Pharmacodynamics and Cardiopulmonary Side Effects of CW002, a Cysteine-reversible Neuromuscular Blocking Drug in Dogs

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ABSTRACT
Background: CW002 is a novel neuromuscular blocking drug with a duration dependent on the rate of cysteine adduction to the molecule. The current study characterized the pharmacodynamics and cardiopulmonary side effects of CW002 in dogs.

Methods: In eight beagles, the dose required to produce 95% neuromuscular blockade (ED95) for CW002 was first determined and cysteine reversibility was confirmed. Five to 7 days later, incrementally larger doses were injected starting with 6.25 × ED95 and doubling the dose every 15 min. Before and after injection, blood was obtained for histamine analysis. Systemic and pulmonary arterial pressures, cardiac output, and left ventricular pressure and volume were recorded along with inspiratory pressure and pulmonary compliance. Ventricular contractility and lusitropy were indexed from pressure and volume data.

Results: The ED95 for CW002 from pooled data was 0.009 mg/kg. At 3 × ED95, onset time was 2.6 ± 0.9 min and duration was 47 ± 9 min. The duration was shortened to 3.7 ± 0.6 min by 50 mg/kg L-cysteine injected 1 min after CW002. At 25 × ED95, CW002 reduced mean arterial pressure with concomitant decreases in systemic vascular resistance, mean pulmonary artery pressure, cardiac output, contractility, and lusitropy, beginning at 50 × ED95. However, even at a dose of 100 × ED95, the average change in any variable was less than 20%. There were no changes in pulmonary vascular resistance or ventilation mechanics at any dose, and histamine release occurred in only two of eight animals.

Conclusions: CW002 is a potent neuromuscular blocking drug that at doses up to 100 × ED95 produces modest hemodynamic effects that are not associated with bronchoconstriction or consistent histamine release.

What We Already Know about This Topic
❖ CW002, a nondepolarizing neuromuscular blocker, can be reversed by intravenous cysteine
❖ Many novel neuromuscular blockers cause histamine release or cardiovascular complications

What This Article Tells Us That Is New
❖ In dogs, CW002 did not consistently cause histamine release and reduced blood pressure and cardiac output only at 25 times or greater the ED95 dose for neuromuscular blockade
❖ Further development of this rapid acting and rapidly reversible neuromuscular blocker is warranted

RECENT data indicate that the nondepolarizing neuromuscular blocking drug gantacurium exhibits an ultrashort duration of action largely dependent on the rate at which endogenous cysteine binds to and inactivates the molecule.1–3 The novel pharmacology of gantacurium has led to development of the nonhalogenated, symmetrical, benzylisoquinolinium fumarate diester compound CW002 (previously known as AV002), a drug that interacts more slowly with endogenous cysteine to produce a longer duration of action4 but can be rapidly reversed by injection of exogenous cysteine, even in the absence of spontaneous muscle recovery.4,5 Accordingly, CW002 may represent a neuromuscular blocking drug with a duration that can be easily tailored to clinical needs.

A limitation to the clinical use of many novel neuromuscular blocking drugs has been an unfavorable cardiopulmonary response profile manifest as histamine release, hypotension, either primary (ganglionic blockade) or secondary (reflex) tachycardia, and/or bronchospasm.6–8 Previous investigation in dogs has demonstrated that gantacurium does not elicit any effect on peak inspiratory pressure or lung compliance but will induce histamine release and a decrease in arterial blood pressure when

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doses in excess of 25 times of that required to produce a 95% neuromuscular blockade (ED95) are administered. The current study was designed to characterize the pharmacodynamic profile of CW002 in dogs and to determine the effect of incrementally larger doses of CW002 on hemodynamics and ventilatory dynamics in the context of simultaneous changes in plasma histamine.

Materials and Methods

Eight adult beagles weighing between 9.0 and 13.0 kg were used for the study after approval of the protocol by the Animal Care and Use Committee of Weill Cornell Medical College (New York, NY). Each animal underwent two procedures: the first to estimate the dose required to produce 95% neuromuscular blockade (ED95) and to confirm cysteine reversal. The second experiment was designed to characterize histamine release and cardiopulmonary effects of incrementally larger doses of CW002 administered as multiples of the ED95. TOF = train-of-four stimulation.

**Fig. 1.** Schematic representation of the study design. Each animal underwent two experiments separated by 5–7 days. The first was to examine the response to multiple submaximal doses of CW002 for estimation of the dose required to produce 95% neuromuscular blockade (ED95) and to confirm cysteine reversal. The second experiment was designed to characterize histamine release and cardiopulmonary effects of incrementally larger doses of CW002 administered as multiples of the ED95. TOF = train-of-four stimulation.

**Fig. 2.** The chemical structure of CW002. The region of the fumeric acid component that interacts with cysteine to inactivate the molecule is designated as the cysteine adduction domain.

**CW002**

CW002, in powder form, was dissolved in 0.9% saline to a concentration of 1 mg/ml.

**Determination of ED95**

After an overnight fast and sedation with subcutaneous acepromazine (0.1 mg/kg), anesthesia was induced with 5 mg/kg intravenous propofol, the trachea was intubated, and the lungs were ventilated with a mixture of 30% oxygen, 70% nitrous oxide, and 1.0–1.5% isoflurane. Inspiratory pressures, volumes, and flow rates were continuously monitored via sidestream spirometry, and inspired and expired gas composition was assessed with infrared analysis (Datex Ultima, Helsinki, Finland). From ventilatory pressures and volumes, pulmonary compliance was calculated on a breath-to-breath basis. Arterial oxygen saturation was continuously measured using oximetry, minute ventilation was adjusted to maintain an end-tidal PCO2 of 30–32 mmHg, and body temperature was maintained at approximately 37.5°C with a water-circulating heating blanket. After placement of electrocardiogram leads, the right femoral artery was cannulated with a 22-gauge catheter for measurement of systemic arterial pressure, and a suture loop was placed around a superficial segment of tendon attached to the left tibialis anterior muscle. The tendon loop was attached to a force transducer (FT10 C; Grass Instruments, Quincy, MA), pre-loaded with 50 g tension, and twitch responses elicited by supramaximal train-of-four electrical stimulation of the peroneal nerve were recorded every 12 s. After preparation, a 15- to 20-min stabilization period was allowed before initiating the protocol. During this time, end-tidal isoflurane concentration was allowed to equilibrate at approximately 1.3%, and consistency of twitch height was confirmed. Potency of CW002 for producing neuromuscular blockade in each animal was then assessed by bolus administration of multiple doses starting at 0.001 mg/kg.
An interval of 60 min was allowed between doses with full recovery of twitch height and verification of normal train-of-four stimulation. For each animal, three doses producing between 2 and 99% block were fit to a log-logit model to estimate individual ED95. Finally, to confirm cysteine reversibility, in four dogs, a dose reflecting 3 × ED95 was administered, followed 1 min later by injection of 50 mg/kg cysteine prepared as the l-cysteine base (Sigma Chemical Company, St. Louis, MO) dissolved in 0.9% saline to a concentration of 100 mg/kg (pH 5.5). After return of neuromuscular function, the animals were awakened, the trachea was extubated, and activity was monitored for clinical signs of recurrent muscle weakness over the next 2 h.

**Evaluation of Cardiopulmonary Side Effects**

After induction of anesthesia and preparation as described earlier for determination of ED95, an additional arterial catheter was inserted for blood sampling. In six of eight dogs, a 5.0-French pulmonary artery catheter was also positioned via the right external jugular vein, and a sternotomy was performed. In these animals, an electromagnetic flow probe (Carolina Medical Instruments, King, SC) was placed around the ascending aorta for continuous blood flow measurement and a conductance/micromanometer catheter (Millar Medical Instruments, Dallas, TX) was inserted into the left ventricle (LV) via an apical stab for continuous measurement of pressure and volume. Offset of the volume signal (parallel conductance) was determined by injection of 5 ml 3% saline via the pulmonary artery catheter, and stroke volume measurements were cross-calibrated with the electromagnetic flow probe. During the procedure, estimated fluid deficits were replaced with lactated Ringer’s solution, which was subsequently maintained at a rate of 5–7 ml·kg⁻¹·h⁻¹ throughout the experiment.

**Study Design and Data Acquisition**

After a 15-min stabilization period, a CW002 dose reflecting 3 × ED95 for that animal was administered as an intravenous bolus. After complete spontaneous recovery of twitch height and a normal train-of-four response, thus allowing for comparison to the time course for cysteine reversal of the same dose in the initial experiment, the cardiopulmonary side effects of incrementally larger boluses of CW002 were determined by first injecting 6.25 × ED95 and then doubling the dose every 15 min up to a dose of at least 100 × ED95. During the incremental CW002 dosing, arterial blood samples were obtained for histamine analysis (immunoassay kit, Immunotech International, Marseille, France) before and 1 min after each dose, and hemodynamic effects were assessed from peak changes that occurred during the 3 min after drug injection. Hemodynamic data were recorded in both analog and digital format. From direct measurements, LV ejection fraction was calculated, the first derivative of LV pressure increase (dP/dt) was normalized to end-diastolic volume as a load-corrected index of LV contractility, and the time constant of isovolumic relaxation (τ) was calculated with the assumption of a nonzero asymptote as an index of lusitropy.

**Statistics and Data Analysis**

For the 3 × ED95 dose of CW002 in each dog, the time to 5, 25, 75, and 95% recovery of first twitch was determined, with the 95% value regarded as duration of neuromuscular blockade. To verify cysteine reversibility, the time to 95% recovery of first twitch after 3 × ED95 CW002 doses given alone or followed after 1 min by cysteine administration (n = 4) was compared by paired t test. Alterations in baseline (preinjection) cardiopulmonary variables over time along with changes from baseline after
each CW002 dose (postinjection) were assessed by two-way ANOVA for repeated measures and the Holm-Sidak test when appropriate. Correlation between changes in plasma histamine concentration and changes in blood pressure was assessed by linear regression analysis. Data are presented as mean ± SD, with a P value of less than or equal to 0.05 considered significant.

Results

Potency, Onset, Duration, and Cysteine Reversibility

In individual animals, estimated ED$_{95}$ ranged from 0.006–0.012 mg/kg. Pooled dose response data subjected to log-logit analysis are shown in figure 3 and demonstrate an ED$_{95}$ of 0.009 mg/kg, with a 95% CI of 0.008–0.11. On
average, $3 \times \text{ED}_{95}$ CW002 had an onset of $2.6 \pm 0.9$ min and a duration of $47 \pm 9$ min. A bolus dose of 50 mg/kg cysteine administered 1 min after injection of $3 \times \text{ED}_{95}$ CW002 rapidly facilitated the return of neuromuscular function, with 95% twitch recovery evident within $3.7 \pm 0.6$ min.

### Cardiopulmonary Side Effects and Histamine Release after Bolus Dosing

Throughout the incremental bolus dosing protocol, end-tidal carbon dioxide, isoflurane concentration, and body temperature remained constant. As shown in figure 4, there were no differences among any of the preinjection baseline values for heart rate, mean arterial pressure (MAP), mean pulmonary arterial pressure, or cardiac output. For MAP, CW002 produced a small but significant reduction using repeated-measures analysis at $25 \times \text{ED}_{95}$. This effect became progressively larger with subsequent doses. Dose-related reductions were also evident in heart rate and cardiac output starting at $50 \times \text{ED}_{95}$ and mean pulmonary arterial pressure at $100 \times \text{ED}_{95}$. Similarly, systemic vascular resistance decreased with increasing CW002 dose (fig. 4), but there were no attendant changes in pulmonary vascular resistance, peak inspiratory pressure, or lung compliance.

The cardiac responses to incremental doses of CW002 are shown in figure 5. There were no changes in preinjection baseline values during the dosing interval. The drug did not significantly alter LV end-diastolic volume, stroke volume, or ejection fraction. For EF, CW002 produced a small but significant reduction using repeated-measures analysis at $25 \times \text{ED}_{95}$. This effect became progressively larger with subsequent doses. Dose-related reductions were also evident in heart rate and cardiac output starting at $50 \times \text{ED}_{95}$ and mean pulmonary arterial pressure at $100 \times \text{ED}_{95}$. Similarly, systemic vascular resistance decreased with increasing CW002 dose (fig. 4), but there were no attendant changes in pulmonary vascular resistance, peak inspiratory pressure, or lung compliance.
fraction at any dose but did decrease dP/dt and dP/dt normalized to end-diastolic volume, consistent with reduced inotropy and increased \( \tau \), indicative of negative lusitropy, at doses of \( 50 \times ED_{95} \) and higher. However, although consistent, changes in inotropy and lusitropy were relatively small.

In that \( ED_{95} \) exhibited a 2-fold range, there was a substantial variation in the absolute CW002 dose in milligrams per kilogram administered for each multiple of \( ED_{95} \). This allowed for plotting the relationships between a wide range of doses and changes in MAP and plasma histamine. As shown in figure 6, regression of the CW002 dose–MAP response relationship estimates that 0.84 mg/kg will produce a 20% decrease in MAP, yielding a safety ratio for this endpoint of 93 (0.84/the \( ED_{95} \) of 0.009 mg/kg).

Discussion

CW002 is a nonhalogenated, symmetrical, benzylisoquinolinium fumarate diester compound with a duration of action largely dependent on the rate at which endogenous cysteine adducts to the molecule. Preliminary data indicate that CW002 (previously known as AV002) produces neuromuscular blockade of intermediate duration in monkeys and cats. The current study demonstrates that CW002 is also a potent neuromuscular blocking drug in dogs (\( ED_{95} \), 0.009 mg/kg), with \( 3 \times ED_{95} \) having an onset and duration of 2.6 ± 0.9 and 47 ± 9 min, respectively. Consistent with molecular inactivation by cysteine, the duration of CW002 can be markedly shortened by intravenous injection of cysteine, with 50 mg/kg administered 1 min after a \( 3 \times ED_{95} \) dose decreasing duration to 3.7 ± 0.6 min.

In that clinical use of many novel neuromuscular blocking drugs has been precluded by an unfavorable cardiopulmonary response profile, the current study was designed to define the effect of incrementally larger doses of CW002 on hemodynamics, ventilation pressures, and pulmonary compliance. Our data indicate that when given at supratherapeutic doses, CW002 produces dose-related effects on hemodynamics, particularly systemic blood pressure. By using a repeated-measures statistical model, even small changes can be significant when they occur consistently from animal to animal, and this was evident from the fact that an average decrease in MAP of only 4 mmHg after administration of \( 25 \times ED_{95} \) was statistically significant. Nonetheless, even at doses as high as \( 100 \times ED_{95} \), the cardiovascular response profile was unchanged.

Fig. 6. The CW002 dose–blood pressure response relationship for all doses administered in the incremental dosing protocol. A regression model was applied in which both the data (solid line) and 95% confidence interval (hatched gray line) were forced through 0. Analysis of this regression predicts that 0.84 mg/kg CW002 will produce a 20% reduction in mean arterial pressure (MAP), yielding a safety ratio for this endpoint of 93 (0.84/the \( ED_{95} \) of 0.009 mg/kg).

Fig. 7. (A) Individual changes in plasma histamine as a function of absolute dose for all doses in all animals (n = 8). Two animals exhibited histamine release, whereas six did not. (B) Percent changes in mean arterial pressure (MAP) plotted as a function of the simultaneous absolute change in plasma histamine. For animals that did not exhibit histamine release (nonreleasers), there was no correlation between plasma histamine and change in MAP. In contrast, for those that did exhibit an increase in plasma histamine (releasers), the two variables were correlated.

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responses to CW002 are relatively modest, reflecting, on average, changes less than or equal to 20%. Furthermore, there was no evidence of bronchoconstriction at any dose.

In contrast to gantacurium, another drug inactivated by cysteine adduction, the cardiovascular response to high doses of CW002 does not seem to be primarily mediated by histamine release; although dose-related changes in MAP occurred in all animals, increases in plasma histamine concentration did not. In addition, although high-dose gantacurium has little effect on heart rate, cardiac output, or myocardial contractility in beagles,10 high-dose CW002 decreased these variables. Accordingly, although the mechanisms behind the hemodynamic effects of CW002 remain unclear, the relationship between changes in blood pressure, blood flow, and cardiac performance at high doses suggests that the response is multifactorial.

Although our data clearly show that the duration of CW002 can be markedly shortened by administration of intravenous cysteine, the study provides little information regarding the cysteine–CW002 interaction or metabolism of the resulting adduction product. Preliminary data from ongoing pharmacokinetic and toxicologic studies indicate that at pH 7.4 and temperature of 37°C, cysteine interacts nonenzymatically with CW002 to produce an adduction product (NB 1043–10) that is 60-fold less potent than the parent molecule as a neuromuscular blocking drug.4,10 Formation of the adduct rapidly occurs in the presence of a slight excess of cysteine in vitro and seems to be relatively unstable under physiologic conditions, undergoing alkaline hydrolysis to two charged, monoquaternary fragments. These in vitro findings are consistent with in vivo observations from the current study, indicating no return of neuromuscular blockade after cysteine reversal of CW002.

Results of the study need to be interpreted in the context of several limitations. First, CW002 is roughly five times more potent in dogs than in nonhuman primates,4 an animal model that may be more appropriate for predicting potency in human. Although the precise reason for this difference in potency is unclear, it is probably not a reflection of species differences in the availability of endogenous cysteine given that the potency of gantacurium is similar in both animal models.2,10 Nonetheless, the fact that a considerably larger dose of CW002 is required to produce the same effect in nonhuman primates raises the question of whether incremental dosing with multiples of the ED$_{95}$ in this animal model would reveal more pronounced cardiovascular effects and histamine release. Accordingly, extrapolation of findings from the current study to predict patient responses to CW002 should be performed cautiously, particularly in light of the supervening clinical influences of advanced age, cardiopulmonary morbidity, and concomitant pharmacotherapy. Second, aspects of the experimental design could have influenced the conclusions of the study. For example, whether the pharmacodynamics of CW002 can be influenced by anesthetic technique remains unknown. Furthermore, given that repeated stimulation of mast cells can lead to depletion of histamine stores, it is possible that high doses of CW002 given as a first dose would have produced a greater increase in plasma histamine in animals that were “releasers” and potentially a demonstrable increase in those that were found to be “nonreleasers” in response to incremental dosing. Finally, although the study confirms that CW002 is reversible by cysteine in dogs, it does not specifically address dose–response relationships or cysteine side effects. Based on previous investigation,10 a dose of 50 mg/kg was chosen for the current study, because this amount was maximally effective in nonhuman primates. However, preliminary data suggest that lower doses are also effective in the dogs5 and may be more clinically attractive given that high-dose cysteine has been reported to elicit a sustained pressor response in dogs and adverse central nervous system effects in rodents.7,10

In summary, our data indicate that CW002 is a potent, cysteine-reversible neuromuscular blocking drug with no apparent cardiopulmonary effects when administered at clinically relevant doses in male beagles. At high doses (> 25 × ED$_{95}$), the compound can produce modest, transient, hemodynamic change that is not associated with any alteration in respiratory mechanics or consistent histamine release. These data suggest that CW002 may represent a worthwhile target for clinical development.

References