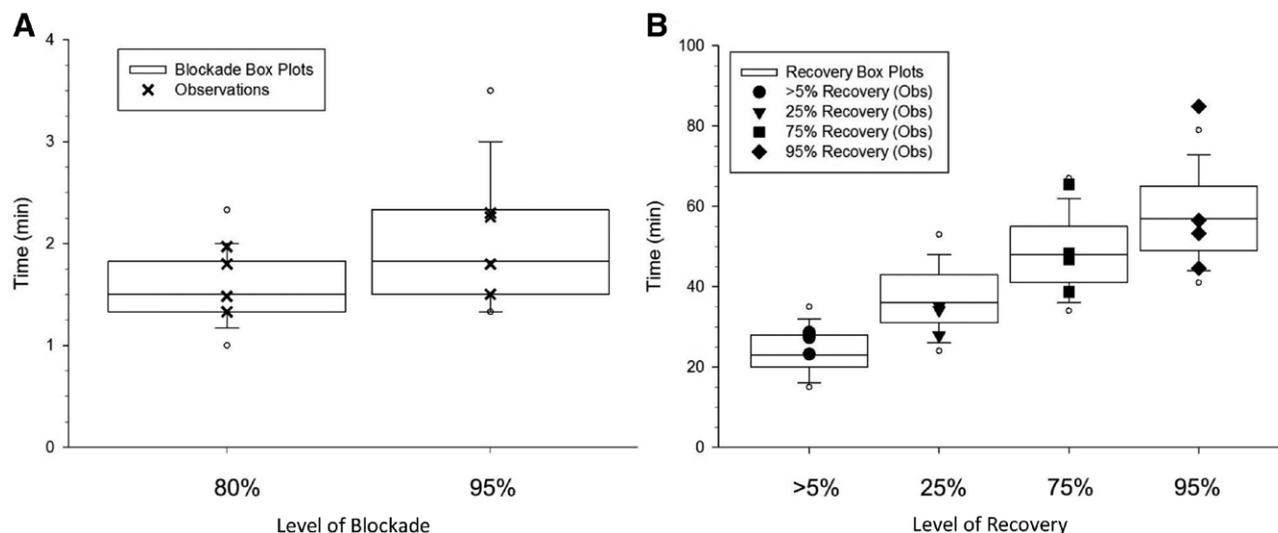


Fig. 3. Predicted *versus* observed time to onset (A) and offset (B). Obs = observed.

Table 4. Predicted Times to Onset and Offset for 2 \times , 3 \times , and 4 \times ED₉₅ Doses

	Dose		
	2 \times ED ₉₅ 0.14 mg/kg (min)	3 \times ED ₉₅ 0.21 mg/kg (min)	4 \times ED ₉₅ 0.28 mg/kg (min)
Time to Onset			
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)

The values are presented as the median (5th and 95th percentile). Recovery was determined by the third observation at or below the threshold 10 min after the dose for each simulation.

models, either as the sample taken at less than or equal to 0.5 min alone or both together. The four-compartment model objective function value was 72 points lower than the three-compartment model when the data taken at less than or equal to 1 min were excluded from fitting. In both models, the average clearance values were still 70 to 100 ml/min lower than those estimated from noncompartmental analysis. The V_{ss} of the four-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 clearance and V_{ss} estimates from the population pharmacokinetic 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 four-compartment model still outperformed the three-compartment model in objective function value and goodness-of-fit plots.

Because 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 three- and four-compartment model. When the central volume of a three-compartment model was fixed to the estimate of plasma volume, the clearance and V_{ss} were closer to the estimates derived from noncompartmental analysis, but the early concentrations were very biased (Supplemental Digital Content 5, <http://links.lww.com/ALN/B663>), 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 Digital Content 6, <http://links.lww.com/ALN/B664>). Although 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 four-compartment model with central volume fixed to the estimate of the plasma volume, interindividual variabilities on clearance and distributional clearances from the central to the first and second peripheral compartments each approached zero; reducing the number of parameters with interindividual 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 elimination.¹² 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, three- and four-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 four-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 clearance 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 clearance from multiple routes. Because the primary goal of this project was to predict neuromuscular blockade at higher doses, we chose the population pharmacokinetic model that best described the observed concentration–time profile to predict the neuromuscular blockade over time. Of the pharmacokinetic/pharmacodynamic models evaluated, the current transit model with the four-compartment population pharmacokinetic 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 pharmacokinetic/pharmacodynamic 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 clearance and V_{ss} , whereas the population pharmacokinetic model is most suitable for predictions of concentrations over time, which can serve as input into the pharmacokinetic/pharmacodynamic model to predict the resulting neuromuscular blocking profile.

The pharmacokinetic/pharmacodynamic model revealed that CW002 is an intermediate duration neuromuscular blocking agent with a rapid onset. Simulations of higher CW002 doses predicted that the onset of complete neuromuscular blockade may be achieved in less than or equal to 60 s 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, whereas a $4 \times ED_{95}$ dose is predicted to result in a duration consistent with long-acting neuromuscular blocking 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. Before 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, pharmacokinetic/pharmacodynamic 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. Because sevoflurane was used instead of balanced anesthesia and because inhalation agents are known to potentiate neuromuscular blockade, one would expect the neuromuscular blocking profile results presented in this article to differ (*i.e.*, shorter duration, slightly slower onset) when CW002 is administered with balanced anesthesia.

It is important to note that the pharmacokinetic/pharmacodynamic 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 propofol.¹⁴ 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 Digital Content 7, <http://links.lww.com/ALN/B666>), regardless of whether a three- or four-compartment population pharmacokinetic model was used. This was surprising given that the effect compartment model was specifically developed for neuromuscular blocking agents⁴ and may be related to the low availability of data at higher doses (*e.g.*, only four 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 4,730 ng/ml, which is much higher than that reported for cisatracurium¹⁵ or rocuronium.¹⁶ The interindividual 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 percentage of blockade observations and predictions *versus* time graphs for the individuals in cohorts 4, 5, 6, and 8 are provided in Supplemental Digital Content 8 (<http://links.lww.com/ALN/B667>).

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 Digital Content 8 (<http://links.lww.com/ALN/B667>). This led to disruption in the pharmacodynamic 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 percentage of blockade and concentration values. This led to intervals where the simulated subjects were demonstrating full blockade, but their predictions fell between

93 to 100% blockade. Importantly, this is consistent with other intermediate neuromuscular blocking agents. Once recovery begins, recovery is relatively rapid. For instance, the simulations showed that the 25 to 75% recovery index is predicted to be independent of dose (12, 11, and 12 min for 2 \times , 3 \times , and 4 \times ED₉₅ doses, respectively), with values similar to other neuromuscular blocking agents such as cisatracurium,¹¹ atracurium,¹⁷ vecuronium,¹⁷ and rocuronium¹⁰ (with 25 to 75% recovery index values of 13, 13, 10, and 13 min, respectively).

In summary, the current article presents the pharmacokinetics, population pharmacokinetics, and pharmacokinetics/pharmacodynamics of CW002, an investigational neuromuscular blocking agent, with a rapid onset and an intermediate duration of action. The pharmacokinetic/pharmacodynamic 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.

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Competing 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, New York). Dr. Owen served as a paid consultant for the population pharmacokinetic analysis.

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