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

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ABSTRACT

Background: CW002, a novel nondepolarizing neuromuscular blocking agent of intermediate duration, is degraded *in vitro* by L-cysteine; CW002-induced neuromuscular blockade (NMB) is antagonized *in vivo* by exogenous L-cysteine.[1] Further, Institutional Animal Care and Use Committee–approved studies of safety and efficacy in eight anesthetized monkeys and six cats are described.

Methods: Mean arterial pressure, heart rate, twitch, and train-of-four were recorded; estimated dose producing 95% twitch inhibition (ED_{95}) for NMB and twitch recovery intervals from 5 to 95% of baseline were derived. Antagonism of 99 to 100% block in monkeys by L-cysteine (50 mg/kg) was tested after bolus doses of approximately 3.75 to 20 × ED_{95} and after infusions. Vagal and sympathetic autonomic responses were recorded in cats. Dose ratios for [circulatory (ED_{20}) or autonomic (ED_{50}) changes/ED_{95} (NMB)] were calculated.

Results: ED_{95}s of CW002 in monkeys and cats were 0.040 and 0.035 mg/kg; L-cysteine readily antagonized block in monkeys: 5 to 95% twitch recovery intervals were shortened to 1.8 to 3.6 min after 3.75 to 10 × ED_{95} or infusions *versus* 11.5 to 13.5 min during spontaneous recovery. ED for 20% *decrease* of mean arterial pressure (n = 27) was 1.06 mg/kg in monkeys; ED for 20% *increase* of HR (n = 27) was 2.16 mg/kg. ED_{50}s for vagal and sympathetic inhibition in cats were 0.59 and >>0.80 mg/kg (n = 14 and 15). Dose ratios for [circulatory or autonomic changes/ED_{95} (NMB)] were all more than 15 × ED_{95}.

Conclusions: The data further verify the neuromuscular blocking properties of CW002, including rapid reversal by L-cysteine of 100% NMB under several circumstances. A notable lack of autonomic or circulatory effects provided added proof of safety and efficacy. **(ANESTHESIOLOGY 2016; 125:732-43)**

CW002 (fig. 1), a novel neuromuscular blocking agent (NMBA), is degraded *in vitro* by L-cysteine adduction (t½ = 11.4 min) followed by alkaline hydrolysis.[1] Neuromuscular blockade (NMB) can be readily antagonized by exogenous L-cysteine in the Rhesus monkey[1] and dog.[2,3]

We classify the duration of CW002 (approximately 30 min in the monkey at approximately 3.75 × ED_{95} for NMB) as intermediate because its duration is about two thirds that of cisatracurium at comparable dosage yet about 3× that of the ultrashort-acting NMBA gantacurium in that species.[1]

CW002 was selected for additional studies of efficacy and safety. The desirable properties of CW002[1–7] are intermediate duration and rapid antagonism of NMB by L-cysteine,[1] and minimal cardiopulmonary effects for both CW002 and L-cysteine.[2,4]

What We Already Know about This Topic

- CW002 is a novel nondepolarizing neuromuscular blocking agent that is degraded *in vitro* by L-cysteine adduction
- CW002-induced neuromuscular blockade is reversed by administration of L-cysteine

What This Article Tells Us That Is New

- L-cysteine caused rapid recovery of twitch from 5 to 95% of baseline within 1.8 to 3.6 min when it was administered to monkeys 1 min after doses of CW002 ranging from approximately 3.75 to 10 times the dose producing 95% twitch suppression (ED_{95}; 0.15 to 0.40 mg/kg)
- The ratios of the doses producing a 20% decrease of mean arterial pressure or a 20% increase in heart rate in monkeys to the ED_{95} for neuromuscular blockade were 27 × ED_{95} and 54 × ED_{95}, respectively

Additional studies included in the current investigations have addressed the following:

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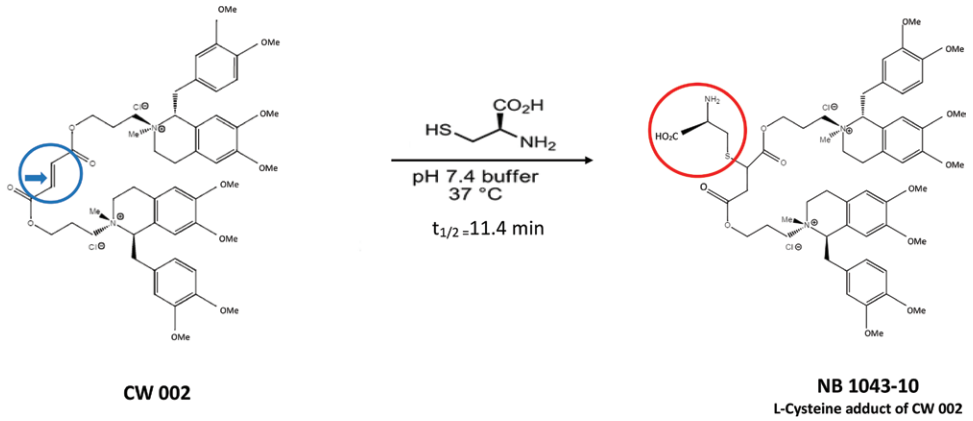


Fig. 1. The chemical formula of CW002. *Blue circle* indicates the fumarate double bond, the locus of L-cysteine adduction. The adduction reaction that converts the active NMBA CW002 to the inactive adduct NB1043-10 is shown. *Red circle* indicates the position of L-cysteine on the adduct. The t½ is the reaction half-time *in vitro*.

a. A comparison of spontaneous recovery from CW002-induced NMB *versus* antagonism by L-cysteine (50 mg/kg) at +1 or +5 min or at 1 to 2% twitch height during early recovery from a dose of 0.15 mg/kg, 3.75 × ED_{95} (estimated dose producing 95% twitch inhibition), or after discontinuation of infusions 30 to 180 min long, and at +1 min after doses of 10 and 20 × ED_{95}.
b. Development of dose ratios comparing ED causing 20% *decrease* in mean arterial pressure (MAP) and/or 20% *increase* in heart rate (HR) *versus* ED_{95} for NMB in the monkey.[8,9]
c. A comparison of changes in MAP and HR after CW002 given to monkeys as a single bolus, as the "first dose of the day," *versus* administration in increments to achieve the same total doses. This comparison is pertinent since in the clinic, a large dose of NMBA is commonly given first.
d. Development of dose ratios in the cat for ED_{50} for vagal block (VB) or sympathetic block (SB) *versus* ED_{95} (NMB).[8,9]

Materials and Methods

All protocols were approved by the Institutional Animal Care and Use Committees (IACUC) of Weill Cornell Medical College (New York, New York) and of Albany Medical College (Albany, New York), where the studies were conducted.

Novel Compounds

CW002 was synthesized at Cedarburg Hauser Pharmaceuticals, a division of Albany Molecular Research, Inc., Grafton, Wisconsin. CW002 was given intravenously at concentrations of 1 to 10 mg/ml in 0.9% NaCl, with pH adjusted to 3.0 with 1 N HCl to improve stability. L-cysteine hydrochloride was purchased from Ajinomoto Pharmaceuticals (USA). In our new formulation for L-cysteine, to maximize stability, vials contained 20 ml (4.0 g at 200 mg/ml) in 0.9% NaCl). The pH was adjusted and buffered to 4.5 to 5.5 using 1 N NaOH and concentrated (10×) phosphate buffer (pH 7.4). The vials were stored at –20°C and thawed before injection. Stability for 8 h, once thawed, has been shown in pilot studies (Laboratory of John J. Savarese, M.D., Weill Cornell Medical College, 2008 to 2015). Solutions of CW002, also stored at –20°C, were freshly thawed on the day of the experiment and kept in an ice bath to further ensure stability.

Studies in Rhesus Monkeys

Experimental Protocol. Eight adult male Rhesus monkeys weighing 8 to 18 kg were studied at intervals of 4 to 6 weeks. Animals were housed in accordance with the Guide for Care and Use of Laboratory Animals (National Research Council, Washington, DC). Care and maintenance of the animals have been described.[1]

Experimental setup was performed as previously described.[1,8] Monkeys received ketamine (7 to 10 mg/kg) intramuscularly, followed by tracheal intubation under topical anesthesia with 2% lidocaine. Ventilation was controlled at V_T 12 to 15 ml/kg at 18 to 20 breaths/min. Isoflurane anesthesia (1.0 to 2.0%) in N_2O/O_2 (2 l/1 l mixture) was maintained during each experiment, and lactated Ringer's solution was given at a rate of approximately 6 ml kg^{-1} h^{-1}. Arterial pressure was monitored directly. HR was measured by tachograph from the arterial waveform. Temperature was kept at 36 to 38°C. Oxygen saturation was kept at more than 96%.

Needle electrodes (25 ga) were placed at the peroneal nerve. Square-wave pulses of 0.2-ms duration at supramaximal voltage were applied to the nerve at 0.15 Hz to elicit twitch responses of the extensor digitorum of the foot; 20% of the tendon was tied to a Grass FT 10 force transducer at a baseline tension of 50 g. Train-of-four (TOF) stimulation (2 Hz for 2 s) was interposed at appropriate time points, especially at 1 to 2 min before injection of CW002 and after recovery of twitch to 95% of baseline.

CW002 dose 1, the "first dose of the day," was given after a more than 15-min stable baseline period as a rapid (5-s) bolus. Dose 2 was given at least 30 min after recovery of TOF ratio to 100% or more after dose 1. Doses 1 and 2 of CW002, given at the beginning of each experiment, were included in data describing neuromuscular blocking potency and duration and recovery. Onset was measured after dose 1 only. At the end of each experiment, analgesics were given per veterinary practice, and the animals were awakened and attended until standing.

Neuromuscular Blocking Properties in the Monkey.

Dose–Response. The dose–response relationship was generated by nonlinear regression of log dose *versus* percentage inhibition of twitch. Doses 1 and 2 from each experiment were used to calculate the dose–response curve. ED_{50} and ED_{95} for twitch inhibition were derived. Onset time from injection to maximum twitch suppression (dose 1 only), total duration from injection to recovery of twitch to 95% of baseline, and the recovery interval from 5 to 95% twitch height (doses 1 and 2) were measured. All recoveries were followed until TOF ratio was at least 95%. (Most Rhesus monkeys have usually shown TOF ratio of 100 to 120% at baseline.[1,8])

Continuous Infusions. Continuous infusions of CW002 were given for durations varying from 30 to 180 min. Two groups of infusions were done: group 1: spontaneous recovery; group 2: antagonism by L-cysteine (50 mg/kg) at the end of infusion. Infusions were initiated by a bolus dose of 0.08 to 0.15 mg/kg; infusion was then begun at recovery to 25% twitch height after the initial bolus, and the infusion rate was thereafter adjusted to maintain 98 to 99% twitch suppression.

At the end of infusions in group 1, spontaneous recovery was measured to 95% twitch height and TOF more than 95%. In group 2, at discontinuation of infusion, the line was flushed with 5 ml lactated Ringer's solution, and L-cysteine (50 mg/kg) was given 1 min later, at 0 to 2% twitch height.

Spontaneous recovery times and L-cysteine–accelerated recovery times, *i.e.*, reversal times, were compared by assessment of the 5 to 95% twitch recovery interval and its relationship to the duration of infusion using linear regression.

Antagonism by L-cysteine. Antagonism of CW002-induced NMB by L-cysteine has been shown to peak in rapidity and completeness at doses of 30 to 50 mg/kg in the Rhesus monkey and dog.[1,2] In the current studies, antagonism by L-cysteine given as a 5-s bolus was evaluated under various circumstances, always administering the same dose (50 mg/kg). Antagonism was evaluated at the following time points: at +1 min (100% block) after CW002 doses of 0.15, 0.40, and 0.80 mg/kg (approximately 3.75 × to 20 × ED_{95}); at +5 min (100% block) after 0.15 mg/kg; at the beginning of twitch recovery (1 to 2% twitch height) after 0.15 mg/kg; and at the termination of infusions in group 2, 1 min after discontinuation of infusion, at 0 to 2% twitch height.

Circulatory Observations. All doses of CW002 were given as rapid (5-s) boluses. Two dose–response relationships were developed for HR and MAP.

A. *Single-bolus method (group 1)*: Data from *the first dose of the day only* were applied to construct the curves. Doses of 0.20 to 2.40 mg/kg (5 to 60 × ED_{95}) were given on separate occasions, spaced 4 to 6 weeks apart. Method A was employed particularly to closely mimic clinical practice where a large dose of NMBA is usually given first.
B. *Cumulative method (group 2)*: Doses from 5 × ED_{95} to 20 × ED_{95} (0.20 to 0.80 mg/kg) were given successively; the cumulative dose was doubled every 10 min to a total of approximately 40 × ED_{95} or 1.60 mg/kg: 5 ×, 5 ×, 10 ×, and 20 × ED_{95}. Maximum changes from the original baseline were noted for each dose increment to yield cumulative dose–response curves.

Curvilinear dose–response curves were calculated for method A, and the EDs for 20% *decrease* of MAP and 20% *increase* of HR after single-bolus doses were derived.

Studies in the Cat

Experimental Protocol and Neuromuscular Blocking Properties. Anesthesia, setup, ventilation, and monitoring were as described in previous studies in Rhesus monkeys and cats.[1,8] Six male cats weighing 4 to 6 kg were studied three times each at intervals of 2 to 4 weeks. About 20% of the tibialis anterior tendon was freed in sterile fashion and tied to an FT 10 transducer at baseline tension of 50 g. Monitoring of neuromuscular function was as described for monkeys. Data obtained were as in monkeys: duration to recovery to 95% of baseline twitch height and 5 to 95% twitch recovery interval.

Onset of NMB was not studied in the cat, nor was continuous infusion. At the end of each experiment, animals were given analgesics per veterinary practice and attended until standing. Cats were offered for adoption after completion of studies.

Antagonism by L-cysteine. Studies were not as extensive as in the Rhesus monkey. Antagonism of approximately 1 × ED_{95} (doses of approximately 0.03 mg/kg) by L-cysteine (50 mg/kg), given at +6 min after CW002 injection, was documented twice.

Autonomic Studies. Inhibition of vagal (parasympathetic, VB) and sympathetic (ganglionic, SB) responses was studied in the third of the three experiments in four of the six cats. The protocol has been described.[8]

The right cervical vagus nerve and sympathetic trunk were exposed under sterile technique and tied off centrally. The nerves were placed on separate electrodes and were stimulated with 10-s trains of square waves (0.5 m at 20 Hz) every 3 to 5 min. Responses recorded were as follows: VB—inhibition of a baseline response of about 50% reduction of HR; SB—block of the contraction of the right nictitating membrane as transduced by a Grass FT-03 instrument.

Dose–response curves were constructed in cumulative fashion: 0.10, 0.10, 0.20, and 0.40 mg/kg boluses of CW002 were given sequentially every 10 min. The total dose studied was therefore 0.80 mg/kg (approximately 23 × ED_{95} in the cat). Nonlinear regression of dose *versus* percentage blockade of the autonomic responses was performed to estimate ED_{50} for VB and SB.

Dose Ratios. From studies in the cat, the following were calculated as indicators of the relative likelihood of VB or SB *versus* NMB potency:

$$[ED_{50}(VB)/ED_{95}(NMB)] = ED_{95} \text{ multiple or } \times ED_{95}$$

$$[ED_{50}(SB)/ED_{95}(NMB)] = ED_{95} \text{ multiple or } \times ED_{95}$$

Similarly, from studies in monkeys (see *Circulatory Observations*), the following were calculated as indicators of the relative likelihood of pertinent deleterious circulatory changes *versus* NMB potency.

$$[ED(20\%\downarrow MAP)/ED_{95}(NMB)] = ED_{95} \text{ multiple or} \times ED_{95}$$

$$[ED(20\%\uparrow HR)/ED_{95}(NMB)] = ED_{95} \text{ multiple or} \times ED_{95}$$

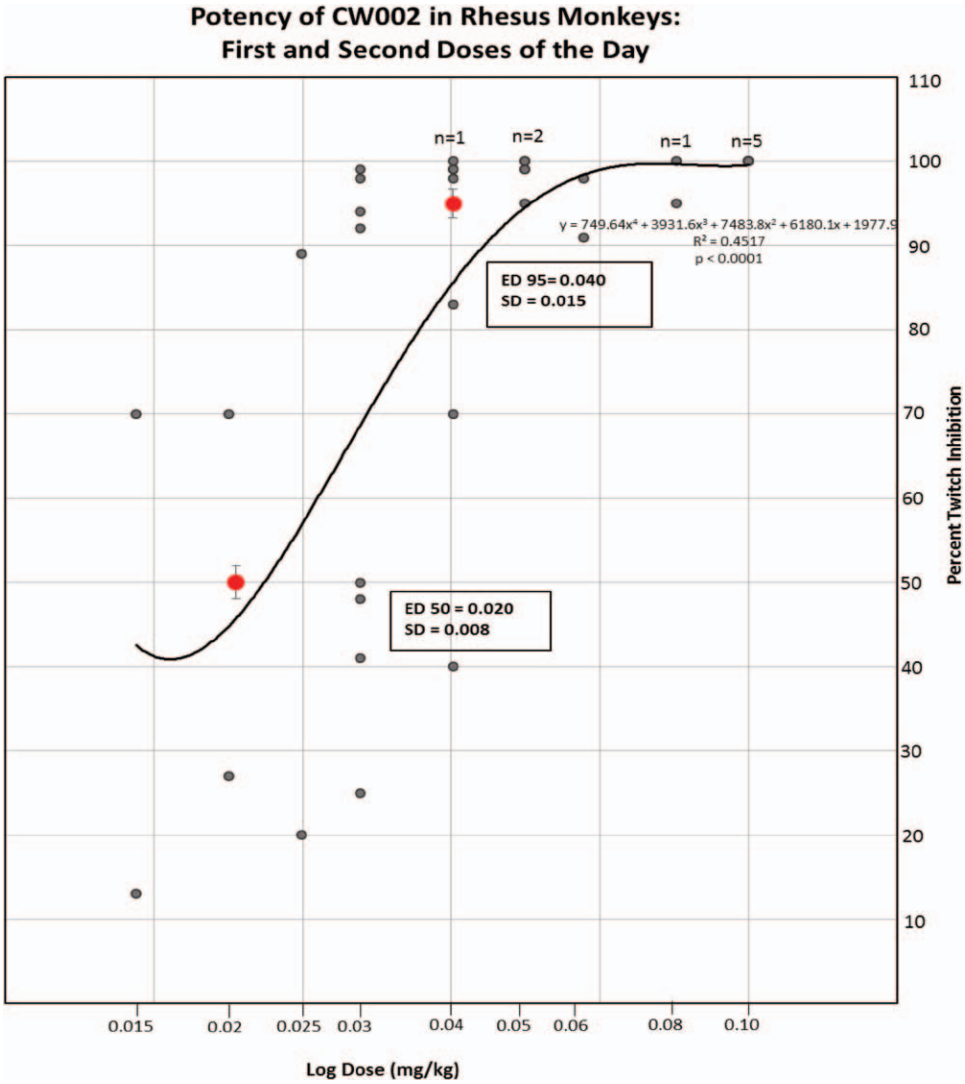


Fig. 2. Nonlinear regression of the dose–response of CW002 for neuromuscular blockade (twitch inhibition) in the isoflurane-anesthetized Rhesus monkey. Scales: x-axis, logarithmic; y-axis, arithmetic. First and second doses of each experiment only were used for the calculations. Twitch was elicited at 0.15 Hz; 33 data points are shown. ED_{50} and ED_{95} (*red points*) indicate effective doses (milligram per kilogram) resulting in 50 and 95% twitch inhibition, respectively. *Vertical bars* indicate SD; n = number of responses (data points) observed at 100% twitch inhibition at each dose of CW002.

Data Analysis

The program Microsoft Excel 2007 (Microsoft Corporation, USA) generated statistical comparisons *via* Student's *t* test (two tailed) or the Tukey–Kramer test (for multiple comparisons).

Noninear regression of the percentage twitch inhibition or block of autonomic responses *versus* log dose was performed using the program GraphPad Prism version 6.0 (GraphPad Software, USA) to generate dose–response curves for neuromuscular or vagal blockade (NMB or VB).

Linear regression was used to evaluate the relation of speed of recovery, spontaneous or accelerated by exogenous L-cysteine, as indicated by the 5 to 95% twitch recovery interval, *versus* the duration of infusion.

SigmaPlot® from Systat Software, Inc. (USA) was used to construct curvilinear regressions for HR and MAP changes in monkeys after single-bolus doses given as the "first dose of the day." $P < 0.05$ was considered significant in all statistical comparisons. Group sample sizes of n = 4 or more were ensured before any statistical comparison. Exact *P* values are given whenever possible (for two-tailed Student's *t* test).

Results

All data are original and have not been previously published. Data are reported as mean (± SD) or as mean with 95% CI.

Neuromuscular Dose–Response: Rhesus Monkey

The calculated ED_{50} and ED_{95} of CW002 for NMB (fig. 2; n = 33) were 0.020 ± 0.008 and 0.040 ± 0.015 mg/kg, respectively.

After "first doses of the day" (n = 5) of 0.03 to 0.06 mg/kg, which produced 95 to 99% block, onset time was 195.0 (18.7) s, total duration to recovery of twitch to 95% of baseline was 17.7 (5.3) min, and the 5 to 95% recovery interval was 12.4 (3.6) min (table 1). After 0.15 mg/kg (approximately 3.75 × ED_{95}), onset was 81.4 (25.3) s, duration was 25.9 (7.0) min, and the recovery interval was 11.5 (4.6) min (n = 7). Larger doses shortened onset time at up to approximately 10 × ED_{95} (0.40 mg/kg), where onset was 37.5 (5.9) s; doses more than 0.40 mg/kg did not shorten onset time significantly (table 1).

Spontaneous recovery intervals (5 to 95% twitch) were not different ($P > 0.05$ by Tukey–Kramer test) over the range 0.05 to 1.60 mg/kg (approximately 1.25 × to approximately 40 × ED_{95}); this interval did not differ significantly during spontaneous recovery after infusions in group 1. Intervals ranged from 11.5 to 16.1 min (tables 1 to 3 and fig. 3).

Continuous Infusion: Rhesus Monkeys

Data are summarized in tables 2 and 3 and figure 3.

In the two groups of experiments (group 1: *spontaneous recovery*, n = 17, and group 2: *L-cysteine antagonism*, n = 8), the rates of CW002 administration required to maintain blockade at 98 to 99% twitch inhibition did not differ: 3.60 (0.9) μg kg^{-1} min^{-1} (group 1) and 3.20 (0.7) μg kg^{-1} min^{-1} (group 2), $P = 0.32$. Infusion duration did not differ (group 1, 103.7 [44.2] min *vs.* group 2, 90.4 [21.6] min), $P = 0.43$.

Antagonism of NMB at end infusion by L-cysteine, 50 mg/kg (group 2), markedly shortened 5 to 95% recovery time *versus* spontaneous recovery: 2.5 (0.4) min *versus* 13.5 (4.0) min ($P < 0.0001$; table 2).

Infusion *duration* had no influence on the rate of spontaneous recovery (5 to 95% recovery interval) or on the accelerated (shortened) recovery induced by L-cysteine (fig. 3); $P = 0.43$ and 0.28, respectively.

Antagonism of CW002-induced NMB by L-cysteine (50 mg/kg) in the Monkey

The data are summarized in tables 2 and 3. L-cysteine caused rapid recovery of twitch from 5 to 95% of baseline within 1.8 to 3.6 min when it was administered 1 min after doses of CW002 ranging from approximately 3.75 × to 10 × ED_{95} (0.15 to 0.40 mg/kg), at which time there was

Table 1. Dose *versus* Time Relationship of CW002-induced Neuromuscular Blockade after ~ED_{95} and Higher Bolus Doses Given as "First Dose of the Day" to Isoflurane-anesthetized Monkeys and Cats: Onset, Duration, and Recovery Intervals

Dose (mg/kg)	~× ED_{95}	NMB (%)	Onset (s)	Total Duration (min)	5–95% Recovery Interval (min)*	n
Monkey						
0.03–0.06	~ 1	97.4 ± 2.2	195.0 ± 18.7	17.7 ± 5.3	12.4 ± 3.6	5
0.15	3.75	100 ± 0	81.4 ± 25.3	25.9 ± 7.0	11.5 ± 4.6	7
0.2	5	100 ± 0	53.1 ± 16.6	28.7 ± 7.4	12.2 ± 3.6	5
0.4	10	100 ± 0	37.5 ± 5.9†	37.1 ± 7.7	12.4 ± 3.2	6
0.8	20	100 ± 0	35.4 ± 5.0†	49.7 ± 10.8†	16.1 ± 5.2	5
1.6	40	100 ± 0	34.1 ± 9.0†	50.5 ± 11.8†	15.1 ± 4.1	5
Cat						
0.025–0.03	~ 1	97.2 ± 4.6		39.7 ± 8.8	27.5 ± 10.8	7
0.1	~ 3.5	100 ± 0		57.2 ± 18.1	21.0 ± 6.4	5
		$P = 0.15$		$P = 0.049$	$P = 0.259$	

Rhesus monkeys or cats under isoflurane/nitrous oxide/oxygen anesthesia. Single-bolus injections given as "first dose of the day," at beginning of experiment. All data are represented as mean ± SD.

*Differences among groups not significant ($P > 0.05$) by Tukey–Kramer test in monkey. †$P < 0.05$ *vs.* 0.15 mg/kg by Tukey–Kramer test.

ED_{95} = dose producing 95% block of twitch; NMB = neuromuscular blockade.

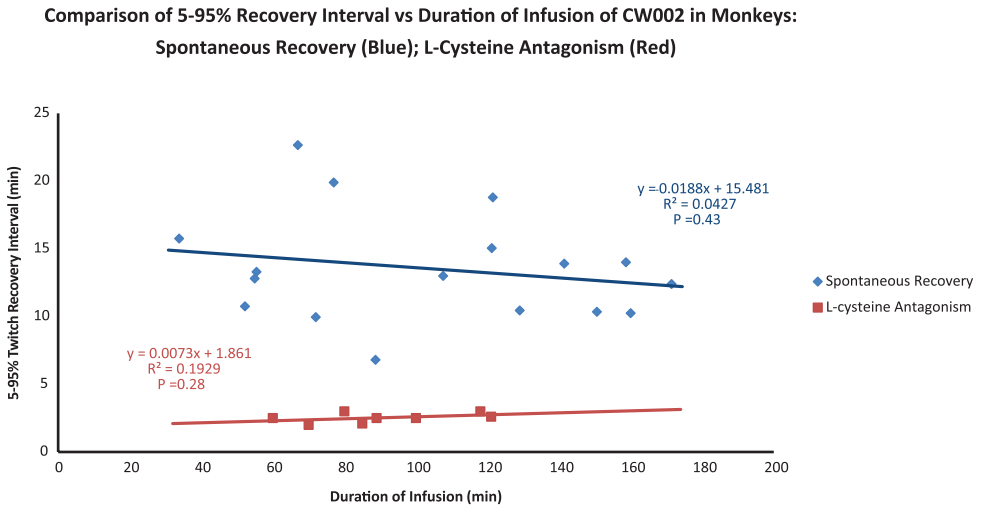


Fig. 3. Comparison of the speed of recovery of neuromuscular blockade in anesthetized Rhesus monkeys (5 to 95% twitch recovery interval), after discontinuation of infusions of CW002, *versus* the duration of infusion. Group 1 (*red*): spontaneous recovery; group 2 (*blue*): L-cysteine (50 mg/kg)—accelerated recovery (reversal) from 0 to 2% twitch height at end of infusion. L-Cysteine was given as a 5-s bolus 1 min after discontinuation of infusion; infusion rates were adjusted to maintain 98 to 99% twitch inhibition. Duration of infusion was not related to speed of recovery in either case.

Table 2. Continuous Infusions of CW002 in Isoflurane-anesthetized Monkeys: Spontaneous Recovery *versus* L-cysteine Antagonism*

	Duration of Infusion (min)	Infusion Rate ($\mu g\ kg^{-1}\ min^{-1}$)†	5–95% Recovery Interval (min)	n
Spontaneous recovery (group 1)	103.7 ± 44.2	3.6 ± 0.9	13.5 ± 4.0	17
L-cysteine 50 mg/kg antagonism† (group 2)	90.4 ± 21.6	3.2 ± 0.7	2.5 ± 0.4	8
P value	0.43	0.32	< 0.0001	

Rhesus monkeys under isoflurane/nitrous oxide/oxygen. Continuous administration was begun by bolus injection of 0.08 to 0.15 mg/kg at t = 0. Infusion was begun at 25% recovery of twitch and adjusted to maintain 98 to 99% twitch inhibition. All data are represented as mean ± SD.

*L-cysteine 50 mg/kg given at +1 min after infusion discontinuation, at 1 to 2% twitch height (98 to 99% block). †Rate required to maintain 98 to 99% block of twitch.

always 100% block of twitch. At two other time points after injection of CW002 (0.15 mg/kg), namely at +5 min and at 1 to 2% twitch height at the beginning of recovery, antagonism by L-cysteine was equally rapid (table 3). Similarly, L-cysteine given at 0 to 2% twitch height, at +1 min after discontinuation of infusions, accelerated the recovery of twitch from 5 to 95% of baseline strength from 13.5 (4.0) min to 2.5 (0.4) min ($P < 0.0001$), during spontaneous *versus* L-cysteine–accelerated recovery (table 2 and fig. 3).

Neuromuscular Blocking Properties: Cat

CW002 was slightly more potent in the cat than in the Rhesus monkey and was longer lasting (table 1). ED_{95} was 0.035 ± 0.01 mg/kg.

After doses inducing 95 to 99% block (approximately 0.03 mg/kg), the duration was 39.7 (8.8) min (n = 7); after 0.10 mg/kg (approximately 3.0 × ED_{95}), the duration was 57.2 (18.1) min (n = 5). The 5 to 95% recovery intervals after doses of approximately 0.03 and 0.10 mg/kg were 27.5 (10.8) *versus* 21.0 (6.4) min, n = 7 *versus* n = 5. They did not differ significantly (table 1), $P = 0.259$.

L-cysteine Antagonism in the Cat

When given at 6 min after CW002 (approximately 0.03 mg/kg), which induced mean 99% block, L-cysteine (50 mg/kg) accelerated the 5 to 95% recovery interval from 27.5 (10.8) min (n = 7) to a mean of 5.8 min (table 3, n = 2).

Circulatory Observations (MAP and HR) and Dose Ratios versus NMB in Rhesus Monkeys

A comparison was made of the circulatory effects of cumulative administration *versus* single-bolus doses, the latter given as the "first dose of the day" (table 4 and figs. 4 and 5). Circulatory data shown in tables and figures are maximum changes from baseline measurements. Any changes noted nearly always occurred within 5 min or less.

Curvilinear regressions done for changes of MAP and HR after the "first dose of the day" show that after the doses of 0.80, 1.60, and 2.40 mg/kg (20, 40, and 60 × ED_{95}), *increases* in HR were dose related, while a *decreasing* trend was seen for MAP (figs. 4 and 5). On the other hand, a *decreasing* trend for *both* HR and MAP was seen during cumulative administration (fig. 4). At 0.80 and 1.60 mg/kg, differences in responses of MAP *versus* baseline were

Table 3. CW002-induced Neuromuscular Blockade in Isoflurane-anesthetized Monkeys and Cats: Spontaneous Recovery *versus* Antagonism by L-cysteine (50 mg/kg)

CW002 Dose (mg/kg)	L-cysteine Dose and Time of Administration*	5–95% Recovery Interval (min)				Total Duration (min)			
		Spontaneous	n	Antagonism (*P* Values)	n	Spontaneous	n	Antagonism (*P* Values)	n
Monkey									
0.15	50 mg/kg at 1 min	11.5 ± 4.6	7	1.8 ± 0.5† (0.001)	4	25.9 ± 7.0	7	3.9 ± 0.6† (0.0002)	4
0.15	50 mg/kg at 5 min	11.5 ± 4.6	7	3.1 ± 0.5† (0.003)	4	25.9 ± 7.0	7	9.5 ± 1.4† (0.0009)	4
0.15	50 mg/kg at 2% recovery	11.5 ± 4.6	7	2.1 ± 0.6†‡ (0.002)	4	25.9 ± 7.0	7	19.9 ± 5.3‡§ (0.17)	4
0.4	50 mg/kg at 1 min	12.4 ± 3.2	6	3.6 ± 1.4† (< 0.0001)	7	37.1 ± 7.7	6	6.3 ± 1.7† (0.0002)	7
0.8	50 mg/kg at 1 min	16.1 ± 5.2	5	7.4 ± 1.3†§ (0.01)	4	49.7 ± 10.8‖	5	10.5 ± 1.8† (0.001)	4
Continuous infusion	50 mg/kg at 1 min after infusion discontinued	13.5 ± 4.0	17	2.5 ± 0.4† (< 0.0001)	8	NA		NA	
Cat									
0.03	50 mg/kg at + 6 min	27.5 ± 8.7	7	5.8	2	39.7 ± 8.8	7	12.5	2

Rhesus monkeys under isoflurane/nitrous oxide/oxygen. All data are represented as mean ± SD.

*Time of L-cysteine administration in monkey = minute after injection of CW002. †$P < 0.05$ *vs.* spontaneous recovery (unpaired Student's *t* test). ‡Previously published data.[1] §$P < 0.05$ *vs.* all other doses (Tukey–Kramer test for multiple comparisons). ‖$P > 0.05$ *vs.* 0.15 mg/kg (unpaired Student's *t* test).

NA = data not available.

significant after bolus ($P = 0.049$ and 0.001) but not after cumulative doses ($P = 0.125$ and 0.096). A table of *P* values is attached to figure 4 and table 4. The ED_{20} with 95% CI for HR *increase* after the "first dose of day" boluses was 2.16 (1.61 to more than 2.40 mg/kg); the ED_{20} for MAP *decrease* was 1.06 (0.94 to 1.21) mg/kg (fig. 5). The dose ratios for ED (20% change in MAP or HR) *versus* ED_{95} for NMB were 54 × ED_{95} for increase in HR and 27 × ED_{95} for decrease in MAP.

Autonomic Blockade and Dose Ratios (VB or SB) versus NMB in the Cat

Data are summarized in table 5. ED_{50} (VB) was 0.59 ± 0.07 mg/kg; ED_{50} (SB) was >>0.80 mg/kg. Dose ratios [ED_{50} (VB)/ED_{95} (NMB)] and [ED_{50} (SB)/ED_{95} (NMB)] were 17 × ED_{95} and >> 23 × ED_{95} for the vagal and sympathetic systems.

Discussion

The studies reported herein were undertaken to assemble a pharmacologic profile of CW002; they provide additional data on *efficacy*: on antagonism of 99 to 100% twitch inhibition by the same dose (50 mg/kg) of L-cysteine under a variety of circumstances and on the pharmacology of continuous infusion of CW002. The minimal circulatory changes after CW002 in large doses (5 to 10 × ED_{95} for NMB) provide information assuring an adequate safety margin in the NMB dose range. An accidental overdose of CW002 (as large as 20 to 60 × ED_{95} for NMB), as suggested in table 1, would likely lengthen its NMB effect less than proportionately due to increased reaction kinetics (the mass action effect), a consideration of both efficacy and safety.

Documentation herein of the circulatory and autonomic side effects of very large doses of CW002 (20 to 60 × ED_{95}) not only provides increased assurance of *safety*, but also suggests upper dose ranges for future evaluations of potential histologic, biochemical, or hematologic pathologic effects.

Definition of dose ratios relating the ED_{50} for autonomic blockade in the cat divided by the neuromuscular blocking potency (ED_{95}) requires the determination of the ED_{95} in that species. Similarly, dose ratios for circulatory changes of 20% decrease in MAP and increase in HR *versus* ED_{95} in the monkey were generated as indicators of safety (table 4 and figs. 4 and 5). Obviously, higher dose ratios suggest greater safety, *i.e.,* less likelihood of occurrence of side effects.

CW002: Neuromuscular Blocking Properties

The numbers of individual animals studied seem appropriate since the numbers are normative to our previously published work in monkeys and cats.[1,8] The dose range chosen for study of CW002 represents the range of potency of published[1] and unpublished compounds (Laboratory of John J. Savarese, M.D., Weill Cornell Medical College, 2008 to 2015). That CW002 has a duration of NMB that is typically "intermediate" in the Rhesus can be inferred from a crossover study showing a ratio of 2:3 of comparative duration of CW002 *versus* cisatracurium.[1]

We are reluctant to compare speed of *onset* of NMB after CW002 *versus* other NMBAs in the monkey model without our own data. In our *unpublished* observations (Laboratory of John J. Savarese, M.D., Weill Cornell Medical College, 2008 to 2015), rocuronium showed approximately 40% the potency of CW002 (ED_{95} approximately 0.10 *vs.* 0.040 mg/kg) and a longer duration of action at approximately 4 × ED_{95}; the onset of effect of CW002 in our monkeys seems longer than the onset of effect of rocuronium and shorter than that of cisatracurium. Onsets of CW002 given in table 1 are intended to show at which dose (0.40 mg/kg) a limit is reached, presumably due to circulation time, and do not imply comparisons with other NMBAs.

Table 4. Circulatory Effects of CW002 in Isoflurane-anesthetized Monkeys: Bolus Doses (First Dose of the Day) *versus* Cumulative Administration*†

"First Dose of the Day" Single Bolus, Mean ± SD				Cumulative Administration, Mean ± SD			
Dose (mg/kg)	MAP % Baseline (*P* Values, Unpaired Student's *t* Test‡)	HR % Baseline (*P* Values, Unpaired Student's *t* Test‡)	n	MAP % Baseline (*P* Values, Unpaired Student's *t* Test‡)	n	HR % Baseline (*P* Values, Unpaired Student's *t* Test‡)	n
0.2	92.50 ± 2.7 (0.135)	99.00 ± 1.5 (0.886)	6	92.83 ± 5.1 (0.570)	6	98.67 ± 2.0 (0.811)	6
0.4	93.17 ± 4.0 (0.432)	99.83 ± 3.1 (0.925)	6	84.80 ± 9.9 (0.134)	10	96.50 ± 3.3 (0.659)	10
0.8	84.57 ± 7.5§ (0.049)	100.29 ± 6.4 (0.346)	7	80.18 ± 9.5 (0.125)	11	94.55 ± 5.0 (0.281)	11
1.6	64.42 ± 9.8‖ (0.001)	107.94 ± 20.4 (0.504)	5	80.40 ± 14.1 (0.096)	5	91.80 ± 3.1 (0.056)	5
2.4	61.45 ± 11.7 (0.127)	131.30 ± 44.0 (0.076)	3	NA		NA	

*See *P* values: two-tailed Student's *t* test; †Maximum changes from baseline measurements (% baseline). ‡Comparisons *vs*. baseline HR or MAP. §*P* = 0.049 *vs.* baseline. ‖*P* = 0.001 *vs.* baseline.

HR = heart rate; MAP = mean arterial pressure; NA = data not available.

Comparisons of onset of effect of NMBAs in humans or other species must require well-controlled anesthetic conditions, measuring onsets obtained only after "first doses of the day."

CW002 Chemistry: Degradation and L-cysteine Antagonism

L-cysteine adduction (fig. 1) initiates a degradation cascade ending in molecular fragments that show only about 0.01 to 0.001 × the potency of CW002 to produce NMB.[1] The key feature of the profile of CW002, antagonism of its NMB effect by exogenous L-cysteine, most likely occurs because the chemistry of degradation is accelerated markedly by a mass-action effect.[1]

The peak effect of L-cysteine, in terms of speed and completeness of reversal, is reached at doses of 50 mg/kg[1,2]; therefore, that dose was given under all conditions reported in this article: L-cysteine was found equally effective at +1 min after CW002 doses of 0.15 to 0.40 mg/kg (approximately 3.75 to approximately 10 × ED_{95}), at +5 min after 0.15 mg/kg, and at 1 to 2% twitch height at the beginning of recovery from a dose of 0.15 mg/kg or from infusions. The 5 to 95% recovery interval was shortened to 1.8 to 3.6 min under all circumstances and did not differ significantly (tables 2 and 3).

Because of its mechanism of reversal, *i.e.*, inactivation and destruction of the NMBA molecule, L-cysteine antagonism of deep CW002-induced NMB is rapidly effective, as shown herein (tables 2 and 3). The competitive mechanism of neostigmine is well understood, on the other hand, to be the reason for its lack of effectiveness in antagonism of deep block caused by other NMBAs[9] as well as CW002.[1]

Plasma Kinetics of L-cysteine

L-cysteine concentrations in plasma remain constant throughout adult life[10]; sources are the diet, cleavage of the –S–S– bond of the dimer (L-cystine), yielding two molecules of L-cysteine, and enzymatic conversion of glutathione. The metabolic pathways sourcing L-cysteine are bidirectional, depending on the local redox state of tissue.[11]

A toxicokinetic study in dogs of L-cysteine (data from Laboratory of John J. Savarese, M.D., Weill Cornell Medical College, 2008 to 2015) reports a plasma half-life of 30 to 40 min, relatively short, possibly because of uptake into various metabolic pools.[11]

The plasma half-life of L-cysteine in humans has been estimated at 30 to 60 min.[11] The plasma half-life of sugammadex is 2 to 4 h in normal subjects.[12] These t½ are pertinent to clinical practice because such agents, which effectively acutely lower the plasma concentration of the NMBA, will show a residual effect, after reversal, of inhibiting the activity of subsequent doses of the NMBA. Such doses given after the reversal agent, to reestablish paralysis and clinical relaxation, would be inhibited for a length of time proportional to the t½ in plasma of the reversal agent.

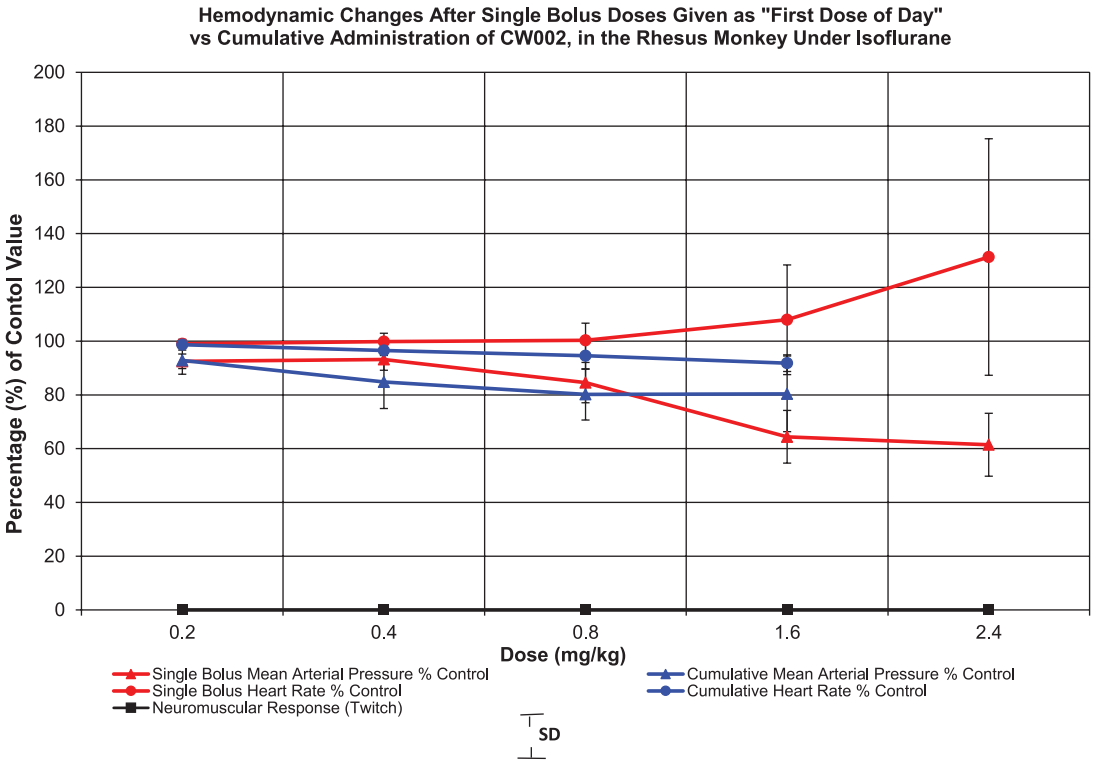
Decreased availability of *endogenous* L-cysteine in practice, such as in malnutrition or cachexia, might slow spontaneous recovery from CW002-induced NMB; the remedy would be administration of exogenous L-cysteine.

Comparison of Degradation and Reversal of CW002 and Atracurium/Cisatracurium

Both L-cysteine adduction and Hofmann degradation are pH dependent.[1,13,14] In Hofmann degradation, hydroxyl ion concentration in living tissue is limited to the narrow range compatible with life and cannot be altered acutely to accelerate recovery from atracurium/cisatracurium; exogenous L-cysteine, however, presumably induces reversal of CW002 by an acute increase in ambient L-cysteine concentration in plasma, markedly accelerating the conversion of CW002 to its inactive adduct (fig. 1).

L-cysteine Antagonism versus Neostigmine, Sugammadex, and Calabadion

Neostigmine is *ineffective* in shortening the timeframe from CW002 injection to complete recovery from CW002-induced blockade in monkeys when given 1 min after 0.15 mg/kg (approximately 3.75 × ED_{95}).[1] An analogous failure was reported when early administration of neostigmine to humans after intubating doses of vecuronium[15] did not shorten the time from injection of vecuronium to complete recovery. This is basis for the commonly advised clinical practice of waiting (ideally) for at least two twitches



P-VALUES (Fig 4), Unpaired t-test‡						
Dose CW002 (mg/kg)		Single Bolus "First Dose of the Day"			Cumulative Administration	
	n	P (HR)	P (MAP)	*n*	P (HR)	P (MAP)
0.2	6	0.886	0.135	6	0.811	0.570
0.4	6	0.925	0.432	10	0.659	0.134
0.8	7	0.346	0.049	11	0.281	0.125
1.6	5	0.504	0.001	5	0.056	0.096
2.4	3	0.076	0.127		—	—

‡ Comparisons vs baseline HR or MAP

Fig. 4. Maximum changes in mean arterial pressure (MAP) and heart rate (HR) ±SD after large doses of CW002 (0.2 to 2.4 mg/kg) or approximately 5 to 60 × effective dose producing 95% block of twitch (ED_{95}) given to Rhesus monkeys under isoflurane. "First dose of the day" bolus (*red*) *versus* cumulative (incremental) administration (*blue*). Change in MAP was significantly different from baseline at 0.8 and 1.60 mg/kg after first bolus doses (P = 0.049 and 0.001); change in HR was not significant after bolus doses of 0.08 or 1.60 mg/kg. Neither change in HR nor MAP was significantly different from baseline at any other point/dose after either bolus or cumulative administration, possibly due to small sample sizes (n = only 3 studied at bolus dose of 2.4 mg/kg; see included table of P values). The major observation is the trend toward ↑ HR and ↓ MAP seen in the case of bolus administration *versus* the trend toward *decrease* in *both* MAP and HR in the case of cumulative administration. See Discussion, Bolus *versus* Cumulative Administration: Circulatory Consequences Are Different, for further details. The table at the *bottom* of this figure shows P values for all comparisons.

to be perceptible on TOF stimulation during recovery from nondepolarizing block before neostigmine administration.[9]

Antagonism of rocuronium-induced blockade may be achieved early, and complete recovery shortened, with sugammadex, by increasing the dose from 2 mg/kg when TOF count is 1 to 4[16,17] to 8 to 16 mg/kg when TOF count is 0.[18–20] Cost may be a consideration in choosing this option.

The new cucurbituril derivative calabadion, like sugammadex, probably complexes with the NMBA (rocuronium or cisatracurium) in antagonizing NMB,[21] and so may also require higher dosage for early reversal.

The same dose of L-cysteine (50 mg/kg) is equally effective in antagonism of CW002-induced NMB at any time after CW002 administration; as shown herein, the interval from 5 to 95% recovery of twitch does not differ significantly under a variety of circumstances during reversal of doses of CW002 from 3.75 to 10 × the ED_{95} for NMB in monkeys (tables 2 and 3). Hypothetically, issues of both convenience

and economics might also be pertinent in future practice with the CW002/L–cysteine combination.

Dose Ratios in the Cat: Autonomic versus Neuromuscular Block

***Vagal (Muscarinic Receptor) Blockade*: [ED_{50} (VB)/ED_{95} (NMB)].** The wide separation of the vagal blocking effect from the NMB property suggested in the dose ratio (17 × ED_{95}, table 5) is desirable. By comparison, the dose ratio of pancuronium in the cat for VB *versus* NMB is only about 2.0 to 3.0 × ED_{95} for NMB, and pancuronium does accelerate HR in clinical practice.[22,23] Rocuronium shows a dose ratio for VB *versus* NMB in the range of 5.0 to 7.0 × ED_{95} in the cat[24] and may cause an increase in HR in humans after doses of 3 to 4 × ED_{95} given for tracheal intubation.[25] The dose ratios for VB *versus* NMB of cisatracurium and vecuronium, which do not accelerate HR,[9] are higher: 25 and 76 to 80.[23,26]

The above dose ratios, obtained from small numbers of experiments, customary in such studies,[8,23,24,26] should be considered approximate, but may suggest increases in HR in humans,[22,25] when the ratio [ED_{50} (VB)/ED_{95} (NMB)] is less than 5.

M2 block in the airway, combined with allosteric sensitization of *M3* receptors, may cause bronchospasm. Rapacuronium was withdrawn from clinical practice likely due to this mechanism,[27,28] demonstrated in a guinea pig model.[29–33] CW002 does not block M2 receptors in the guinea pig.[4]

***Sympathetic (Ganglionic) Nicotinic Receptor Blockade*: [ED_{50} (SB)/ED_{95} (NMB)].** CW002 has a high dose ratio (>>23 × ED_{95} for NMB) *versus* inhibition of nicotinic ganglionic responses. In fact, this mechanism is really no longer a consideration, in terms of safety, and has been "engineered" out of current NMBAs.[9,23,26]

Dose Ratios in Monkeys: Circulatory Changes versus Neuromuscular Block

Bolus *versus* Cumulative Administration: Circulatory Consequences Are Different. Large doses of CW002 (approximately 20 or more × ED_{95}, or 0.80 to 2.40 mg/kg), given to monkeys as the "first dose of the day," result in dose-related *decreases* in MAP and *increases* in HR, which are *not* noted after incremental (cumulative) administration (table 4 and figs. 4 and 5). After bolus injection of 2.4 mg/kg (approximately 60 × ED_{95}), flushing was noted in each of three monkeys, but was *not* observed during cumulative administration. This contrast has also been observed in the dog, where plasma histamine levels increased after 0.40 to 0.60 mg/kg (approximately 40 to 60 × ED_{95}) only after single-bolus doses; decreases in MAP and increases in HR were also noted (unpublished, Paul M. Heerdt, M.D., Ph.D., Weill Cornell Medical College, 2010 to 2013).

Before clinical trials, protocols evaluating the potential of NMBAs to cause circulatory and/or histaminoid changes in dogs or monkeys should measure the effects of single-bolus

Table 5. Autonomic Blockade by CW002 and Dose Ratios *versus* Neuromuscular Blockade in the Cat*

Autonomic Effect or Dose Ratio	Result (± SD)†
VB	ED_{50} = 0.59 ± 0.07 mg/kg
SB	ED_{50} = >> 0.80 mg/kg
NMB‡	ED_{95} = 0.035 ± 0.01 mg/kg
Dose ratio: ED_{50} (VB)/ED_{95} (NMB)‡	17 × ED_{95} (NMB)
Dose ratio: ED_{50} (SB)/ED_{95} (NMB)‡	>> 23 × ED_{95} (NMB)

*Cats under isoflurane anesthesia. Right vagus nerve and sympathetic trunk were stimulated with 10-s trains of square waves (0.5 ms × 20 Hz). †ED_{50} and ED_{95} determined from dose–response curves generated by nonlinear regression. ‡Peroneal nerve stimulated at 0.15 Hz to elicit twitch of the tibialis anterior.

ED_{50} = dose producing 50% block of vagal or sympathetic response (see Methods, Studies in the Cat, Autonomic Studies); ED_{95} = dose producing 95% block of twitch; NMB = neuromuscular blockade; SB = sympathetic blockade; VB = vagal blockade.

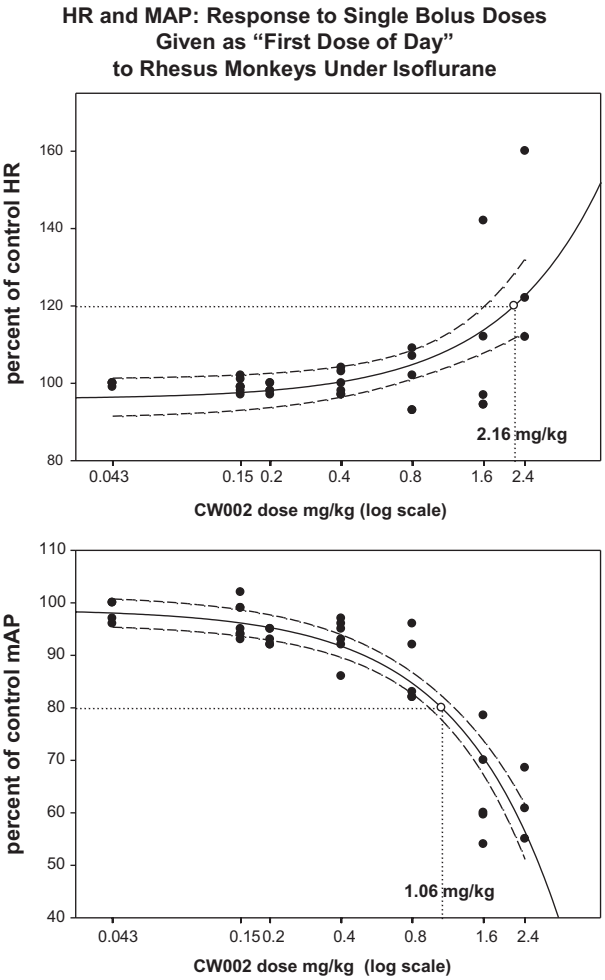


Fig. 5. Curvilinear regressions for maximum changes in mean arterial pressure (MAP) and heart rate (HR) in isoflurane-anesthetized Rhesus monkeys after large bolus doses of CW002 given as "first dose of the day." Effective dose causing 20% change (ED_{20}) for MAP *decrease* was 1.06 mg/kg; ED_{20} for HR *increase* was 2.16 mg/kg. 95% CI was 0.94 to 1.21 mg/kg for MAP and 1.61 to more than 2.4 mg/kg for HR. ED_{20}s are shown as *open circles* on the curves. See Discussion, Bolus *versus* Cumulative Administration: Circulatory Consequences Are Different, for further details.

doses given as the "first dose of the day" to better mimic future clinical practice, where a large dose of NMBA is commonly given first; cumulative administration may underestimate the circulatory effects of NMBAs later observed in humans.

For example, in dogs, gantacurium caused histaminoid responses during cumulative administration at about 12.5 to 25 × ED_{95}[34]; in monkeys, mivacurium and gantacurium elicited such responses at approximately 12 and 50 × ED_{95}, respectively, when given cumulatively.[8] After "first doses of the day" given to humans, histaminoid changes resulted after 2.5 × ED_{95} for mivacurium (0.20 mg/kg), and 3 to 4 × ED_{95} for gantacurium (0.50 to 0.70 mg/kg).[35,36]

Summary

Additional properties of the novel NMBA CW002, including its rapid reversal by L-cysteine under various circumstances and its relative lack of circulatory or autonomic effects, are described herein. The data further support its safety and efficacy.

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Competing Interests

Drs. Savarese and McGilvra declare inventorship. The other authors declare no competing interests.

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