Preclinical Pharmacology in the Rhesus Monkey of CW 1759-50, a New Ultra-short Acting Nondepolarizing Neuromuscular Blocking Agent, Degraded and Antagonized by L-Cysteine

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ABSTRACT

Background: Structure–activity studies were performed to identify a new neuromuscular blocking agent retaining the ultra-short acting characteristics of gantacurium, including degradation and reversal by L-cysteine, but lacking its histaminoid properties in man. CW 1759-50 has emerged from this program.

Methods: Adduction of CW 1759-50 with L-cysteine was studied by high-performance liquid chromatography and mass spectrometry. Institutional Animal Care and Use Committee–approved comparisons of CW 1759-50 to gantacurium were performed in rhesus monkeys. ED95 for neuromuscular blockade was established. Spontaneous recovery was compared to reversal by L-cysteine in paired studies of boluses or infusions. In addition, changes in mean arterial pressure and heart rate after very large doses of 15 to 60 × ED95 were compared.

Results: The half-time of adduction of L-cysteine to CW 1759-50 *in vitro* was 2.3 min. The ED95 of CW 1759-50 was 0.069 ± 0.02 mg/kg; ED95 of gantacurium was 0.081 ± 0.05 mg/kg (P = 0.006). Duration of action (recovery to 95% twitch height after 98 to 99% blockade) was as follows: CW 1759-50, 8.2 ± 1.5 min; and gantacurium, 7.4 ± 1.9 min; (n = 8 and 9, P = 0.355). Administration of L-cysteine (30 mg/kg) shortened recovery (*i.e.*, induced reversal) from CW 1759-50 after boluses or infusions (P always less than 0.0001). Recovery intervals (5 to 95% twitch) ranged from 6.1 to 6.7 min (and did not differ significantly) after boluses of 0.10 to 0.50 mg/kg, as well as control infusions (P = 0.426 by analysis of variance). Dose ratios comparing changes of 30% in mean arterial pressure or heart rate to ED95 for neuromuscular blockade (ED 30% Δ [mean arterial pressure or heart rate]/ED95) were higher for CW 1759-50 than for gantacurium.

Conclusions: CW 1759-50, similar to gantacurium, is an ultra-short acting neuromuscular blocking agent, antagonized by L-cysteine, in the monkey. The circulatory effects, however, are much reduced in comparison with gantacurium, suggesting a trial in humans. **(ANESTHESIOLOGY 2018; 129:970-88)**

WE have described novel nondepolarizing neuromuscular blocking agents that are degraded nonenzymatically by adduction of L-cysteine under physiologic conditions, yielding inactive water-soluble derivatives.[1] The half-time of the adduction/degradation reaction *in vitro* is directly related to the duration of neuromuscular blockade in the anesthetized rhesus monkey.[1] Half-time is defined[1] as the time required *in vitro* for the consumption of 50% of the remaining amount of reactant (neuromuscular blocking agent) in the adduction reaction with L-cysteine at any time. Reversal of 100% twitch inhibition after 3 × ED95 doses of the compounds described[1] (gantacurium, CW 002, and CW 011) is rapidly induced by exogenous L-cysteine (30 mg/kg iv). If L-cysteine is given 1 min after such doses, recovery of twitch from 100% block to at least 95% of baseline occurs within 3 min or less.[1] Structures with half-times of L-cysteine adduction *in vitro* of less than 3 min (such as gantacurium) are ultra-short acting neuromuscular

Editor's Perspective

What We Already Know about This Topic

- Gantacurium is an ultra-short acting nondepolarizing neuromuscular blocking agent in the monkey and in man that is degraded nonenzymatically by adduction of L-cysteine under physiologic conditions
- Administration of gantacurium results in decreased mean arterial pressure and increased heart rate

What This Article Tells Us That Is New

- CW 1759-50 is a new nondepolarizing neuromuscular blocking agent that may have a clinical profile that is superior to that of gantacurium
- Studies in rhesus monkeys comparing CW 1759-50 with gantacurium found both of them to be ultra-short acting because of their rapid degradation by L-cysteine adduction
- The effects of CW 1759-50 on mean arterial pressure and heart rate were substantially less than those of gantacurium

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blocking agents, with durations of action at ED95 doses, from injection to spontaneous recovery of twitch to at least 95% of baseline, of less than 10 min in the rhesus monkey.[1]

Gantacurium underwent phase I study in humans; the duration to spontaneous recovery of twitch to at least 95% of baseline at approximately 2 to 3 × ED95 (0.4 to 0.5 mg/kg) was 11 to 14 min.[2] Transient histaminoid phenomena, such as facial flushing, decreased mean arterial pressure (MAP), and increased heart rate (HR), were noted, together with increased plasma histamine concentrations.[2] These changes were not considered unsafe, however, and the compound was approved for phase III studies by the US Food and Drug Administration. Development was subsequently halted by the developers because of the aforementioned side effects and especially due to intubation scores unequal to those of succinylcholine at 60 s from injection. Lack of financing was also a factor (personal verbal communications, Avera Pharmaceuticals, San Diego, California, 2002 to 2006).

We have since continued to seek ultra-short nondepolarizing neuromuscular blocking activity, minus the abovementioned shortcomings of gantacurium, during a program (2012 to 2015) that explored new structure–activity relationships. Gantacurium has been the standard of comparison against which we have evaluated new compounds throughout these investigations because of its ultra-short duration of action in the monkey[3,4] and in man.[2] Antagonism of 100% twitch inhibition by exogenous L-cysteine and reduction of the histaminoid side effects of gantacurium were considered essential.

A new compound, CW 1759-50 (fig. 1), which has emerged during these investigations, displays a profile superior to that of gantacurium. We describe comparative studies done in the rhesus monkey model between 2012 and 2015, which we feel well anticipate the human pharmacology.

Materials and Methods

Synthesis of CW 1759-50 and Gantacurium

CW 1759-50 was synthesized by J. McGilvra at Cedarburg Hauser Pharmaceuticals, Grafton, Wisconsin, a division of Albany Molecular Research, Inc., Albany, New York (appendix 1). Briefly, the quaternary benzylisoquinolinium amino alcohol 1972-25 was first esterified with maleic anhydride in acetonitrile and triethylamine to give the monoester 1972-38 and then again esterified with the morpholinium alcohol 1972-11 to yield the desired bisquaternary diester neuromuscular blocking agent CW 1759-50. The synthesis of gantacurium has been described.[3] The synthetic scheme for gantacurium (appendix 1) is analogous to that of CW 1759-50. New batches of CW 1759-50 and gantacurium were synthesized during the study period.

Formulation of Neuromuscular Blocking Agents

CW 1759-50 or gantacurium (solid materials) were freshly weighed on the morning of each experiment and dissolved in 0.9% NaCl at concentrations of 1.0 to 10.0 mg/ml; pH was adjusted to 3.0 with 1 N HCl, and the compounds were kept in an ice bath to help preserve stability for the day.

Formulation of L-Cysteine

The solution and its stabilization have been described.[1,5] L-Cysteine hydrochloride was dissolved at concentrations of 100 to 200 mg/ml in 0.9% NaCl, with pH adjusted to about

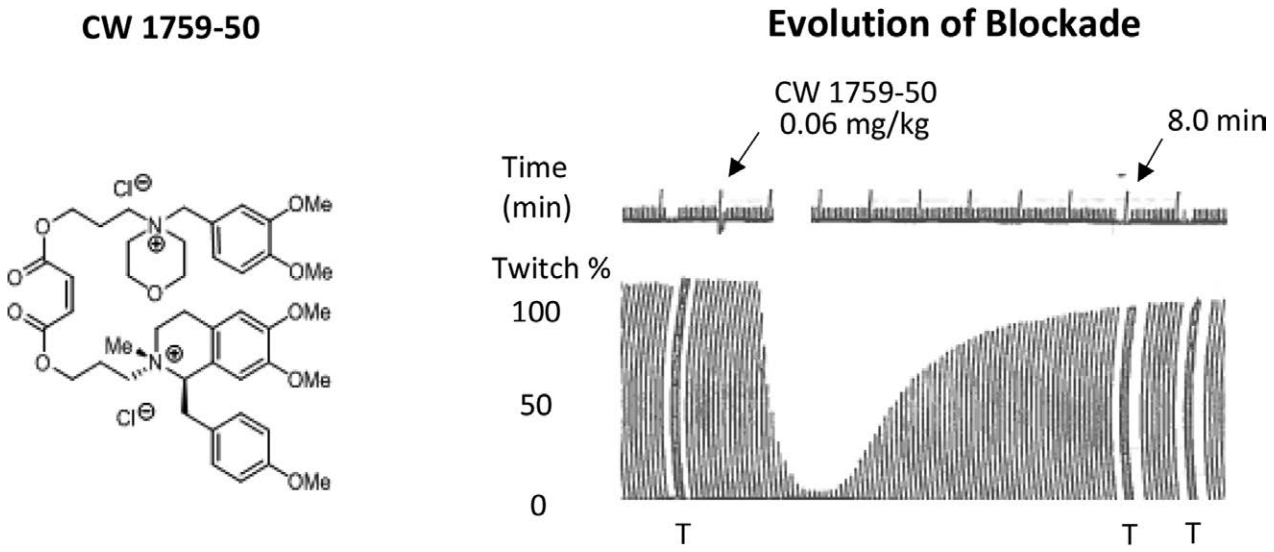

Fig. 1. The chemical formula of CW 1759-50, together with a recording showing the evolution of neuromuscular blockade from an anesthetized rhesus monkey. A dose of 0.06 mg/kg yielded 99% block with onset at 91 s and a duration to recovery of twitch to 95% of baseline of 8.0 min. Twitch of the Achilles tendon was elicited at 0.15 Hz, and train-of-four stimulation (T) was interposed before dosing and after recovery of twitch. Note that train-of-four ratio has recovered to 100% at the time of twitch recovery to 95% of baseline.

5.5 using concentrated (10×) phosphate or Tris buffer and 1 N NaOH or 1N HCl.

Rate of Degradation of CW 1759-50 In Vitro

Background Alkaline/Aqueous Hydrolysis and L-Cysteine Adduction. CW 1759-50 was dissolved in phosphate buffer (pH 7.4) at a concentration of 1,000 μg/ml. Baseline chromatograms were obtained, monitoring the concentration of CW 1759-50 at 37°C over the course of 3.5 h. A plot of the concentration of CW 1759-50 over time allowed the calculation of an alkaline hydrolysis half-time using the plot trend line equation (appendix 2).

For analysis of the adduction rate of L-cysteine to CW 1759-50, freshly prepared buffered solutions of CW 1759-50 were charged with a 5 mol% excess (above stoichiometric) of an aqueous solution of L-cysteine hydrochloride monohydrate to give a final concentration of 200 μg/ml of CW 1759-50 at pH 7.4 in the reaction mixture. The concentration of CW 1759-50 remaining at specific time points after the addition of L-cysteine was determined by analysis of undiluted reaction samples by high-performance liquid chromatography (appendix 2). A half-time of the adduction reaction of L-cysteine to CW 1759-50 was calculated by graphical determination of the reaction rate constant for the second order reaction of L-cysteine adduction to CW 1759-50. A proposed pathway was developed for the degradation of CW 1759-50 in the presence of L-cysteine. Mass spectrometry (appendix 2) was used to identify the products (adducts) of the adduction reaction.

Studies in Anesthetized Rhesus Monkeys

Protocols were approved by the institutional animal care and use committees of Weill Cornell Medical College (New York, New York) and of Albany Medical College (Albany, New York), where the experiments were performed. The data reported in this article on both CW 1759-50 and gantacurium were obtained from the same colony of animals during the same time period of the study from batches of CW 1759-50 and gantacurium, which were synthesized during that time.

Experimental Setup and Protocol

Eight adult male rhesus monkeys weighing 10 to 18 kg were studied at 4- to 6-week intervals. Care and maintenance of the animals and experimental setup have been described.[1,4,5] The animals were given ketamine (7 to 10 mg/kg intramuscular) for induction of anesthesia; the trachea was intubated under topical anesthesia with 2% lidocaine; isoflurane (1 to 2%) in N_2O/O_2 (70/30 mixture) was given for maintenance, and ventilation and monitoring were as previously described.[1,4,5]

Twitch of the Achilles tendon was elicited through the popliteal branch of the sciatic nerve at 0.15 Hz[1,4,5] and recorded *via* a Grass FT 10 (Grass Instruments, USA) force transducer. Train-of-four stimulation was interposed at several time points between 10 min and 1 min before neuromuscular blocking agent administration as a baseline for train-of-four ratio responses and every 1 to 2 min after recovery of twitch to 95% of baseline to show further recovery to a train-of-four ratio of 90% or more. Examples of recordings showing this procedure have been published.[1,4,5] An example is shown here in figure 1.

At the end of each experiment, the animals were given analgesics per veterinary practice and attended under direct observation until they were awake, standing, and climbing. At the end of the study period, all monkeys were sent to primate retirement colonies, in good condition, healthy and uninjured.

Details of Experimental Procedures

The duration of data collection averaged about 8 h per each experimental day. Two protocols were followed to fit this time frame. The two protocols are described and diagrammed below (fig. 2). In general, experimental procedures during which data were gathered were preceded by a baseline period of approximately 15 to 20 min where MAP, HR, twitch, and train-of-four ratio did not vary by more than 5%.

Protocol I: The Dose–Response Protocol. Three doses of the neuromuscular blocking agent under study, the first three doses of the day (only one neuromuscular blocking agent on any experimental day), were given at the beginning of the experiment, to (ideally) generate three points on the dose–response curve, giving approximately 10 to 99% inhibition of the single twitch. The dosing was sequential, not random (see the dose–response section below). Additional doses of 0.20 and/or 0.50 mg/kg were subsequently given to acquire more dose-duration data. Then, after a new baseline period of 20 to 30 min, continuous infusion studies were done; if infusions were paired, the second infusion of the pair was ended with reversal by L-cysteine (30 mg/kg). Alternatively, paired bolus studies were done at doses of 0.20 or 0.50 mg/kg, with the second dose of the pair undergoing reversal with 30 mg/kg of L-cysteine. Reversal was always the last measurement of any day's experiment.

Protocol II: The Large-dose Protocol. Large doses (1.0 to 4.0 mg/kg) or approximately 15 to 60 × ED95, were given as a single bolus over 10 to 15 s as the "first dose of the day": one dose per day. Spontaneous recovery was then recorded for all parameters, to single twitch at least 95% of baseline and train-of-four ratio of more than 90% and to stabilization of HR and MAP. A new baseline period of at least 30 min (approximately 15 × half-time of the adduction/degradation reaction *in vitro*) was then established, after which paired bolus studies or paired infusion studies were done to compare spontaneous recovery with reversal by L-cysteine. Reversal was the final measurement of the experiment.

Neuromuscular Blocking Properties

Dose–Response Curves. One neuromuscular blocking agent was studied per day. A stable baseline period of 15 to 20 min before and between all doses was established where single twitch, train-of-four ratio, MAP, and HR did not vary by more than 5%. The first three doses of the day were given

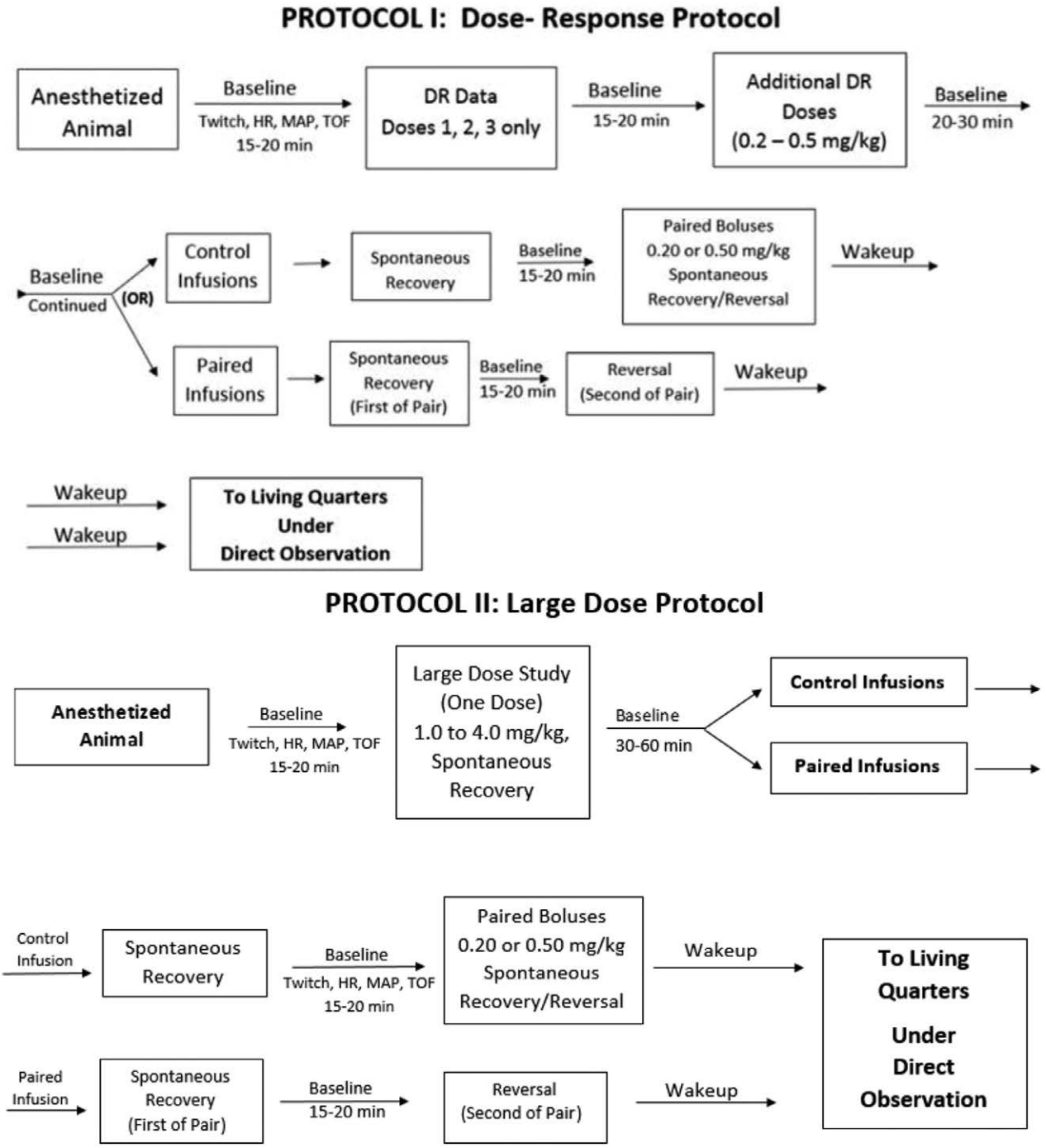

Fig. 2. Diagrams of experimental protocols 1 and 2. *Boxes* indicate periods of data collection. *Arrows* indicate baseline periods. Data for dose–response measurements or circulatory data from administration of large doses (1.0 to 4.0 mg/kg) were obtained at the beginning of the experiments. Infusions were studied in the middle, and reversals were the final measurements.

sequentially, in increasing amounts. The doses were given as a 5-s rapid bolus. Dosing was not randomized; however, an interval of 15 to 20 min was interposed between recovery of the previous dose to single twitch at least 95% of baseline and a train-of-four ratio of 90% or more, before giving a subsequent dose. New baselines of twitch, train-of-four ratio, MAP, and HR were obtained during each interval between doses.

Data only from the first three doses of each experiment were entered into calculation of the dose–response curves. The curves for CW 1759-50 and gantacurium were generated by nonlinear regression of dose (log scale) *versus* the percentage twitch inhibition on an arithmetic scale; ED50 and ED95 were derived from the curves as previously described[5]; the program GraphPad Prism 7.0 was used (GraphPad Software, USA). The curve for gantacurium was generated from

new data obtained during the same time period and from the same animals from which data was collected for CW 1759-50. A dose–response curve for gantacurium, done by log-logit regression, has been previously reported.[4]

The duration of neuromuscular blockade was measured from the point of drug injection to recovery of twitch to 95% of baseline as well as to a train-of-four ratio of 90% or more. The 5 to 95% twitch and 5% twitch to train-of-four ratio of 90% recovery intervals were measured after doses that yielded 95% block or more of the single twitch.

Dose–Duration Relationship Regression for CW 1759-50. The durations (min ± SD) to recovery of twitch to 95% of baseline after doses of CW 1759-50 ranging from 0.05 up to 1.0 mg/kg (0.8 to 15 × ED95) were plotted as a regression of dose (log scale) *versus* duration (arithmetic scale). We determined whether the regression was linear and the slope was constant, because if they are, a change in duration *versus* a constant change in dose would likely have predictive value.

Onset of Neuromuscular Blockade. The databases for CW 1759-50 and gantacurium were screened to select only doses that achieved exactly 98 to 99% block of single twitch (but not 100%). Onset (s) was measured from injection to the point of maximum block. These doses were chosen because 99% block is, in our opinion, the last point readily measurable during onset of twitch suppression as an indicator of onset of neuromuscular blockade, without causing 100% twitch inhibition.

Antagonism of Bolus Doses of CW 1759-50 by L-Cysteine. Pilot studies had established that L-cysteine (30 mg/kg) effectively and rapidly antagonized 100% twitch inhibition produced by CW 1759-50. This dose of L-cysteine is in the same range of 30 to 50 mg/kg found to be optimal for reversal of the intermediate-duration compounds CW 002 and CW 011 in the same colony of monkeys[1,5] in previously reported work, as well as in the dog.[6,7] L-Cysteine 30 mg/kg was administered for reversal in all studies of reversal described in "Reversal at +1 min after Bolus Doses of CW 1759-50: Paired Comparisons of 0.20 and 0.50 mg/kg" and "Continuous Infusion Maintaining 99% Block: Spontaneous Recovery Compared with Antagonism by L-Cysteine: Paired Infusions (n = 11)," which were performed as paired comparisons, where the first dose of the pair underwent spontaneous recovery. Reversal of the second dose by L-cysteine was then always done at the end of the day's studies for two reasons: (1) to mimic clinical practice and (2) especially because we have noted, in the dog[6,7] as well as in the monkey (laboratory of John J. Savarese, 2010 to 2015, unpublished), that reversal of a prior dose of a cysteine-reversible neuromuscular blocking agent, will markedly inhibit and very much shorten the neuromuscular blocking effect of any following dose. Therefore, data on reversal was always gathered as the final measurement at the end of any day's studies.

Reversal at +1 min after Bolus Doses of CW 1759-50: Paired Comparisons of 0.20 and 0.50 mg/kg. At the end of either experimental protocols I or II, after a stable baseline period of at least 15 to 20 min, a control dose of CW 1759-50 of either 3 × ED95 (0.20 mg/kg; n = 8) or 7 × ED95 (0.50 mg/kg; n = 4) was given and allowed to recover spontaneously to more than 95% twitch height and train-of-four ratio of 90% or more. Fifteen to twenty minutes (approximately 10 × half-time *in vitro*) after recovery from the control dose, during which new baselines were established, the same dose was repeated, followed 1 min later by L-cysteine (30 mg/kg) at a time when there was always 100% block of twitch. Duration to 95% twitch recovery and 90% train-of-four ratio, 5 to 95% twitch recovery, and 5% twitch–90% train-of-four ratio recovery intervals were obtained during spontaneous recovery, as well as during reversal by L-cysteine. For the reasons described in "Antagonism of Bolus Doses of CW 1759-50 by L-Cysteine," only one such paired comparison was done per day, as the last determination of the experiment. Data from control bolus doses that recovered from neuromuscular blockade spontaneously in paired studies of reversals of bolus doses were not included in dose–response or dose–duration calculations.

Continuous Infusion: Control Infusion Study (n = 32). Control infusions of CW 1759-50 were given to establish the rate of administration of CW 1759-50 (ED 99 Inf) required to maintain 99% twitch inhibition over a wide range of durations of infusion. Infusion was initiated with a bolus of 0.20 mg/kg; continuous administration *via* a syringe pump was begun when single twitch had recovered to 25% of baseline strength and maintained at a rate that kept neuromuscular blockade at 99% inhibition of the single twitch (ED 99 Inf). Infusion duration was varied randomly from 20 to 130 min to gain a broad experience of spontaneous recovery times after widely varying durations of infusions (to ascertain whether the recovery rate varied with the duration of infusion). Spontaneous recovery was followed in all control infusions, to a train-of-four ratio of at least 90%.

Continuous Infusion Maintaining 99% Block: Spontaneous Recovery Compared with Antagonism by L-Cysteine: Paired Infusions (n = 11). These studies were designed such that spontaneous recovery was compared with reversal by L-cysteine on the same day, at the end of paired infusions where spontaneous recovery was measured at the end of the first infusion (infusion A); reversal with L-cysteine (30 mg/kg) was then measured at the end of the following second infusion of the same duration (infusion B). Infusion durations were varied randomly from day to day, but A and B were controlled to be given over the same duration on a particular day.

Infusion A was initiated with a dose of 0.20 mg/kg of CW 1759-50. Upon recovery of single twitch to 25% of baseline, infusion was begun and continued at a rate maintaining 99% block of twitch (ED 99 Inf). At the end of infusion A, spontaneous recovery of twitch was measured from 5 to 95% of baseline and from 5% twitch to train-of-four ratio of 90%. After spontaneous recovery of infusion A, a 15- to 20-min stable baseline interval of less than 5% change of train-of-four ratio or twitch was maintained before initiation of infusion B.

Infusion B was begun and continued using the same procedure as described for infusion A; the duration of infusion B was identical to that of infusion A. Upon discontinuation of infusion B, the iv line was flushed with 5 ml of Ringer's lactate, and 1 min later, L-cysteine (30 mg/kg) was given at a point where 99 to 100% twitch inhibition was always present. The recovery intervals of single twitch from 5 to 95% of baseline and from 5% twitch to 90% train-of-four ratio were compared after spontaneous recovery (infusion A), with L-cysteine–accelerated recovery (reversal, infusion B). As in comparisons of bolus doses, reversal at the end of the second infusion (B) was the last measurement of the day.

Paired Infusions at Dose of 2 × ED99, Maintaining 100% Twitch Inhibition: Spontaneous Recovery Compared with Reversal by L-Cysteine (n = 6). In a second series of paired infusion studies, the infusion rate of CW 1759-50 required to maintain 99% twitch suppression in the individual animal was first determined on the day of the experiment and then doubled on that same day to a rate of (2 × ED99 Inf) (infusion C). Infusion C was then continued (100% twitch inhibition being maintained for 20 to 75 min). Spontaneous recovery after infusion C was then measured. Fifteen to twenty minutes after recovery to a train-of-four ratio of 90% or more after infusion C, infusion was begun again (infusion D) with a dose of 0.20 mg/kg and maintained at the same infusion rate (2 × ED99 Inf) and for a duration (79.6 ± 24.1 min) not differing from that of infusion C (83.3 ± 21.7 min), $P = 0.786$. One minute after discontinuation of infusion D, L-cysteine (30 mg/kg) was given (100% block of twitch always present), and the 5 to 95% single twitch recovery and the 5% twitch–90% train-of-four recovery intervals were recorded during reversal under these artificial conditions of continuous intentional overdose. Reversal of infusion D was per protocol the last measurement of the day.

Comparisons of 5 to 95% Recovery Intervals during Spontaneous Recovery

One of the more important observations to be made during the program of evaluation of the neuromuscular blocking properties of CW 1759-50 was to ascertain whether the rate of spontaneous recovery from neuromuscular blockade (the 5 to 95% twitch recovery interval) is affected by the size of a bolus dose or by the duration of continuous administration (infusion). We therefore compared by analysis of variance the 5 to 95% recovery intervals measured during spontaneous recovery from bolus doses of 0.10 mg/kg (1.5 × ED95, n = 37); 0.20 mg/kg (3 × ED95, n = 69); 0.50 mg/kg (7 × ED95, n = 47); and all control infusions (n = 32). We also compared by linear regression the effect of duration of infusion on the rate of recovery (5 to 95% twitch recovery rate) during spontaneous recovery from infusion A or during reversal at discontinuation of infusion B.

Comparisons to Gantacurium: Neuromuscular Blockade and Circulatory Effects

Neuromuscular Blocking Properties.

Dose–Response Analysis. The previously reported ED95 of gantacurium[4] was recalculated from new data obtained during the time period of the present studies, in the same animals that were under study for CW 1759-50. The dose–response curve was plotted this time using nonlinear regression of log dose *versus* percentage neuromuscular blockade on an arithmetic scale. The ED95 of gantacurium derived from nonlinear regression was compared with that of CW 1759-50 also obtained by nonlinear regression. The data were derived from study of the same animals and were compared using the unpaired *t* test; we did not attempt to pair the dose–response studies of the two compounds.

Onset and Duration of Neuromuscular Blockade. New dose–response data for CW 1759-50 and gantacurium yielding doses producing exactly 98 to 99% (but not 100%) twitch inhibition were collected. Onset time (measured in seconds) from the point of injection to the point of maximum block was measured and compared by unpaired *t* test. The durations (measured in min) to 95% twitch recovery of gantacurium and of CW 1759-50 after the above doses were also compared by unpaired *t* test. Comparisons of duration were also made after doses of 0.20 mg/kg (2.5 or 3.0 × ED95 of gantacurium and CW 1759-50, respectively).

Circulatory Effects. Comparative maximum changes, from baseline, of MAP and HR resulting after large boluses of CW 1759-50 or gantacurium (1.0 to 4.0 mg/kg) given over 10 to 15 s as the "first dose of the day"[5] (protocol II) were analyzed by unpaired *t* test. The data were expressed as curvilinear dose–response curves and effective doses for 30% change (ED 30% Δ) in MAP (decrease) and HR (increase) were derived, using the program GraphPad Prism version 7.0. The dose ratios [ED 30% Δ HR/ED95 NMB] and [ED 30% Δ MAP/ED95 NMB] were calculated for both CW 1759-50 and gantacurium.

Statistics

Dose–Response Curves for Neuromuscular Blockade. The curves for neuromuscular blockade (twitch inhibition) to determine the potency of CW 1759-50 and gantacurium were plotted by nonlinear regression using the program GraphPad Prism version 7.0 as done previously.[5] These curves represent fifth-order polynomial expansions from which ED50 and ED95 were extracted. The equation for the calculation of the curve for CW 1759-50 is shown together with the curve.

Linear Regressions. Linear regressions were done using GraphPad Prism version 7.0 to compare (1) dose *versus* duration of effect of CW 1759-50, (2) the duration of continuous infusions *versus* the rate of spontaneous recovery from neuromuscular blockade, as well as infusion duration *versus* the rate of recovery after L-cysteine (30 mg/kg) administration for reversal, and (3) the half-time *in vitro versus* duration (spontaneous recovery)

from neuromuscular blockade after bolus doses of 2 × ED95 in man, using already published data[2,8] for gantacurium and CW 002 in humans. In this estimation, the point on the regression where the known half-time *in vitro* of L-cysteine adduction to CW 1759-50 (2.3 min) was identified, and the duration was estimated from the corresponding ordinate (duration axis). A forecast of the duration of neuromuscular blockade that may result after CW 1759-50 doses of 2 × ED95 is given—in the future—to humans, was obtained (appendix 3).

Intergroup Comparisons of Neuromuscular Blockade. Comparisons were done by two-tailed Student's *t* test for unpaired values; in paired comparisons (such as comparison of spontaneous recovery to reversal), the paired *t* test was used (Microsoft Excel Analysis Toolpak, Microsoft Corporation, USA). Analysis of variance was done to compare selected grouped data where spontaneous recovery from 5 to 95% of baseline had been measured: namely, after 1.5, 3.0, and 7.0 × ED95, *i.e.*, bolus doses of 0.10, 0.20, and 0.50 mg/kg and the grouped data from the control infusions. The program used was GraphPad Prism 7.0. This comparison was done to ascertain whether rate of recovery is related to dose within a dose range that might be used clinically.

Intergroup Comparisons: Circulatory Changes. For analysis of changes in MAP and HR caused by large bolus doses of CW 1759-50 or gantacurium, given as "first dose of the day,"[5] curvilinear dose–response curves were constructed using GraphPad Prism 7.0. ED for 30% change (decrease) from baseline was derived for change in MAP [ED 30% Δ MAP]; ED for 30% change (increase) in HR [ED 30% Δ HR] was similarly derived.

Statistical Significance

In all comparisons, $P < 0.05$ was considered to be statistically significant. In this article, we report exact *P* values (except where $P < 0.001$).

Power Analysis

Power analyses were not done in advance to guide appropriate group sizing. Rather, we relied on previous experience to adjust group sizes; for example, where n = 32 for control infusions; n was then decreased to n = 11 and subsequently to n = 6 in later paired studies of infusions comparing spontaneous recovery to reversal of neuromuscular blockade. Particularly in the case of continuous infusions, we wanted to obtain a broad experience over a wide range of infusion durations, to note the effect of duration of administration upon both spontaneous recovery and reversal at the end of infusions. This was felt to be key information to have in hand before administration to humans, where in clinical practice a short-acting drug such as CW 1759-50 might be given preferentially by continuous infusion rather than by repetitive boluses.

Results

Degradation of CW 1759-50 In Vitro

The half-time of the reaction of alkaline hydrolysis in the breakdown of CW 1759-50 (fig. 3, reaction B) was estimated at 240 min based on a plot of data derived from high-performance liquid chromatography, with respect to time (appendix 2). When a 5 mol% excess of L-cysteine was added to a solution of CW 1759-50, the single peak for CW 1759-50 was fully converted to two new peaks within less than 10 min. The half-time for L-cysteine reaction (adduction) with CW 1759-50 was calculated to be 2.3 min at the initial concentration of 200 μg/ml of CW 1759-50 (appendix 2). The two new peaks were identified by mass spectrometry as having the higher molecular weights consistent with the formulae of the adducts proposed in figure 3, reaction A. Because the single peak representing CW 1759-50 is absent after the two new peaks are formed, the adduction reaction must include consumption of CW 1759-50. Note (fig. 3) that because adduction of L-cysteine can occur on either side of the maleate double bond, the two adducts are regioisomers having the same molecular weight.

Neuromuscular Blocking Properties

Potency and Duration of CW 1759-50 and Gantacurium. The ED95 of CW 1759-50 was calculated to be 0.069 ± 0.02 mg/kg; the ED95 of gantacurium was 0.081 ± 0.05 mg/kg. CW 1759-50 is more potent than gantacurium ($P = 0.006$). The dose–response curve is shown in figure 4. Table 1 summarizes grouped dose–response and dose–duration data for CW 1759-50 after bolus doses ranging from 0.02 mg/kg (0.3 × ED95) to 4.0 mg/kg (approximately 60 × ED95). Doubling of dose resulted in an increase in average duration of 1.9 to 4.9 min over the dose range (0.05 to 1.0 mg/kg). The relationship of dose to duration over this range in the monkey can be shown to be linear (fig. 5), where the slope of the line is 5.4 (slope = [min/mg/kg]). The line shows a rather constant increase (or decrease) in duration of about 3 min as dose is doubled (or halved). Half-time for the adduction reaction *in vitro* is similar (2.3 min). These results together suggest that degradation/adduction in plasma by endogenous L-cysteine should be a major pathway of degradation *in vivo*, as well as *in vitro.*

Spontaneous Recovery versus *L-Cysteine-accelerated Recovery (Reversal): Bolus Doses of CW 1759-50*

Comparisons were made of spontaneous recovery and reversal by L-cysteine after the bolus doses of CW 1759-50 of 0.20 (n = 8) and 0.50 (n = 4) mg/kg (3 and 7 × ED95). The data are summarized in table 2. In these paired comparisons of bolus doses, recovery was accelerated significantly by L-cysteine: the duration from injection to recovery of twitch to 95% of baseline was markedly shortened ($P < 0.0001$ in all comparisons). For example, in paired bolus studies, duration after spontaneous recovery from doses of 0.20 mg/kg averaged 12.5 min (n = 8); L-cysteine shortened that duration to 4.5 min (n = 8) when given 1 min after CW 1759-50. Similarly, a dose of 0.50 mg/kg showed duration of 16.1 min (n = 4) after spontaneous recovery; the duration was shortened

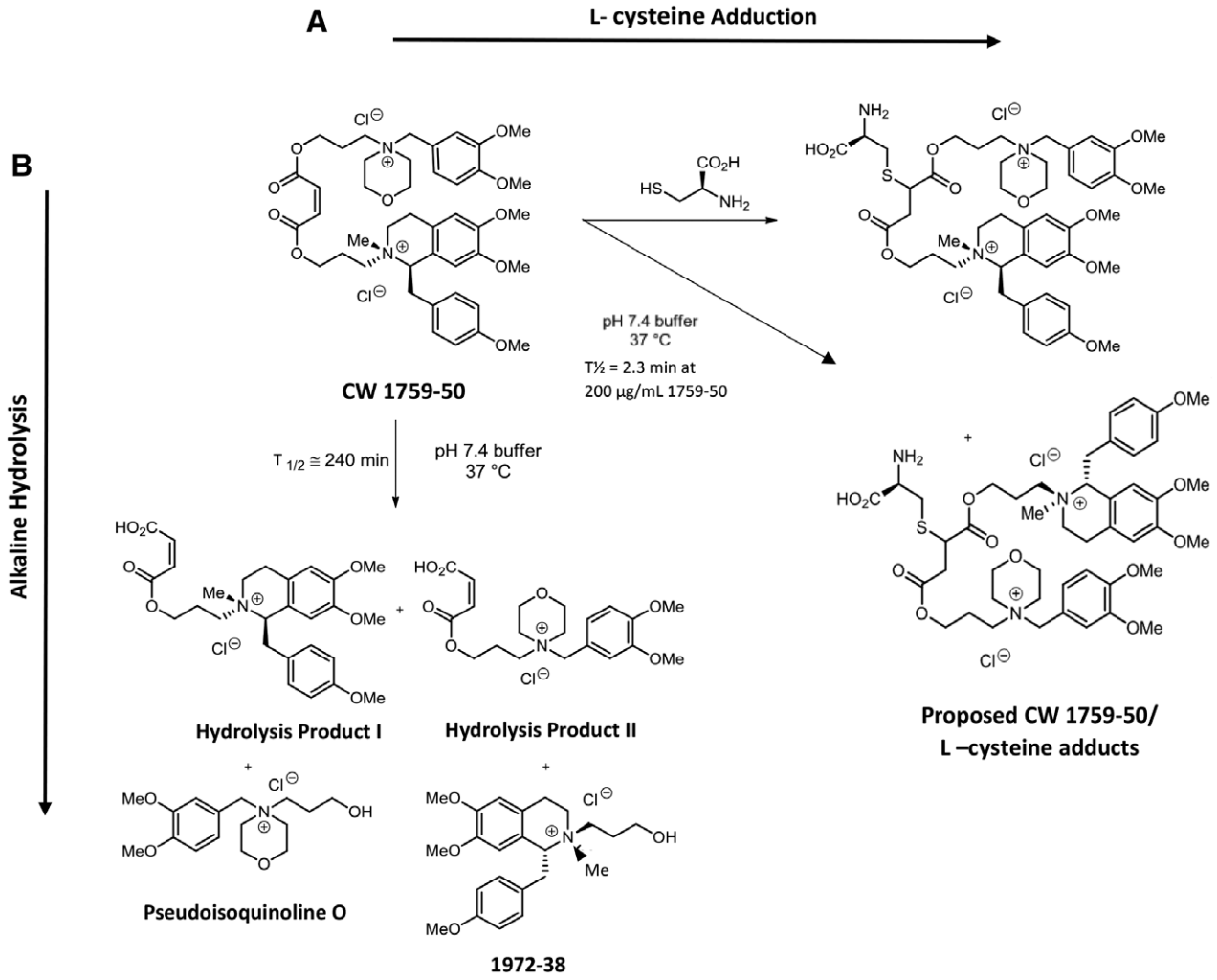

Fig. 3. Proposed degradation *in vitro* of CW 1759-50. Addition of L-cysteine (reaction A) to a solution of CW 1759-50 at physiologic pH and temperature rapidly converts the parent molecule (CW 1759-50) to two adducts with a reaction half-time of 2.3 min. The adducts were identified by mass spectrometry as having the higher molecular weights compatible with the proposed products (adducts) of reaction A. Alkaline hydrolysis (reaction B) also occurs, at a much slower rate (half-time = 240 min), to the hydrolysis products shown. The products of alkaline hydrolysis are monoesters (hydrolysis products I and II) and amino alcohols (pseudoisoquinoline O and 1972-38). The degradation products of both reaction A and reaction B are positively charged quaternary ammonium compounds that are usually readily soluble in water and rapidly excreted in urine.

to 5.6 min (n = 4) after reversal by L-cysteine. Figure 6A shows an original record of a comparison of spontaneous recovery to reversal by L-cysteine after paired doses of CW 1759-50 (0.20 mg/kg), given sequentially as the last two doses of the experiment.

In all paired comparisons of bolus doses, reversal of CW 1759-50 by L-cysteine was always tested on the second dose of the pair, not only to mimic clinical practice but also because reversal of a previous dose would leave a residual inhibitory effect of L-cysteine upon the second dose of the pair.[6]

Spontaneous Recovery Compared with L-Cysteine-accelerated Recovery: Infusions of CW 1759-50

Control infusions of CW 1759-50 were given over periods of 20 to 130 min and required administration at rates of 19.1 ± 5.8 µg/kg/min (ED 99 Inf ± SD) to maintain 99% twitch inhibition. After discontinuation of infusion, spontaneous recovery (5 to 95% twitch recovery interval) remained constant over time at 6.1 ± 1.3 min. The mean recovery interval after the latter control infusions did not differ significantly from that interval measured after bolus doses of 0.10, 0.20, and 0.50 mg/kg (1.5 – 7 × ED95); P = 0.426 by analysis of variance (table 3).

When infusions were paired on the same day (n = 11 pairs, figs. 6B and 7), the dose (ED99 Inf) for the first infusion of the pair, infusion A, required to maintain 99% block of single twitch (20.6 ± 5.0 µg/kg/min) did not differ from the control group of infusions (19.1 ± 5.8 µg/kg/min), P = 0.449 (table 3). When L-cysteine was given for reversal 1 min after discontinuation of the second infusion (infusion B), comparative recovery intervals were

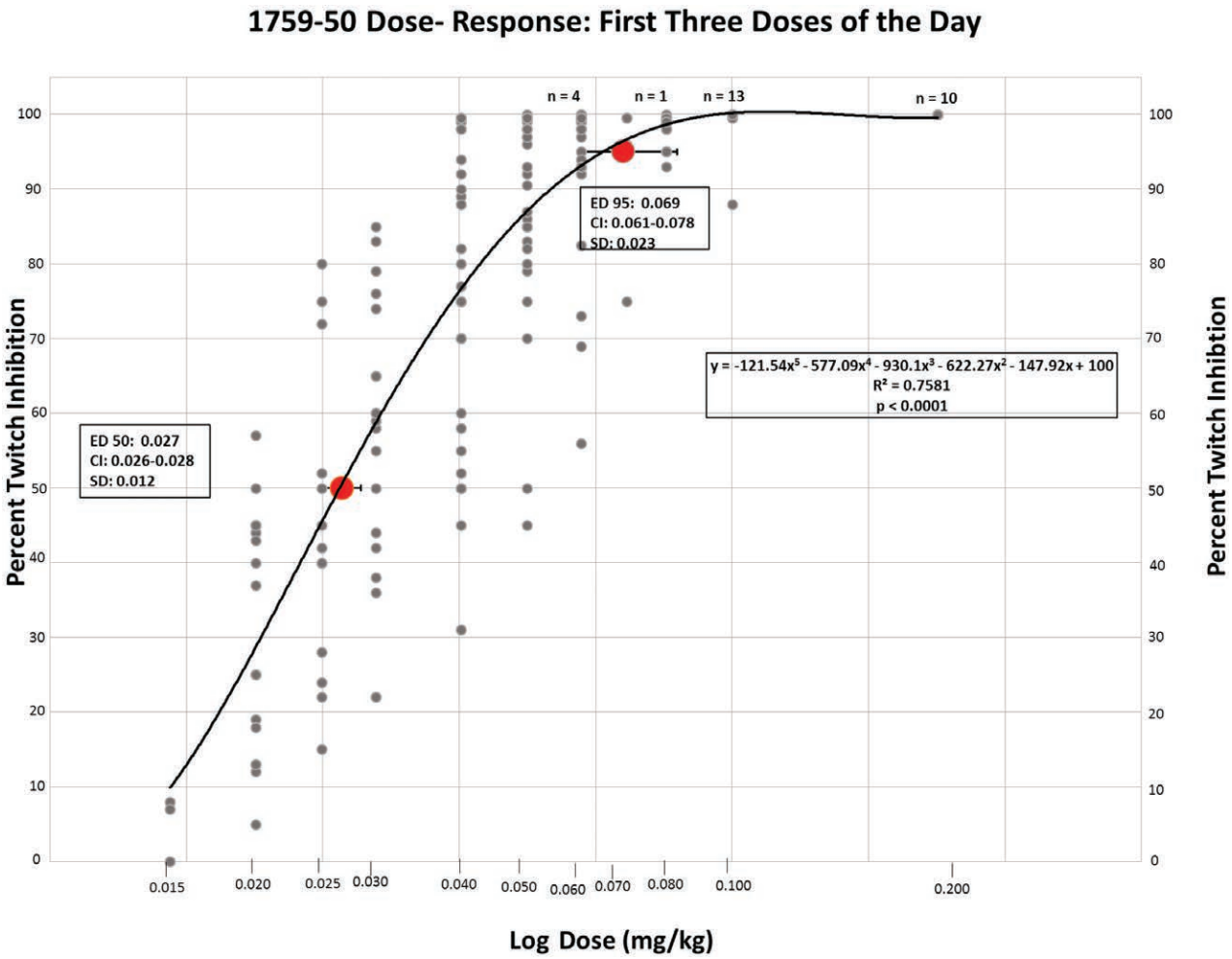

Fig. 4. The dose–response curve of CW 1759-50 in rhesus monkeys under isoflurane. The results of nonlinear regression of log dose *versus* twitch inhibition are shown. Nonlinear regression has been used previously[5] to analyze the curve for the similar neuromuscular blocking agent CW 002. ED95 and ED50 are shown on the graph in *red* with SD and CI in *boxes*; 199 data points are included; $P < 0.0001$; the n values at the top of the *curve* show the number of trials producing 100% twitch inhibition at doses indicated. The equation shown is a fifth order polynomial, from which the ED50 and ED95 were extracted.

Table 1. Neuromuscular Blocking Properties of Bolus Doses of CW 1759-50

Bolus Dose, mg/kg	Approximate ED95 Multiple	n	Twitch Inhibition ± SD, %	Duration, Injection to 95% Twitch, min ± SD	Duration Injection to 90% Train-of-four Ratio, min ± SD	5 to 95% Twitch Recovery Interval, min ± SD	5% Twitch: 90% Train-of-four Ratio Interval, min ± SD
0.02	0.3	27	37.4±20.1	4.9±1.8	N/A	N/A	N/A
0.04	0.6	36	77.0±22.5	7.1±2.5	N/A	N/A	N/A
0.05	0.8	35	89.8±12.7	7.6±1.8	N/A	N/A	N/A
0.06	1.0	37	93.1±15.3	7.1±1.7	8.3±1.9	5.0±1.3	6.2±1.5
0.08	1.2	19	98.5±1.8	7.9±1.4	9.2±1.5	5.3±1.1	6.6±1.2
0.10	1.5	36	99.9±0.6	10.3±2.6	11.9±3.1	6.1±1.6	7.7±2.1
0.20	3.0	69	100.0	12.0±2.7	14.7±3.2	6.4±1.9	9.1±2.4
0.50	7.0	47	100.0	15.7±2.9	17.7±3.3	6.7±2.4	8.5±2.8
1.00	15	15	100.0	21.6±4.6	23.9±4.6	8.1±2.9	10.4±2.9
2.00	30	6	100.0	28.8±5.6	31.4±6.8	10.8±3.4	13.4±4.6
3.00	45	23	100.0	35.3±5.7	41.6±10.4	12.7±3.3	19.0±8.0
4.00	60	21	100.0	39.4±6.3	46.0±9.3	13.0±3.4	20.0±6.4

N/A, not applicable.

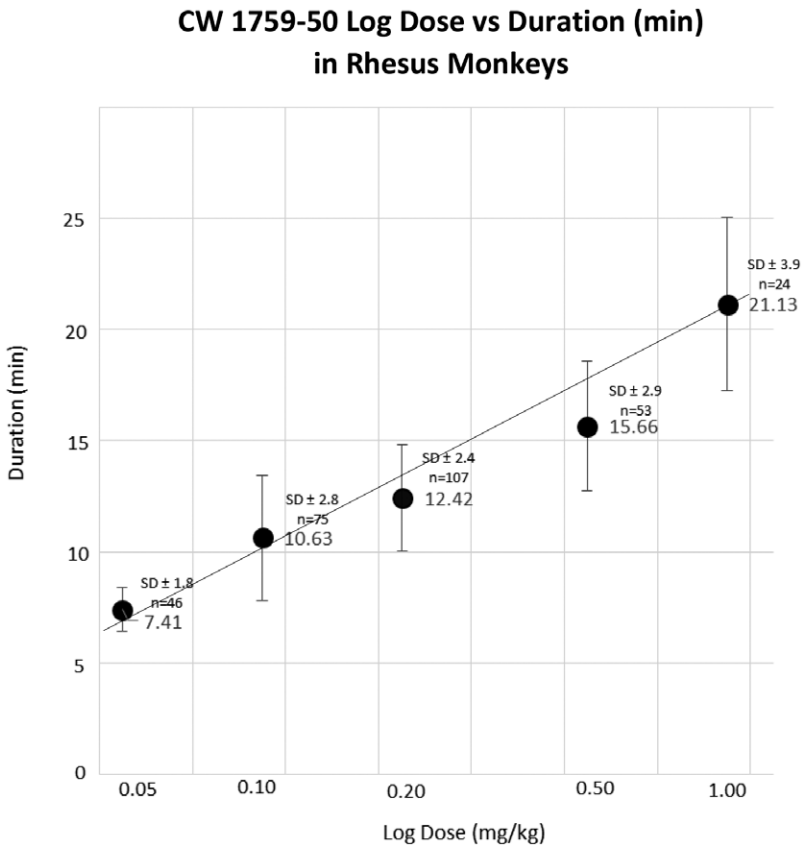

Fig. 5. Linear regression of the duration of action (arithmetic scale) of CW 1759-50, from injection to 95% twitch recovery, in the anesthetized rhesus monkey, *versus* dose (log scale). The slope of this line is 5.4 min/mg/kg. The *line* shows that doubling the dose of CW 1759-50 results in an increase in duration of approximately 3 min over the dose range of 0.05 to 1.00 mg/kg (0.7 to 15 × ED95); conversely, halving the dose over that range results in a decrease in duration of about 3 min.

Table 2. Paired Studies of Reversal by L-Cysteine (30 mg/kg) after CW 1759-50, 0.20 and 0.50 mg/kg

Bolus Dose (Point of Reversal), mg/kg	Approximate ED95 Multiple	n	Twitch Inhibition ± SD, %	Duration, Injection to 95% Twitch, min ± SD	Duration Injection to 90% Train-of-four Ratio, min ± SD	5 to 95% Twitch Recovery Interval, min ± SD	5% Twitch: 90% Train-of-four Ratio Interval, min ± SD
0.20 (spontaneous recovery)	3	8	100.0	12.6 ± 1.8	14.1 ± 2.1	6.5 ± 0.5	8.0 ± 0.8
0.20 (reversal at +1 min)	3	8	100.0	4.5 ± 0.9*	5.4 ± 1.2	2.0 ± 0.5*	2.9 ± 0.8*
0.50 (spontaneous recovery)	7	4	100.0	16.7 ± 3.2	18.2 ± 3.7	7.1 ± 0.6	8.6 ± 1.1
0.50 (reversal at +1 min)	7	4	100.0	5.6 ± 0.7*	6.8 ± 0.9	3.0 ± 0.7*	4.2 ± 0.9*

*P < 0.0001, Comparisons of reversal to spontaneous recovery by paired t test.

significantly shorter compared to spontaneous recovery (2.1 ± 0.2 min *vs.* 5.2 ± 1.1 min; P < 0.0001; table 3). At the time of reversal, there was always 99 to 100% twitch inhibition (fig. 6B). The 5 to 95% twitch recovery interval, whether after spontaneous recovery (infusion A) or reversal by L-cysteine (infusion B), remained constant over time and was not related to the duration of infusion (fig. 7).

In a second set of paired infusions, continuous overdoses (infusions C and D) of CW 1759-50 were given at twice the rate (2 × ED99 Inf) required for 99% twitch inhibition derived from infusions A and B. Rates of 41.3 ± 14.5 and 37.8 ± 12.7 μg/kg/min were required for maintenance of 100% block of twitch during infusions C and D, respectively. Comparative recovery intervals were significantly shorter (3.0 ± 0.6 min) after reversal by L-cysteine (infusion D) than after spontaneous recovery after infusion C: 7.3 ± 1.4 min, P < 0.0001 (table 3).

Comparisons of CW 1759-50 with Gantacurium

Neuromuscular Blockade: Potency, Onset of Action, and Duration of Action. CW 1759-50 is more potent than gantacurium in the rhesus monkey (ED95 is 0.069 ± 0.02 mg/kg *vs.* 0.081 ± 0.05 mg/kg, P = 0.006). The onsets of block are not different at doses causing exactly 98 to 99% twitch inhibition: (CW 1759-50 = 94 ± 9 s; gantacurium = 97 ± 12 s, n = 8 and 9, respectively, P = 0.573). Neither are the respective

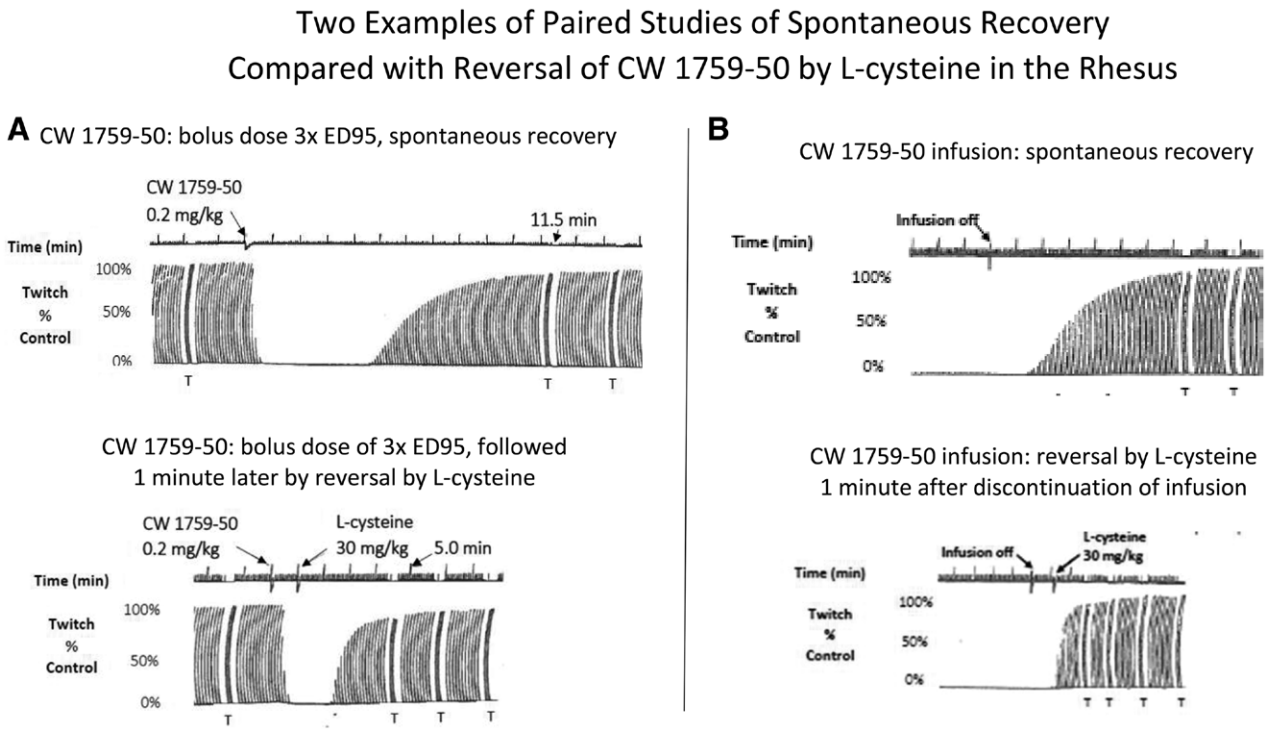

Fig. 6. Two examples of recordings from paired studies in rhesus monkeys under isoflurane, where spontaneous recovery is compared with L-cysteine reversal of CW 1759-50. (*A*) Paired bolus doses of 0.20 mg/kg (3 × ED95) showing spontaneous recovery (*top*) and reversal by L-cysteine (30 mg/kg; *bottom*). (*B*) Paired infusions (120 min each) maintaining 99% twitch suppression, showing spontaneous recovery (*top*) and L-cysteine reversal (*bottom*). Twitch was elicited from the Achilles tendon at 0.15 Hz. Train-of-four stimulation was interposed at T. Dosing as indicated (above time scales). Note that train-of-four ratio recovers to a ratio of 90% or more within approximately 2 min after recovery of twitch to 95% of baseline.

durations of action different at the above doses: CW 1759-50, 8.2 ± 1.5 min and gantacurium, 7.4 ± 1.9 min, $P = 0.355$.

Circulatory Properties

The effects of CW 1759-50 and gantacurium on HR and MAP after doses of up to 60 × ED95, given as "first dose of the day," are shown in figure 8, A and B. The comparative trends are strikingly different: gantacurium (fig. 8B) causes dose-related, increasingly greater, circulatory changes than does CW 1759-50 (fig. 8A). The difference between the two compounds is most notable in the effect on HR, where a change (increase) *versus* baseline of 30% in HR after gantacurium is estimated by curvilinear regression to occur at 3.1 (CI 2.7 to 3.3) mg/kg, whereas no trend of change in HR is noted after doses of CW 1759-50 as high as 4.0 mg/kg (approximately 60 × ED95). The dose ratio (ED 30% Δ HR/ED95 NMB) for an increase in HR caused by gantacurium is 3.1/0.081 or approximately 38 × ED95. No trend of change in HR, however, was noted for CW 1759-50 after doses as high as 4.0 mg/kg. Consequently, no dose ratio (ED 30% Δ HR/ED95 NMB) can be calculated for CW 1759-50, except to note that the ratio must be greater than 58 × ED95 (4.0/0.069).

By curvilinear regression, ED for 30% decrease of MAP after gantacurium (ED 30% Δ MAP) is 3.6 (CI 3.3 to 3.8) mg/kg compared with 6.9 (CI 6.8 to 7.1) mg/kg for CW 1759-50. The comparative dose ratios for decrease of MAP (ED 30% Δ MAP/ED95 NMB) are 3.6/0.081 (44 × ED95) for gantacurium compared with 6.9/0.069 (100 × ED95) for CW 1759-50. The much higher dose ratios for CW 1759-50 for both ΔHR/ED95 and ΔMAP/ED95 suggest greater circulatory safety of CW 1759-50 compared to gantacurium.

Discussion

The profile of CW 1759-50 has characteristics that we consider desirable in a short-acting neuromuscular blocking agent.

Breakdown to Inactive Derivatives in a Chemical Reaction

Degradation at a rapid rate at physiologic pH and temperature in a chemical reaction by adduction of L-cysteine is desirable because this reaction is nonenzymatic, likely little affected by pathologic changes, and converts this type of neuromuscular blocking agent into inactive derivatives (adducts), which are approximately 70 to 100 times less potent as neuromuscular blocking agents than the parent compounds.[1] For example, if the ED95 of CW 1759-50 in man for neuromuscular blockade were 0.10 mg/kg, then the corresponding ED95 dose of the adduct for neuromuscular blockade (which would have to be given as a bolus) would be approximately 7 to 10 mg/kg. Cysteine adduction could be the rate-limiting step governing the pharmacokinetics and dynamics of CW 1759-50 *in vivo*. Because the plasma concentration of L-cysteine remains

Table 3. Continuous Infusions of CW 1759-50: Spontaneous Recovery *versus* L-Cysteine Reversal

Infusions, Maintenance Dosage ± SD = ED99 Inf ± SD	*n*	5 to 95% Twitch Recovery Interval, min ± SD		5% Twitch - 90% Train-of-four Recovery Interval, min ± SD	Duration of Infusion	
		Spontaneous Recovery	L-Cysteine Reversal at 1 min after Infusion Off		Spontaneous Recovery, min ± SD (range)	L-Cysteine Reversal, min ± SD (range)
Control infusions (ED99 Inf)	32	6.1 ± 1.3	N/A	7.7 ± 1.7	69.4 ± 32.7 (20–128) (NS)	N/A
19.1 ± 5.8 μg/kg/min						
Paired infusion A (ED99 Inf)	11	5.2 ± 1.1	N/A	6.9 ± 1.5	73.6 ± 33.1 (30–125)	N/A
20.6 ± 5.0 μg/kg/min						
Paired infusion B (ED99 Inf)	11	N/A	2.1 ± 0.5	2.9 ± 0.6	N/A	73.6 ± 33.1 (30–125)
21.5 ± 5.6 μg/kg/min						
Paired infusion C	6	7.3 ± 1.4	N/A	11.2 ± 3.2	83.3 ± 21.7 (50–115)	N/A
(2 × ED99 Inf) 41.3 ± 714.5 μg/kg/min						
Paired infusion D	6	N/A	3.7 ± 0.6	4.9 ± 0.8	N/A	79.6 ± 24.1 (50–109)
(2 × ED99 Inf) 37.8 ± 12.7 μg/kg/min						

Compared *P* values: 5 to 95% recovery, Inf A *versus* Inf B, $P < 0.0001$; 5 to 95% recovery, Inf C *versus* Inf D, $P < 0.0002$; and duration of infusions, Inf C and Inf D, $P = 0.786$.

Inf, infusion; N/A, not applicable; NS, not significant.

Analysis of Variance Comparing 5 to 95% Recovery Intervals for Three Bolus Doses and Control Infusions of CW 1759-50

Dose	n	5 to 95% Interval (min ± SD)	*P* Value
Control infusions	32	6.1 ± 1.3	0.426
0.10 mg/kg	36	6.1 ± 1.6	
0.20 mg/kg	69	6.4 ± 1.9	
0.50 mg/kg	47	6.7 ± 2.4	

The values for 5 to 95% recovery intervals for three bolus doses (0.10, 0.20, and 0.50 mg/kg) are from table 1, and the values for control infusions of CW 1759-50 are from table 3.

relatively constant throughout life,[9] we may speculate that a consistent duration of neuromuscular blockade at various ages in man might be anticipated. This speculation requires proof in future studies.

Yet to be studied also is the effect of potential hydrolysis by plasma cholinesterase (pseudocholinesterase) on the ester linkages of CW 1759-50 and on the duration of neuromuscular blockade. The location of the half-time of L-cysteine adduction to CW 1759-50 (at 2.3 min), directly on the line of regression previously shown[1] (appendix 2), suggests that the adduction mechanism, not enzymatic hydrolysis, is the major determinant mechanism of breakdown and likely the principal factor responsible for the short duration of CW 1759-50. Certainly a lack of dependence on pseudocholinesterase, with its surrounding clinical issues, would be desirable. However, this and other possible mechanisms of metabolism, elimination, or degradation of CW 1759-50 have not yet been studied.

The circulatory effects of L-cysteine in the dog have been published.[6] The data show that in doses required for rapid reversal in this species (20 to 30 mg/kg), L-cysteine has only minor circulatory effects that should not be an issue in clinical practice. The circulatory effects of L-cysteine in that study, measured at doses of up to 100 mg/kg, or 3 to 5 times the dose required for rapid reversal, caused less than 10% change in hemodynamic parameters.[6] We have not studied the effect of larger doses (overdoses) on the circulation in the dog.

Correlation of Reaction Rate In Vitro *with Characteristics of Neuromuscular Blockade* In Vivo

The correlation of the duration of neuromuscular blockade of CW 1759-50, CW 002, and other related compounds in the rhesus *versus* half-time of adduction with L-cysteine remains significant (appendix 3) when data for CW 1759-50 are added to a previously published regression.[1] The half-time (2.3 min) for adduction of L-cysteine to CW 1759-50 is one fifth that of CW 002[1] and ten times that reported for gantacurium,[1] yet the comparative duration in the rhesus is only 1.0 to 1.5 times longer than that of gantacurium[1,4] and one third the duration of CW 002.[1,5] The regressions shown

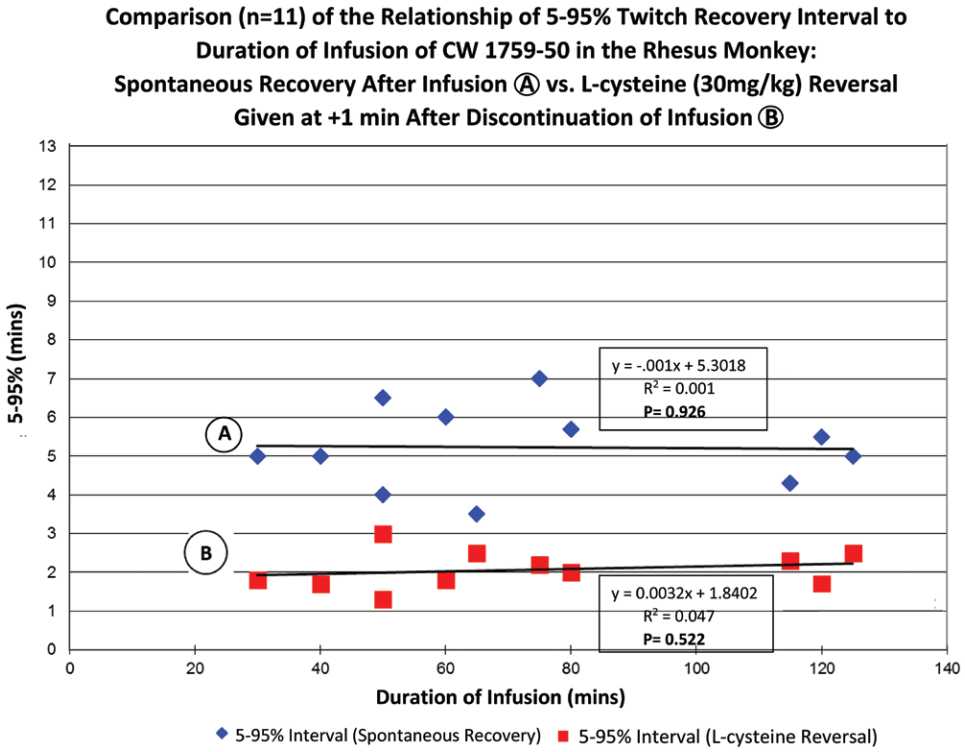

Fig. 7. Relationship of 5 to 95% recovery interval to duration of infusion of CW 1759-50 in rhesus monkeys under isoflurane. Importantly, neither the rapidity of spontaneous recovery (*A*) nor the speed of reversal (*B*) is affected by the duration of the infusion; in (*A*) $P = 0.926$ and in (*B*) $P = 0.522$.

in appendix 3 suggest that a similar comparative duration may be likely in humans as well.

An estimation of the anticipated duration of action of CW 1759-50 in man can be made by correlation of the half-time of L-cysteine adduction *in vitro* of CW 1759-50, gantacurium, and CW 002 with the duration of action already measured at 2 × ED95 in human subjects during phase I studies of gantacurium[2] and CW 002[8] (appendix 3). The duration is estimated to be approximately 20 min.

Rate of Adduction Not too Fast or Slow

In selecting a candidate for a short-acting neuromuscular blocking agent in man, we were not necessarily interested in compounds undergoing adduction with L-cysteine as quickly as gantacurium because very rapid breakdown might tend to reduce potency, requiring larger amounts of material to be given during continuous administration, a possible pharmacoeconomic factor favoring the more potent material. Greater neuromuscular blocking potency and appropriately slower degradation would reduce the comparative amount of CW 1759-50 required for maintenance of neuromuscular blockade, in comparison with gantacurium.

Much past discussion comments on the inverse relationship of the speed of onset to the potency of neuromuscular blocking agents.[10–12] When considerations include clearance or breakdown (no matter the mechanism), rapid onset and short duration are favored by low potency and high clearance (the latter preferably by destruction of the molecule).[10–12] High potency and low clearance without chemical or enzymatic breakdown conversely predict slow onset, long duration, and relatively slow excretion by organs of elimination. The requirement for rapid breakdown is more important if the duration is to be short in a potent neuromuscular blocking agent.[10] The article by Bowman *et al.*[12] shows the inverse relationship of onset to potency in a series of steroidal neuromuscular blocking agents where low potency correlates with both rapid onset and short duration in the cat; although some structural correlates were made in the article, a correlation of onset and duration with the overall clearance or with the rate of chemical or enzymatic breakdown was not done in that study.[12]

Economics of Maintenance of Neuromuscular Blockade by Infusion

A short-acting neuromuscular blocking agent rapidly degraded and showing speedy spontaneous recovery from deep neuromuscular blockade (such as within approximately 10 min in man from one twitch perceptible on train-of-four stimulation to a train-of-four ratio of 90%), might more usually be given for maintenance of neuromuscular blockade as a continuous infusion in clinical practice, more often than by repeated boluses. Accordingly, much effort was spent in the experiments reported herein to define the infusion characteristics of CW 1759-50 in the rhesus model (table 3).

The data in tables 1 and 3 and figure 7 show that spontaneous recovery from deep neuromuscular blockade (99% twitch inhibition) is unaffected after bolus doses of 1 to 3 × ED95

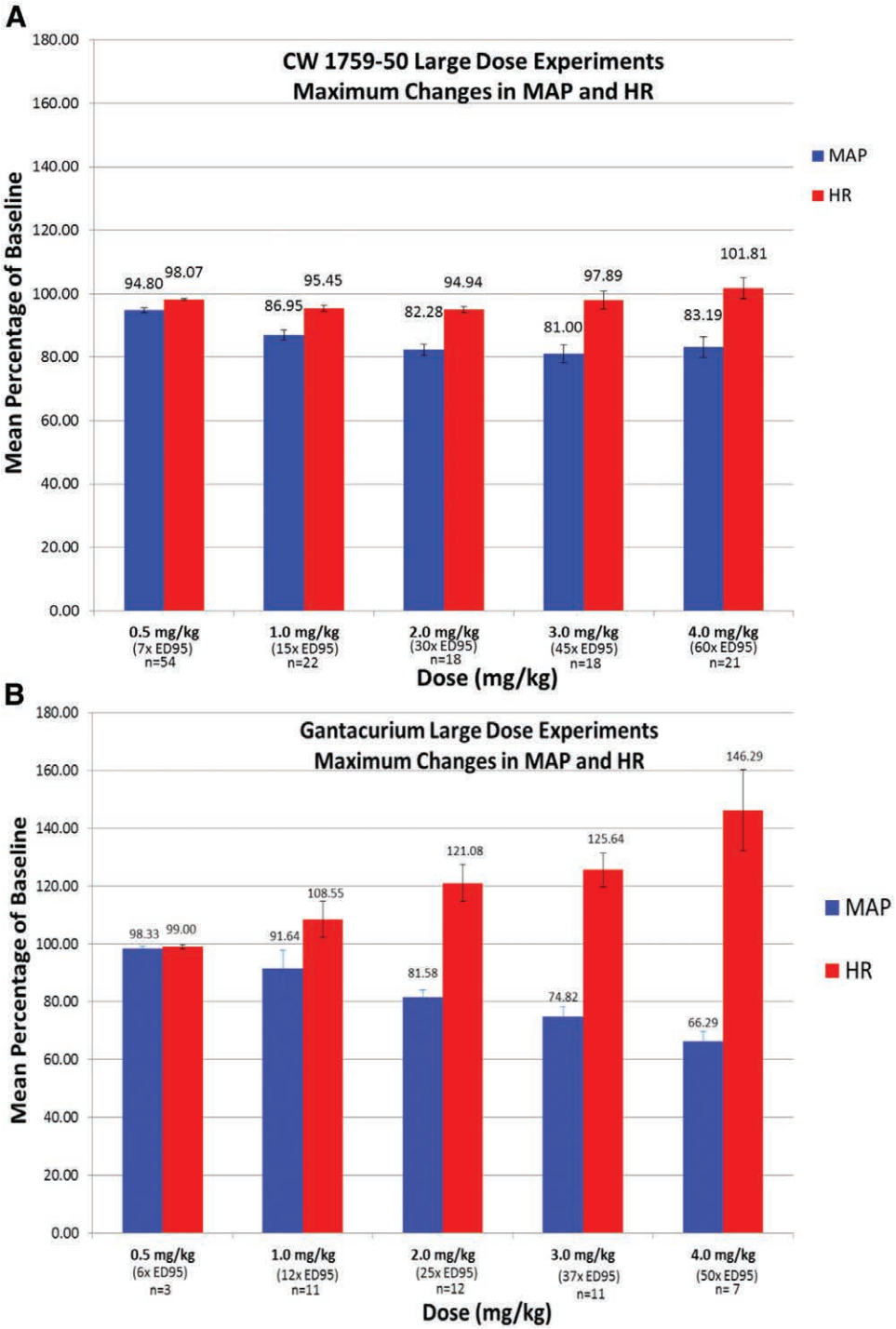

Fig. 8. Changes in HR and MAP from baseline after large boluses of CW 1759-50 (*A*) or gantacurium (*B*), given as first dose of the day. A progressively greater decrease in mean arterial pressure (MAP) and increase in heart rate (HR) is seen after increasingly larger doses of gantacurium; no trend is apparent in the case of CW 1759-50, where the heart rate remains unchanged from baseline over the entire dose range shown, and mean arterial pressure changes also show no dose–response relationship.

or by the duration of infusion (5 to 95% recovery interval is approximately 6 min; figs. 6 and 7 and tables 1 and 3) where analysis of variance shows no significant differences in the various recovery intervals. Neither is antagonism by L-cysteine affected (5 to 95% interval is decreased to approximately 2 min after reversal after discontinuation of infusions of any duration maintaining 99% twitch inhibition; fig. 7 and table 3). Reversal of boluses of 0.10 to 0.50 mg/kg (1.5 to 7.0 × ED95) results in similar shortening of the 5 to 95% interval (table 2).

Infusion characteristics were tested most rigorously (table 3) when the dose required to maintain 99% block of twitch (ED 99 Inf) in the rhesus was doubled to (2 × ED99 Inf). Spontaneous

recovery (infusion C) after infusions of (2 × ED99 Inf) required only an additional approximately 2 min in comparison to spontaneous recovery from (1 × ED99 Inf), *i.e.*, infusion A. Lengthening of 5 to 95% recovery by approximately 2 min in the above comparisons (where infusion rate is doubled) is compatible with both the half-time *in vitro* of CW 1759-50 being 2.3 min and also is compatible with the increase (or decrease) in duration of about 3 min when the bolus dose is doubled (or halved; fig. 5).

Unchanging recovery rate over time (fig. 7) is presumably due to the continued efficiency of the L-cysteine adduction/degradation reaction, as well as the generation of inactive breakdown products.[1] This point might apply to either spontaneous recovery (promoted by endogenous L-cysteine) or induced recovery (reversal) by administration of exogenous L-cysteine to accelerate the adduction reaction.[1,5,6,13–15] The latter point would likely be true in humans as well, because the route of inactivation is a chemical reaction involving no enzymes[1] or other biologic mechanisms (fig. 3).

Comparisons with Gantacurium Suggest Reduced Circulatory Effects

A pattern of divergence in the response of HR (increase) and MAP (decrease) is not evident after large doses of up to 60 × ED95 or 4.0 mg/kg of CW 1759-50 (fig. 8A); it is obvious, however, after gantacurium, especially in doses above 12.5 × ED95 or 1.0 mg/kg (fig. 8B), given as "first doses of the day." In contrast, in a previous study where gantacurium was given sequentially, such a divergence occurred only at larger doses above 3 mg/kg,[4] indicating that dose–response studies of circulatory changes caused by neuromuscular blocking agents should be based preferably on first doses[5] for greater accuracy of predictive value.

The data of figure 8 suggest that possible histaminoid side effects of CW 1759-50 are reduced in comparison with gantacurium in the monkey, implying that such changes might occur less often in humans as well. Studies in the dog (laboratory of Paul M. Heerdt, 2013 to 2014, unpublished) suggest a similar reduction of the histaminoid property in that species. This is the most important improvement in comparison with gantacurium: the decreased cardiovascular effect of CW 1759-50 should allow higher ED95 multiples to be given to man in comparison to gantacurium and better facilitation of early tracheal intubation, *e.g.*, within 60 s. The short duration and rapid recovery would presumably be maintained due to the short half-time (2.3 min) of the L-cysteine adduction reaction.

The divergence of HR and MAP described in "Results, Circulatory Properties" in the case of gantacurium is typical of the class of benzylisoquinolinium neuromuscular blocking agents. It has been described for metocurine (human),[16] mivacurium (monkey[4] and man[17]), gantacurium (monkey[4] and man[2]), and CW 002 in the monkey[5] but not as yet in man.[8] The exception is cisatracurium for which absence of histaminoid responses has been documented in the cat[18] and human.[19] Much higher dose ratios [ED 30% Δ HR/ED95] neuromuscular blockade and [ED 30% Δ MAP/ED95] neuromuscular blockade have been shown for CW 1759-50 than for gantacurium in the present study. The dose-related changes shown for gantacurium are absent for CW 1759-50 (fig. 8), anticipating greater circulatory safety of CW 1759-50 in comparison with changes already noted after gantacurium in humans.[2]

Potential Pharmacoeconomic Benefit of CW 1759-50

Infusion rates in the rhesus of approximately 30 to 50 μg/kg/min to maintain 95% block of twitch have been observed but not reported for gantacurium (laboratory of John J. Savarese, 1996 to 2000, unpublished). The lower (ED99 Inf) rates of approximately 19 μg/kg/min reported herein for CW 1759-50 (table 3) might have economic implications regarding reduced maintenance dose in humans. Adduction of L-cysteine to CW 1759-50 suggests that degradation is fast enough to limit its duration as dose is increased (table 1). As shown in paired studies herein, spontaneous recovery, already rapid, may readily be further accelerated by administration of exogenous L-cysteine.

Conclusions

CW 1759-50 has been chosen as a candidate for human study as a potential short-acting neuromuscular blocking agent because of its balance of high potency, fast rate of breakdown in a chemical reaction (L-cysteine adduction), rapid onset and recovery, and reduced circulatory effects in comparison with gantacurium. Reversal by administration of the same dose of L-cysteine under most circumstances that have been tested herein, greatly accelerating the chemical reaction that inactivates this neuromuscular blocking agent, is a novel feature.

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Competing Interests

Drs. Savarese and McGilvra claim inventorship of CW 1759-50. Drs. Savarese and Heerdt claim co-inventorship of L-cysteine as reversal agent.

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Address correspondence to Dr. Savarese: Weill Medical College of Cornell University, 1300 York Avenue, New York, New York 10065. jjsavare@med.cornell.edu or jjsavarese@icloud.com. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

Appendix 1. Syntheses of CW 1759-50 and Gantacurium Chloride (GW280430A)

The synthesis of CW 1759-50 from an advanced isoquinolinium intermediate is shown in figure A1.1. CW 1759-50 assayed at 96% area peak purity by high-performance liquid chromatography. The product identity was confirmed based on mass spectrometric analysis and by analogy to synthesis of other well-characterized analogs prepared using minor variations on the scheme.[1,5]

The scheme for CW 1759-50 shows esterification of the amino alcohol 1972-25 with maleic anhydride in triethylamine (Et3N) and acetonitrile to yield the monoester 1972-38. Treatment of the monoester with the (second) amino alcohol 1972-11 in oxalyl chloride and 1,2-dichloroethane yields the diester CW 1759-50.

The synthesis of gantacurium is also shown in figure A1.1.3 The scheme is analogous to the scheme for CW 1759-50, showing the final steps from advanced intermediates to the desired product, gantacurium.

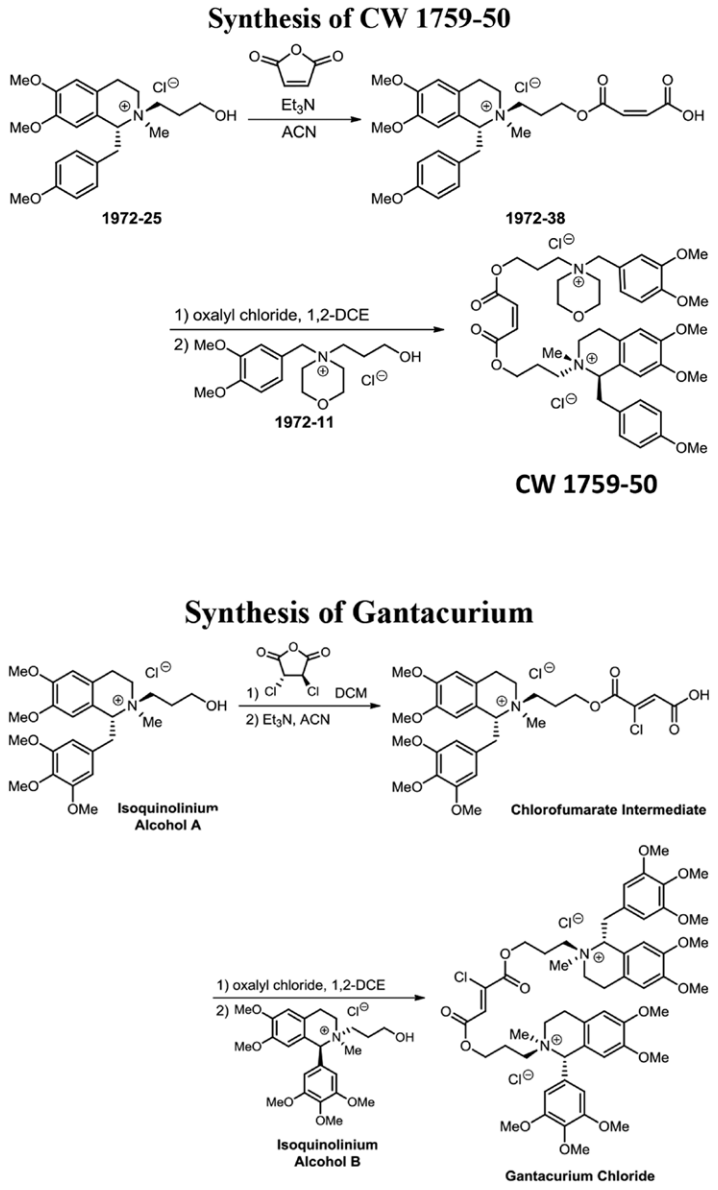

Fig. A1.1. Synthesis of CW 1759-50: The scheme for CW 1759-50 shows esterification of the amino alcohol 1972-25 with maleic anhydride in triethylamine (Et3N) and acetonitrile to yield the monoester 1972-38. Treatment of the monoester with the (second) amino alcohol 1972-11 in oxalyl chloride and 1,2-dichloroethane yields the diester CW 1759-50. Synthesis of gantacurium chloride (GW280430A): The synthesis of gantacurium has been described.[3] The scheme shown is analogous to the scheme for CW 1759-50, showing the final steps from advanced intermediates to the desired product, gantacurium.

Appendix 2. *In Vitro* Degradation of CW 1759-50

Methods

High-Performance Liquid Chromatography Analyses were performed using an Agilent 1050 series module controlled by Chemstation software. Details for the method used for all analyses are as follows: Agilent Zorbax Eclipse XDB-C8 4.6 × 250-mm 5-μm column (Agilent P/N 990967-906); 235-nm detector wavelength; 10-μl sample injection volume; 27-min run time at ambient column temperature; 1.0 ml/min flow rate; mobile phase A composed of 1 ml of trifluoroacetic acid mixed with 1000 ml of MilliQ water, mobile phase B composed of 1 ml of trifluoroacetic acid mixed with 1000 ml acetonitrile; gradient program of 75% mobile phase A/25% mobile phase B from minute 0 to 4, ramp to 20% mobile phase A/80% mobile phase B from minute 4 to 20, hold at 20% mobile phase A/80% mobile phase B from minute 20 to 21, ramp to 75% mobile phase A/25% mobile phase B from minute 21 to 22 and hold at 75% mobile phase A/25% mobile phase B from minute 22 to 27. Approximate retention times of analytes for this method are as follows: 1972-11 morpholinium alcohol = 4.1 min, 1759-50 L-cysteine adducts = 9.7 min, 1972-25 isoquinolinium alcohol = 9.5 min, 1972-38 isoquinolinium maleate = 11.3 min and 1759-50 = 12.0 min.

Alkaline Hydrolysis. Alkaline hydrolysis of 1759-50 was monitored by high-performance liquid chromatography for loss of 1759-50 peak area counts over time compared to a time zero standard. A time zero standard was prepared by diluting 5.0 mg of 1759-50 to volume in a 25-ml volumetric flask using 1 M aqueous HCl. The standard was analyzed in triplicate with no further dilution before injection to establish area counts for a 1 mg/ml solution of 1759-50. Samples analyzed for hydrolysis of 1759-50 over time in a pH 7.4 aqueous phosphate buffer at 37°C were prepared by diluting 25.0 mg of 1759-50 to volume in a 25-ml volumetric flask with an aqueous pH 7.4 buffer. The sample was heated at 37°C in a temperature controlled water bath, and samples were pulled for analysis of 1759-50 area counts at T = 0, +19 min, +73 min, and +123 min, with immediate injection for analysis of 1759-50 with no dilution of sample before injection. Peaks were observed in the analysis of hydrolysis samples, with retention times matching those for 1759-50, 1972-38, 1972-25, and 1972-11.

L-Cysteine Adduction. Cysteine adduction of 1759-50 was monitored by analysis using high-performance liquid chromatography for loss of 1759-50 peak area counts compared to a time zero standard. A time zero standard was prepared by diluting 5.0 mg of 1759-50 to volume in a 25-ml volumetric flask using 1 M aqueous HCl. The standard was analyzed in triplicate without further sample dilution before injection to establish area counts for a 0.2 mg/ml solution of 1759-50. Samples analyzed for cysteine adduction of 1759-50 over time in a pH 7.4 aqueous phosphate buffer at 37°C were prepared at a starting 1759-50 concentration of 0.2 mg/ml by combining a solution of 6.0 mg of 1759-50 diluted to volume in a 25-ml volumetric flask with aqueous pH 7.4 buffer and 1.3 mg of L-cysteine diluted to volume with aqueous pH 7.4 buffer in a 5-ml volumetric flask. The samples were maintained at 37°C in a temperature-controlled water bath, and samples were pulled for analysis of 1759-50 area counts at T = 0, +73 s, +111 s, and +147 s, with immediate injection for analysis of 1759-50 with no dilution of samples before injection. Peaks were observed in the analysis of cysteine adduction samples with retention times matching those for 1759-50 and 1759-50 L-cysteine adducts (m/z = 441.7, liquid chromatography–mass spectrometry electrospray ionization mode, consistent with L-cysteine addition to 1759-50).

Mass Spectrometry. Liquid chromatography–mass spectrometry analysis was performed using a Thermo Scientific Dionex Ultimate 3,000 system coupled to a MSQ21033 Plus module with instrument control using Chromeleon software. This portion of the analysis was performed using the same column, mobile phases, and operating conditions described for analyses of degradation and adduction reactions. Mass spectrometric analysis was performed in electrospray ionization mode. A cysteine adduction reaction sample was prepared for identification of the adduct peak. A solution of 25 mg of 1759-50 dissolved in 1 ml of deionized water was mixed with 3.0 mg of L-cysteine (0.8 equivalents with respect to 1759-50; Aldrich P/N 168149) at room temperature.

Results

Degradation *In Vitro*

Alkaline Hydrolysis. A reaction half-time, *i.e.*, time required *in vitro* for the initial concentration of CW 1759-50 to decrease by 50% during alkaline hydrolysis of CW 1759-50, was estimated at 240 min based on plotting of high-performance liquid chromatographic analysis–derived data with respect to time (reaction B, fig. 3 in text).

L-Cysteine Adduction. When a 5 mol% excess of L-cysteine was added to CW 1759-50 at biologic pH and temperature, the neuromuscular blocking agent (CW 1759-50) peak was fully converted to two overlapping neuromuscular blocking agent/L-cysteine adduct peaks within 10 min (see reaction A, fig. 3, in text). The peaks were identified by mass spectrometry as having molecular weights compatible with the structures proposed as the adducts (fig. 3). The calculated half–time for CW 1759-50/L-cysteine adduction was 2.3 min at an initial concentration of CW 1759-50 of 200 μg/ml. The peak presentation obtained for the 1759-50/L-cysteine adduction is consistent with cysteine adduction occurring on either side of the maleate

alkene functional group, giving a mixture of the two adducts proposed in the text (fig. 3). The adducts were shown by mass spectrometry to have the same molecular weights, consistent with the regioisomerism shown in the scheme of figure 3.

Mass Spectrometry. Analysis of a reaction aliquot returned an m/z value of 441.7 for the overlapping peaks present at 9.6 min. Liquid chromatography retention time was consistent with the expected $[M]^{+2}$ (molecular weight of the cation) of 883.43 for the 1759-50 cysteine adduct regioisomers. The 1759-50 peak at a retention of 12.0 min returned an m/z value of 381.1, consistent with the expected $[M]^{+2}$ of 762.41.

Table A2.1. L-cysteine Adduction Reaction Half Time *In Vitro*

Neuromuscular Blocking Agent	k_a ($M^{-1}s^{-1}$)*	$[NMBA]_0$ (M)**	$t_{1/2}$ (min)***
CW 1759-50	30.4	0.000240	2.3

*k_a: Reaction Rate constant
** $[NMBA]_0$ (M): Molar concentration of CW 1759-50 at Time 0
*** $t_{1/2}$: Half-time of the adduction reaction

Appendix 3. Discussion: Regressions Showing Correlation of L-Cysteine Adduction *In Vitro* with Duration of Action of New Neuromuscular Blocking Agents in Monkeys and in Man

Correlation of the duration of action at 3 × ED95 in anesthetized monkeys of structurally related fumarate or maleate diester compounds with the rate of L-cysteine adduction *in vitro*. The duration of action of CW 1759-50 in monkeys is directly related to the half-time of L-cysteine adduction *in vitro* ($P < 0.0001$; fig. A3.1).

A Forecast of the Duration of Action of CW 1759-50 in Humans

A forecast of the duration of CW 1759-50–induced NMB in humans at approximately 2 × ED95 is shown in figure A3.2. The half-time (2.3 min) of the adduction reaction of L-cysteine with CW 1759-50 *in vitro* is indicated on the regression. The durations of NMB of gantacurium and CW 002 in man are published.[2,8] Predicted duration of CW 1759-50 at approximately 2 × ED95 is approximately 20 min.

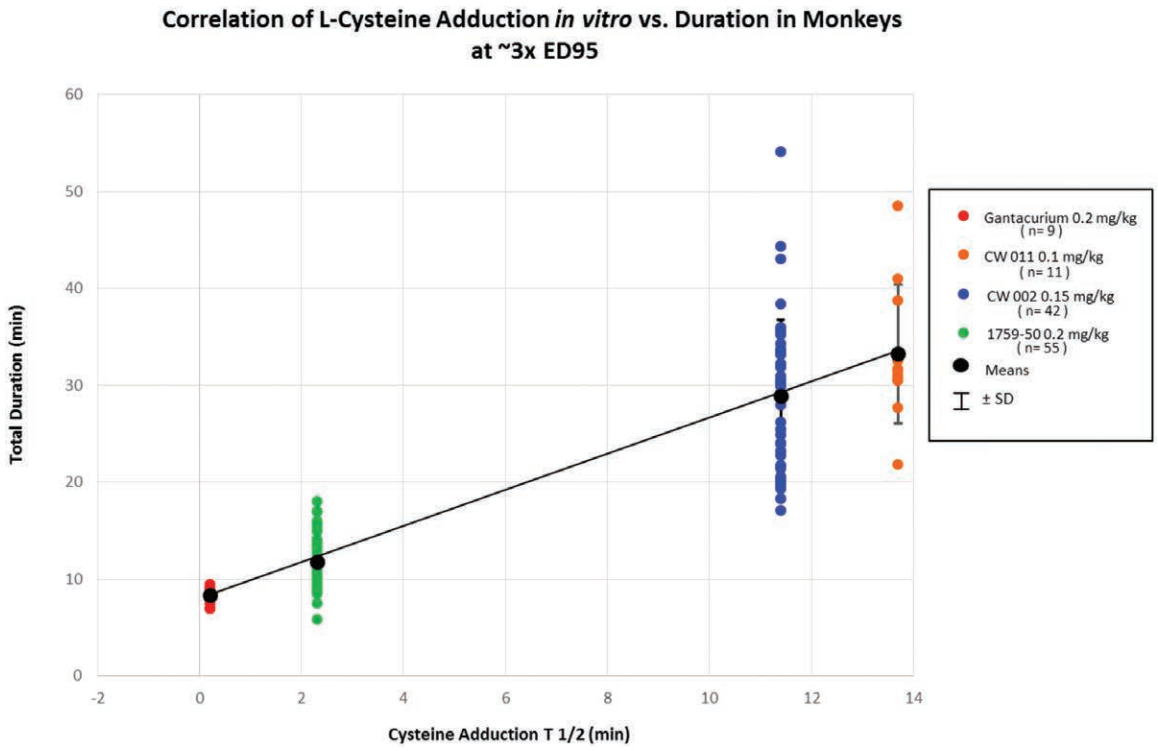

Fig. A3.1. This regression is published.[1] Data for CW 1759-50 (*green*) are added. Compounds at *lower left*, with T 1/2 *in vitro* less than 3 min are ultra-short acting in monkeys (duration 10 to 12 min at 3x ED95). Compounds at *upper right* are of medium (intermediate) duration (30 to 35 min).

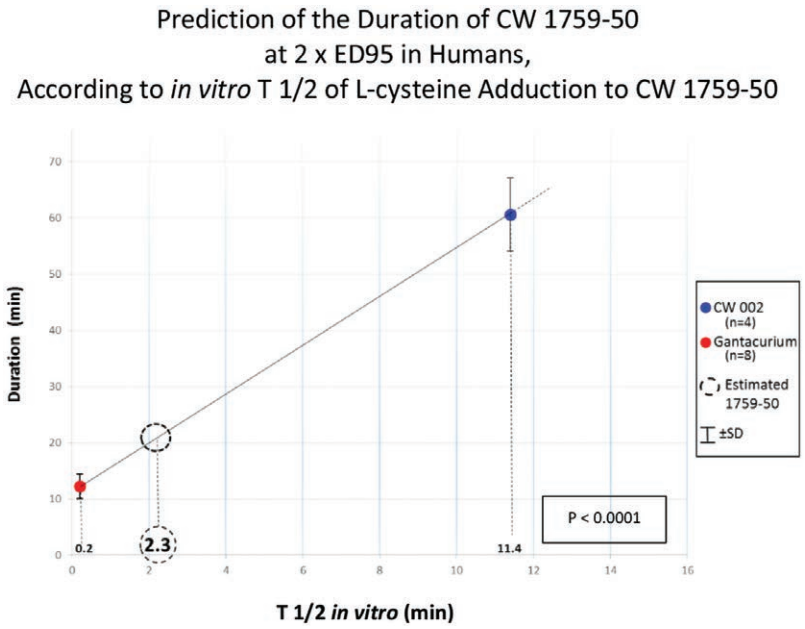

Fig. A3.2. This new regression shows a correlation of T 1/2 of L-cysteine adduction with duration of action of gantacurium and CW002 in humans after 2 × ED95 from already published data.[2,8] Addition of *in vitro* data for CW 1759-50 (T 1/2 of 2.3 min) forecasts duration in humans of about 20 min (*dotted circles*).

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