Dose–response and Cardiopulmonary Side Effects of the Novel Neuromuscular-blocking Drug CW002 in Man

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ABSTRACT

Background: CW002 is a benzylisoquinolinium nondepolarizing neuromuscular-blocking drug found to be inactivated by cysteine in preclinical studies. The current study represents a dose escalation clinical trial designed to describe CW002 potency, duration, cardiopulmonary side effects, and histamine release.

Methods: Healthy subjects anesthetized with sevoflurane/nitrous oxide were divided into five groups (n = 6), each receiving a fixed CW002 dose (0.02, 0.04, 0.06, 0.08, or 0.10 mg/kg), and one group (n = 4) receiving 0.14 mg/kg. Blood pressure and heart rate were continuously recorded along with airway dynamic compliance. Neuromuscular blockade was assessed with mechanomyography at the adductor pollicis. Arterial blood was obtained before and after CW002 injection for analysis of plasma histamine concentration. Potency was estimated from a baseline sigmoid Emax model.

Results: ED_{50} was found to be 0.036 mg/kg (95% CI, 0.020 to 0.053 mg/kg) and ED_{95} 0.077 mg/kg (95% CI, 0.044 to 0.114 mg/kg). At 0.14 mg/kg (1.8 × ED_{95}), 80% twitch depression occurred in 94 ± 18 s with complete block in 200 ± 87 s. Clinical recovery (25% of maximum twitch) occurred in 34 ± 3.4 min, with a 5 to 95% recovery interval of 35.0 ± 2.7 min. The time to a train-of-four ratio greater than 0.9 ranged from 59 to 86 min. CW002 did not elicit histamine release or significant (greater than 10%) changes in blood pressure, heart rate, or dynamic airway compliance.

Conclusions: In healthy subjects receiving sevoflurane/nitrous oxide, CW002 at 1.8 × estimated ED_{95} produces a clinical duration less than 40 min, elicits no histamine release, and has minimal cardiopulmonary side effects. **(ANESTHESIOLOGY 2016; 125:1136-43)**

PRECLINICAL studies have shown that CW002 is one of a series of nondepolarizing benzylisoquinolinium neuromuscular-blocking drugs that are irreversibly inactivated by interaction with cysteine.[1–4] These animal studies demonstrated that the administration of cysteine at any point during CW002-induced neuromuscular blockade (*i.e.*, before any muscle recovery is established) can facilitate rapid restoration of muscle strength.[2,5] Evaluation in multiple species has demonstrated that CW002 is a relatively potent neuromuscular-blocking drug with an intermediate duration of action, ranging from 27 ± 7 min in monkeys to 47 ± 9 min in dogs.[1,6,7] In a clinically relevant dose range of 2 to 4 × the dose required to produce 95% neuromuscular blockade (ED_{95}), these studies found CW002 to have little potential for histamine release, minimal cardiovascular and autonomic nervous system effects, and no bronchoconstrictive properties.[1,6–8] The current investigation was a phase 1, first-in-man study designed to provide clinical data on CW002 potency and neuromuscular blockade onset and recovery, along with dose-related effects on plasma histamine concentration, blood pressure, heart rate (HR), and ventilation dynamics. For this preliminary trial, the specific role of cysteine in the clinical pharmacology of CW002 was not evaluated.

What We Already Know about This Topic

- The benzylisoquinolinium nondepolarizing neuromuscular-blocking drug CW002 had an ED_{95} of 0.01 to 0.04 mg/kg in a variety of animal models and an intermediate duration of action
- CW002 had minimal cardiopulmonary side effects and caused no histamine release in animal models until doses much larger than the ED_{95} were administered

What This Article Tells Us That Is New

- The ED_{95} of CW002 in man is approximately 0.077 mg/kg
- The clinical onset time of CW002 in man at a dose that was 1.8 × the ED_{95} was approximately 90 s, and its mean clinical duration was 34 min
- The ED_{95} of CW002 in man produced minimal cardiopulmonary side effects and no histamine release

This article is featured in "This Month in Anesthesiology," page 1A. The work was performed at Department of Anesthesiology, Weill Medical College of Cornell University, New York, New York.

The findings of this study were presented, in part, at the 2013 annual meeting of the International Anesthesia Research Society in San Diego, California, May 4–7, 2013, and the 2013 annual meeting of the American Society of Anesthesiologists in San Francisco, California, October 12–16, 2013.

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Materials and Methods

Study Recruitment

This open-label, ascending dose study (ClinicalTrials.gov NCT01338935) was approved by the Institutional Review Board of Weill Cornell Medical Center, New York, New York. Thirty-four healthy subjects (17 male and 17 female) between the ages of 18 and 55 yr were recruited for the study between October 2012 and October 2013 and gave written, informed consent before participation. Exclusion criteria included significant chronic disease, tobacco use, weight 30% greater than or less than ideal body weight, a history of drug use, allergies, and a history of difficult tracheal intubation. Female participants of childbearing potential were using an acceptable method of birth control and were not pregnant. All study subjects underwent a screening clinical assessment within 2 weeks 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 complete blood count). All laboratory tests were repeated the morning of the exam, and for female participants, a negative urine pregnancy test was confirmed.

Subjects were assigned sequentially into five equal groups of 6, each receiving a single CW002 dose (0.02, 0.04, 0.06, 0.08, or 0.10 mg/kg). Once enrollment in a study group was completed, enrollment in the next one was begun, with men and women included in each. After initial estimates of potency (quantified as the effective dose producing 50 and 95% neuromuscular blockade or ED_{50} and ED_{95}, respectively), an additional cohort of four subjects received 0.14 mg/kg CW002. Data from one participant who received 0.08 mg/kg were omitted due to technical difficulties.

Study Protocol

On the day of each study, subjects were kept *non per os* after midnight, and after morning admission to the Weill Cornell Clinical Research Center, hydrated with intravenous lactated Ringer's solution. Each study began in the afternoon after confirmation of no change in physical status or laboratory values. Once in the operating room, electrocardiogram leads along with a blood pressure cuff and pulse oximeter were applied, and the study subject's lungs were preoxygenated with 100% O_2. Anesthesia was induced with propofol (3 to 5 mg/kg) and sevoflurane, and 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 H_2O positive end-expiratory pressure (PEEP). Respiratory rate was adjusted to maintain end-tidal CO_2 at 32 to 40 mmHg. Anesthesia was maintained with N_2O (70%) and sevoflurane (0.8 to 1.2% end-tidal).

A radial arterial catheter was inserted for blood sampling and continuous blood pressure recording. Throughout the study, peak inspiratory pressure (PIP), PEEP, tidal volume, esophageal temperature, and the bispectral index were continuously recorded. The depth of neuromuscular block was assessed at the adductor pollicis using stimulation of the ulnar nerve and mechanomyography. Supramaximal stimuli were delivered *via* surface electrodes placed over the ulnar nerve at a frequency of 0.10 Hz from a Grass (Grass Instruments, USA) model S88 stimulator in conjunction with a Grass Stimulation Isolation Unit (Grass Instruments). The strength of contraction of the adductor pollicis in response to stimulation was measured using a Grass Model FT-10 force displacement transducer (Grass Instruments) applied to the thumb. These methods were consistent with previously described guidelines.[9]

At least 30 min after achieving a steady end-tidal concentration of sevoflurane and 20 min after beginning single twitch (T1) neuromuscular stimulation, CW002 was administered as a bolus over 5 s into a freely running intravenous catheter. The adductor pollicis response to ulnar nerve stimulation was continuously monitored for maximal suppression of T1. Once initial recovery of neuromuscular function was evident, the stimulation mode was changed to train-of-four (TOF; 0.2-ms square waves at 2 Hz for 2 s) applied every 20 s and continued until the ratio of T4 to T1 (TOFR) was greater than or equal to 0.9. Subjects were then allowed to emerge from anesthesia, the trachea extubated, and monitoring continued in the postanesthesia care unit for a minimum of 2 h. Adequacy of strength, as determined by head lift, hand grip, ability to oppose incisors and negative inspiratory force was assessed every 15 min after tracheal extubation until discharge from the postanesthesia care unit. Study participants were then transferred to a holding area for observation for an additional 1 to 2 h before discharge home with a responsible adult.

Data Acquisition and Cardiopulmonary Effects

Continuous mechanomyography data along with arterial pressure and the electrocardiogram (lead II) were recorded to disc (LabChart; ADInstruments, Australia), and a peak detection algorithm was used to discriminate the individual components of the TOF response. Simultaneously, samples at 15-s intervals from an automated record keeping system (CompuRecord; Philips Healthcare, USA) were used to quantify end-tidal gas concentrations, airway pressures, tidal volume, arterial oxygen saturation, the bispectral index, and dynamic airway compliance (DC), calculated as tidal volume/(PIP – PEEP). During each study, a technician at the bedside observed mean arterial pressure (mAP), HR, and DC at regular intervals before and after CW002 injection, noting conditions under which any apparent changes occurred (*i.e.*, coughing). Events flagged as potentially significant in individual subjects, defined as changes greater than 10%

from pre-CW002 baseline, were then further assessed after the study from continuously recorded data and written records.

Potency Determination

A dose–T1 depression curve for CW002 was constructed from all measurements using a baseline sigmoid Emax model with a maximum of 100 and minimum of 0 (GraphPad Prism 7.0; GraphPad Software, USA). From the curve parameters, the doses corresponding to 50 and 95% T1 depression (ED_{50} and ED_{95}, respectively) were calculated with 95% CI. The study was designed under the assumption that at least 24 measured responses would be greater than 0 and less than 100, meeting sample size recommendations for assessing clinical potency of a neuromuscular-blocking drug.[10]

Onset and Recovery Times

From doses producing more than 95% block, *clinical onset* was defined as the time from CW002 injection to 80% depression of T1, and *complete onset* defined as the time to three consecutive responses of the same twitch height less than 5% of baseline, or complete loss. Recovery was monitored as the time required for the return of T1 amplitude to 5, 25, 75, and 95% of maximal recovery, as well as the TOFR reaching 0.9. These data were derived by plotting T1 and T4/T1 as continuous variables *versus* time and solving the best fit line for each recovery point (SigmaPlot 13; Systat Software, Inc., USA). The 5 to 95% and 25 to 75% recovery intervals in minutes were then calculated. The *clinical duration* of CW002 was defined as the time from bolus administration to 25% recovery of T1, and *total duration* defined as the time from administration to a TOFR greater than or equal to 0.9.

Plasma Histamine Analysis

Arterial blood samples obtained 1 min before and 1 and 3 min after the administration of CW002 were analyzed for histamine concentration (Immunoassay kit; Immunotech International, France) as previously described.[1] These time points were based upon the reported half-life of infused histamine in humans (approximately 4 min),[11] and previous studies showing that histamine release by benzylisoquinolinium molecules, and high-dose CW002 in particular, is an acute event, with maximal effects achieved in the first few minutes after injection.[1,3] Each subject served as his/her own control, with either a five-fold change from baseline or an absolute value in excess of 2,000 pg/ml regarded as a positive response. These parameters were chosen in light of the high degree of intrasubject variation over time described for plasma histamine, and data indicating that high plasma levels of histamine are generally required to elicit systemic effects.[12,13]

Statistics and Data Analysis

Within each cohort, changes in mAP, HR, and DC from baseline (the mean of data during the 2 min before CW002 injection) to times 0 (immediately before injection), 1, 3, 5, and 10 min after administration were assessed by analysis of variance for repeated measures and the Bonferonni test after verification of normality (Shapiro–Wilk test). This time window was derived from preclinical studies showing the time course for cardiopulmonary responses to high-dose CW002.[1,2] Repeated-measures analysis of variance was also applied to plasma histamine concentrations 1 and 3 min after CW002 injection relative to measurements from blood sampled 1 min before injection. Statistical analysis was done within SigmaPlot 13 (Systat Software) using the "analysis" module. Data are presented as mean ± SD, and for all analyses, $P \leq 0.05$ was considered significant.

Results

Demographics

As shown in table 1, study subjects ranged in age from 19 to 51 yr and were equally distributed between men and women. Across all study groups, racial distribution was 29% white, 53% black, and 18% "other," with mean body mass index ranging from 26.8 ± 2.8 to 23.5 ± 2.0 kg/m^2.

Potency

Individual dose–response data for CW002 are shown in figure 1, highlighting the range of responses observed at each dose. Plotting all data points to a baseline sigmoid Emax model with a maximum of 100 and minimum of 0 yielded a Hill slope of 2.90 (95% CI, 2.19 to 3.97), an ED_{50} of 0.036 mg/kg (95% CI, 0.032 to 0.040 mg/kg), and an ED_{95} of 0.099 mg/kg (95% CI, 0.078 to 0.129 mg/kg). Given that the derived Hill slope was below the 3.6 to 5.1 range reported by Kopman *et al.*[14], for other nondepolarizing neuromuscular-blocking drugs, a robust nonlinear regression model (GraphPad 7.0; GraphPad Software) was applied to compensate for any impact of outlying data.[15] As shown in figure 1, this analysis increased the Hill slope to 3.81, had no effect on ED_{50}, (0.036 mg/kg; 95% CI, 0.020 to 0.053 mg/kg), and determined ED_{95} to be 0.077 mg/kg (95% CI, 0.044 to 0.114 mg/kg). Given the more appropriate Hill slope and no change in potency as defined by ED_{50}, the 0.077-mg/kg value was regarded as the more accurate estimate of ED_{95}.

Onset and Recovery

Three subjects receiving 0.08 mg/kg CW002 and all receiving 0.10 and 0.14 mg/kg developed more than 95% neuromuscular block. Individual onset and recovery times for these subjects are shown in table 2. At the highest dose of 0.14 mg/kg (1.8 × estimated ED_{95}), clinical onset (80% twitch depression) ranged from 75 to 111 s (mean, 94 ± 18 s) with complete onset achieved on average in 200 ± 87 s. Clinical duration (time to 25% T1 recovery) ranged from 28.8 to 36.1 min (mean, 33.8 ± 3.4 min), while 95% recovery of T1 occurred in 60.6 ± 6.5 min (range, 52 to 67 min), with a T4/T1 ratio greater than 0.9 reached in 72.8 ± 11.3 min (range,

Table 1. Volunteer Demographics

	CW002 Dose, mg/kg					
	0.02 (n = 6)	0.04 (n = 6)	0.06 (n = 6)	0.08 (n = 6)	0.10 (n = 6)	0.14 (n = 4)
Age, yr						
Mean (SD)	37.8 ± 10.4	36.3 ± 9.6	31.5 ± 8.7	33.0 ± 8.9	39.7 ± 8.7	34.0 ± 13.1
Range	24–51	25–49	26–49	22–48	30–50	19–46
Sex (n)						
Male	3	3	3	4	3	1
Female	3	3	3	2	3	3
Race (n)						
White	1	2	1	3	3	0
Black	3	2	4	2	3	4
Other	2	2	1	1	0	0
Body mass index, kg/m^2						
Mean (SD)	26.8 + 2.8	26.6 ± 4.0	23.5 ± 2.03	24.7 ± 2.9	26.6 ± 1.45	26.1 ± 3.16
Range	22–30	20–30	21–26	21–28	25–29	22–29

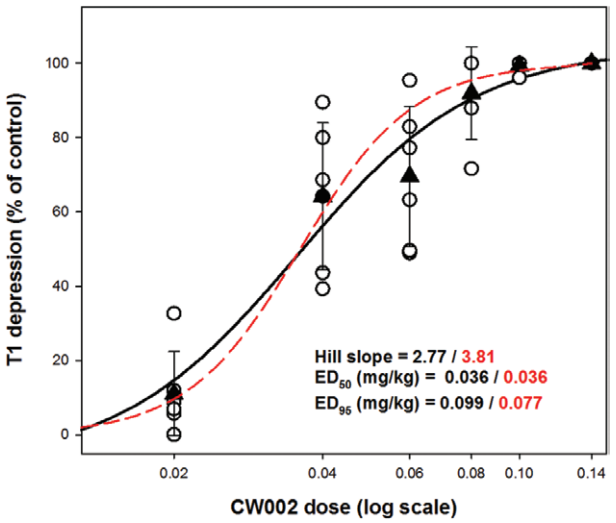

Fig. 1. The dose-muscle twitch (T1) depression response relationship for CW002 using both standard (*solid black line*) and robust (*hatched red line*) regression models. Individual responses at each dose are shown as *open circles* with mean ± SD as *closed triangles*. Numerical results for robust regression are shown in *red* and demonstrate no difference in the estimated effective dose for producing 50% neuromuscular blockade (ED_{50}) but a lower effective dose for producing 95% neuromuscular blockade (ED_{95}).

59 to 86 minutes). As also shown in table 2, the T1 recovery intervals for doses producing more than 95% neuromuscular blockade were relatively constant and independent of the CW002 dose, with 25 to 75% occurring, on average, in 14 to 15 min and 5 to 95% in 35 to 40 min.

Cardiopulmonary Side Effects

Data for mAP, HR, and DC from individual subjects in the three highest dose cohorts (0.08, 0.10, and 0.14 mg/kg) along with their mean are shown in figure 2. With data pooled, there were no differences relative to baseline for mAP at any dose, and none of the individual subjects exhibited a change in mAP exceeding 10% during the observation interval. In contrast, after injection of 0.10 and 0.14 mg/kg CW002, there was a small (no more than 7 beats/min) but consistent increase in HR. This effect was present 3, 5, and 10 min after 0.10 mg/kg and 1, 3, 5, and 10 min after 0.14 mg/kg. In only one of the volunteers was the increase from baseline more than 10% (10.8%) at any time point. On average, there was no change in DC from baseline at any time point for any dose. However, single subjects in the 0.08, 0.10, and 0.14 mg/kg groups exhibited a decline in DC of more than 10% for one post-CW002 injection time point. These observations were targeted for the secondary review of both written records and continuous data recordings. For the subject that received 0.08 mg/kg CW002, the measured decline in DC occurred 10 min after drug injection and was coincident with ventilator manipulations made to offset a rising end-tidal carbon dioxide tension. In the study subject that received 0.10 mg/kg CW002, there was a brief decline in DC observed in response to coughing before the full onset of neuromuscular blockade. The secondary review of written documents and continuous data recordings for the single subject with a greater than 10% decline in DC after 0.14 mg/kg CW002 indicated a response that was slow in onset and largely coincident with both the onset of neuromuscular blockade and a rise in HR.

Histamine Release

Consistent with the high inter- and intrasubject variation in plasma histamine previously reported,[12] measured baseline values ranged from 131 to 739 pg/ml, 1 min post-CW002 values ranged from 103 to 866 pg/ml, and 3-min post-CW002 values from 175 to 840 pg/ml. Focusing on the three doses at or above ED_{95} (0.08, 0.10, and 0.14 mg/kg; fig. 3), none of the subjects showed greater than or equal to five-fold increase from pre-CW002 baseline or an absolute value exceeding 2,000 pg/ml at any time point. There were no differences from baseline values at either 1 or 3 min after administration of any CW002 dose.

Table 2. Onset and Recovery Data

	0.08 mg/kg				0.10 mg/kg				0.14 mg/kg			
	Individual Subjects			Mean ± SD	Individual Subjects			Mean ± SD	Individual Subjects			Mean ± SD
80% T1 suppression, s	134	118	106	119 ± 14	135	115	119	100	107	118 ± 12	75	111	108	82	94 ± 18
Maximal T1 suppression, s	260	263	305	276 ± 25	249	283	311	252	228	268 ± 29	127	318	212	141	200 ± 87
5% T1 recovery, min	11.1	12.3	13.2	12.2 ± 1.1	10.3	20.9	20.3	17.7	15.6	16.2 ± 4.9	25.6	19.9	25.8	28.5	25.0 ± 3.6
25% T1 recovery, min	24.8	19.5	21.5	22.0 ± 2.7	20.0	31.0	28.8	26.6	20.3	24.8	25.3 ± 5.0	34.5	28.8	35.9	36.1	33.8 ± 3.4
75% T1 recovery, min	46.5	29.4	34.2	36.7 ± 8.8	32.5	48.8	45.9	41.3	33.7	40.0	40.5 ± 7.2	48.3	41.9	51.0	50.9	48.0 ± 4.3
95% T1 recovery, min	54.7	38.2	47.8	46.9 ± 8.3	44.6	68.5	66.7	52.2	44.6	56.1	55.3 ± 11.6	60.2	51.8	63.5	67.0	60.6 ± 6.5
T4:T1 > 0.9, min	60.6	44.1	53.8	52.8 ± 8.3	48.9	83.9	89.1	64.3	54.8	64.4	68.2 ± 17.7	68.7	59.3	77.3	85.8	72.8 ± 11.3
5–95% recovery interval, min	43.6	25.9	34.6	34.7 ± 8.9	34.3	47.6	46.4	34.5	32.9	40.5	39.4 ± 6.5	34.5	31.9	37.7	38.5	35.6 ± 3.1
25–75% recovery interval, min	21.7	9.8	12.7	14.7 ± 6.2	12.5	17.7	17.1	14.8	13.5	15.2	15.1 ± 2.0	13.8	13.1	15.1	14.8	14.2 ± 0.9

T1 = the twitch response to a single stimulus or the first response to a sequence of four stimuli delivered over 2 s; T4 = the fourth twitch response to a sequence of four stimuli delivered over 2 s.

Discussion

CW002 is a benzylisoquinolinium neuromuscular-blocking drug that has undergone preclinical evaluation in rabbits, cats, guinea pigs, dogs, and nonhuman primates.[1,2,5–8] In these studies, CW002 was found to have an ED_{95} of 0.01 to 0.04 mg/kg with an intermediate duration of action. Dose escalation research with CW002 in animal models has indicated minimal cardiopulmonary side effects and no histamine release until doses far in excess of ED_{95} are administered. The current study demonstrates that CW002 is less potent in humans compared with animal models (ED_{95} approximately 0.077 mg/kg). At 0.14 mg/kg (1.8 × estimated ED_{95}), CW002 exhibits a clinical onset time of roughly 90 s and a mean clinical duration of 33.8 min (range, 28.8 to 36.1 min), while eliciting minimal cardiopulmonary side effects and no histamine release. Although the relatively small sample size precludes definitive conclusions, recovery intervals for T1 after CW002 doses producing more than 95% neuromuscular blockade appear to be dose independent consistent with drug inactivation in the plasma,[16] a finding in agreement with preclinical studies.[5]

The first neuromuscular-blocking drug in the class of *bis*- and mixed-tetrahydroisoquinolinium chlorofumarates was gantacurium, an asymmetrical mixed-onium chlorofumarate with an ultrashort duration of action.[4,17,18] While preclinical evaluation of gantacurium was generally favorable,[19,20] a clinical trial suggested a greater potential for histamine release than anticipated,[21] and further development has been suspended. Nonetheless, early work in animal models established that the complete paralysis present just minutes after the administration of gantacurium could be rapidly reversed by intravenous injection of L-cysteine.[5] This unique pharmacology led to the development of CW002, a nonhalogenated symmetrical benzylisoquinolinium fumarate diester that interacts *in vitro* more slowly with cysteine and, in experimental animals, can be reversed at any time by intravenous injection of L-cysteine.[2,3,5,7]

For the current study, anesthesia with sevoflurane and nitrous oxide was chosen due to concerns that the high plasma propofol concentrations achieved during total intravenous anesthesia may interfere with the liquid chromatography-tandem mass spectrometry analysis of CW002 for pharmacokinetic profiling (data not presented). The administration of sevoflurane, however, undoubtedly influenced CW002 potency and duration.[22–24] Overall, study results indicate that with an ED_{50} of 0.036 mg/kg in healthy volunteers, CW002 is less potent than either cisatracurium or vecuronium,[23,24] and more potent than rocuronium,[25,26] in patients receiving sevoflurane. Despite the use of volatile agent anesthesia, CW002 in the current human trial did not elicit prolonged neuromuscular blockade at 1.8 × estimated ED_{95}, producing an average clinical duration of 34 min, a 25 to 75% recovery interval of 14 min, and a TOFR of 0.9 in 73 minutes. For comparison, in a methodologically similar study (sevoflurane/nitrous oxide anesthesia, single bolus

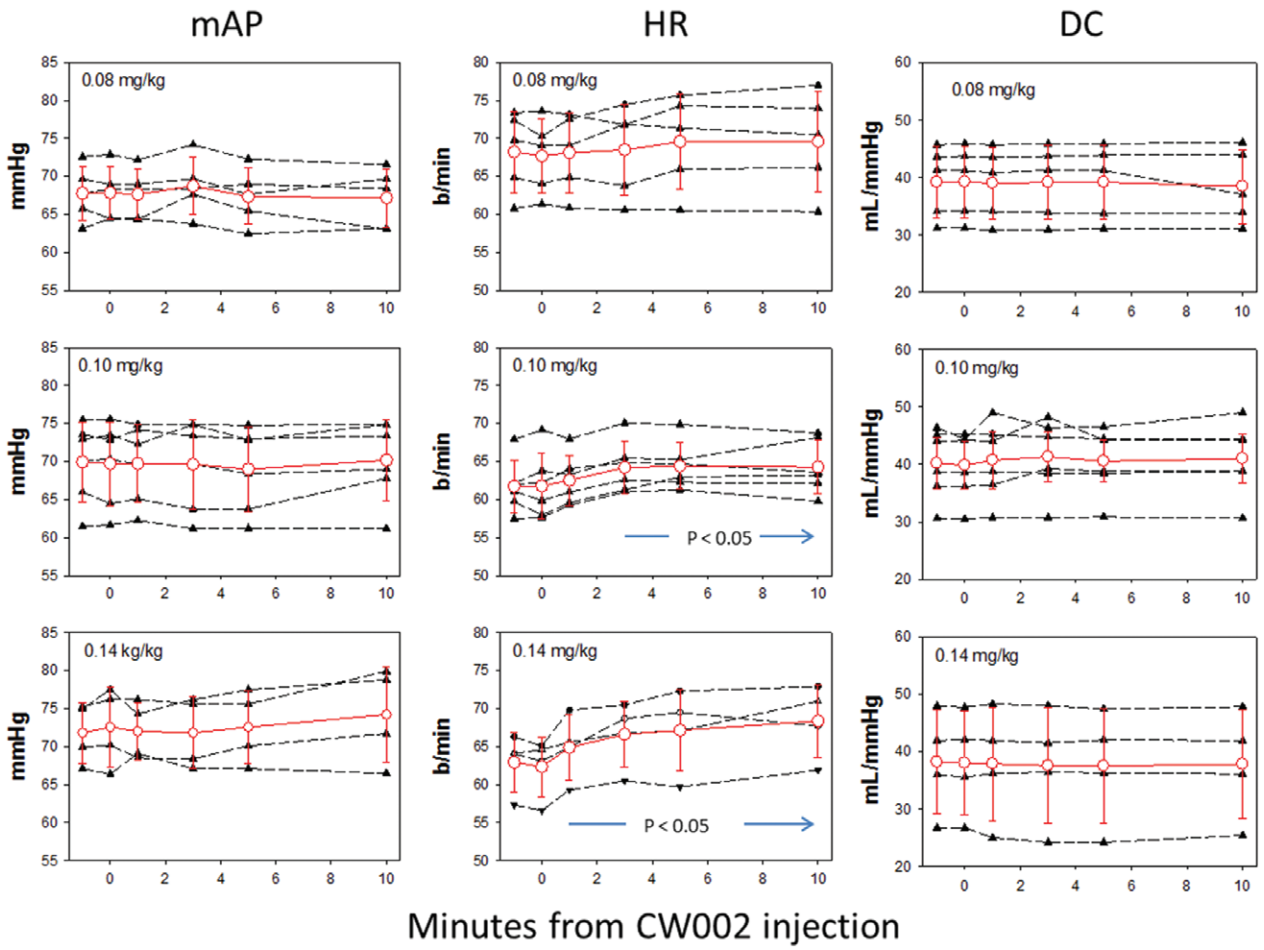

Fig. 2. Mean arterial pressure (mAP), heart rate (HR), and airway dynamic compliance (DC) measured before CW002, at injection (0 time) and 1, 3, 5, and 10 min after administration of the three highest doses. *Triangles* connected by *dashed lines* depict data for individual volunteers; *open circles* connected by *solid lines* represent mean ± SD for the cohort. HR was increased relative to baseline ($P \leq 0.05$) during a range of time points after injection of both 0.10 and 0.14 mg/kg CW002 (designated by *arrows*).

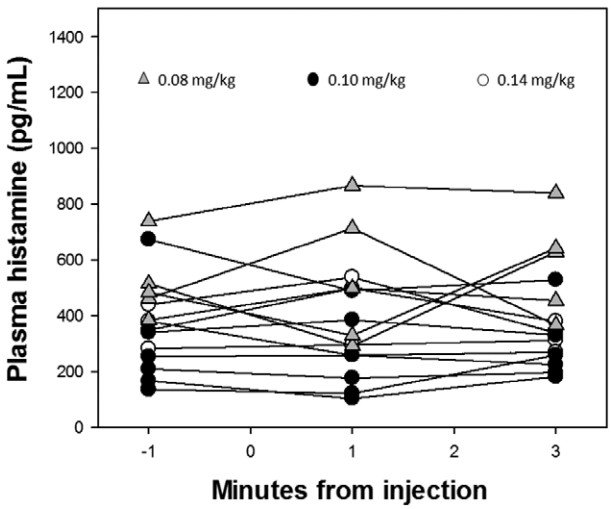

Fig. 3. Plasma histamine concentrations 1 min before and 1 and 3 min after CW002 injection at the three highest doses (0.08, 0.10, and 0.14 mg/kg).

drug dosing, and force displacement twitch recording), Lowry *et al.*[26] reported that 2.3 × ED_{95} rocuronium had a clinical duration of 45 min, a 25 to 75% recovery interval of 26 min, and a TOFR of 0.8 in 103 min. In the current study, recovery intervals after CW002 at 0.08, 0.10, and 0.14 mg/kg remained essentially unchanged. This response is similar to that reported for other benzylisoquinoliniums and the ultrashort-acting chlorofumarate molecule gantacurium, reflecting the lack of dependence on renal or hepatic elimination.[3,27,28]

Within the dose range used (0.02 to 0.14 mg/kg), the data are consistent with preclinical investigation in terms of no demonstrable effects on blood pressure or plasma histamine concentration. Current results do suggest that CW002 in doses at or above 0.10 mg/kg can increase HR in healthy humans. While small (less than or equal to 7 beats/min), this response was consistent among subjects receiving 0.10 and 0.14 mg/kg and probably reflects a weak vagolytic

effect[7] demonstrable in healthy subjects with low resting HRs. Pooled data did not indicate any consistent effect on ventilatory dynamics at any dose. However, one subject in each of the 0.08-, 0.10-, and 0.14-mg/kg groups exhibited a DC decline greater than 10% relative to baseline at some point after CW002 injection, prompting further analysis of the simultaneous measurements used to calculate DC (PIP, PEEP, and tidal volume). This assessment incorporated two main considerations. First, that DC is affected by factors both physiologic (airway resistance, chest wall stiffness, diaphragmatic tone, and atelectasis) and technical (ventilator settings and in-line humidifiers). Second, that small changes in PIP produced by any cause can alter calculated DC, especially when incorporated with the normal breath-to-breath variation in measured PEEP and tidal volume. In this light, the transient DC decreases measured in single subjects from the 0.08- and 0.10-mg/kg groups were regarded as resulting from technical factors (ventilator changes) and cough, respectively. In contrast, the modest DC decline (12% at 5 min) in one subject receiving 0.14 mg/kg was not associated with ventilator changes and persisted after onset of paralysis (*i.e.*, no coughing). There were neither clinical signs of bronchoconstriction (wheezing) nor biochemical evidence of concomitant histamine release as a potentially precipitating factor. While a previous study showing that CW002 is devoid of significant effects on airway muscarinic receptors modulating bronchial smooth muscle tone[8] lessens the likelihood of this mechanism contributing to the small effect noted in the subject, further study of higher CW002 doses should include careful evaluation of changes in airway resistance and compliance.

Results of the study need to be interpreted in the context of certain limitations. The first relates to the scope of this preliminary investigation. Although preclinical studies have shown CW002 to be inactivated by endogenous cysteine and neuromuscular blockade rapidly reversed by cysteine injection, the current clinical investigation did not specifically assess any aspect of this pharmacology. Second, the relatively small sample size, and range of responses to 0.06 mg/kg CW002 in particular (fig. 1), may have influenced the estimation of ED_{95}, a variable unique to neuromuscular pharmacology and less robust as an index of potency than ED_{50}.[14] While the sample size included 33 measurements and all were included in the analysis, only 20 were between the fixed limits of 0 and 100, potentially influencing the impact of outliers on the distribution of responses. Consistent with this possibility was derivation of the Hill slope of 2.90, a value below the reported range of 3.6 to 6.1 for other nondepolarizing neuromuscular-blocking drugs.[14] Applying a robust nonlinear regression method designed to identify the undue impact of data outliers[15] increased the Hill slope to 3.81, and while not altering the ED_{50} (conventional potency), it reduced the calculated ED_{95} from 0.099 to 0.077 mg/kg, a value more consistent with the responses observed after 0.08 (3 of 5 with 100% block) and 0.10 mg/kg (6 of 6 with more than 95% block). If the common definition of intermediate duration neuromuscular blockade is applied—return of T1 to 25% of baseline within 40 min after a dose of at least 2 × ED_{95}—these first-in-man study results are insufficient to definitively make this distinction for CW002. Nonetheless, the data do suggest that CW002 is an intermediate duration drug in man.

In summary, this phase 1 study of CW002 demonstrated it to be a potent neuromuscular-blocking drug (ED_{50} = 0.036 mg/kg) that at 1.8 × estimated ED_{95} exhibits a clinical onset time of about 90 s and a clinical duration less than 40 min. Recovery intervals for T1 after CW002 appear to be dose independent consistent with drug inactivation in the plasma. When administered as a single intravenous bolus in doses up to 0.14 mg/kg, CW002 elicits minimal cardiopulmonary side effects and no histamine release.

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Competing Interests

Dr. Savarese is the inventor of CW002. The patent for this molecule is held by Weill Cornell Medical College, New York, New York. Drs. Heerdt and Savarese are the inventors of cysteine formulations for reversal of CW002. The patents for these drugs are held by Weill Cornell Medical College, New York, New York. The other authors declare no competing interests.

Reproducible Science

Full protocol available from Dr. Lien: calien@med.cornell.edu. Raw data available from Dr. Lien: calien@med.cornell.edu.

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