# Rapid Chemical Antagonism of Neuromuscular Blockade by L-Cysteine Adduction to and Inactivation of the Olefinic (Double-bonded) Isoquinolinium Diester Compounds Gantacurium (AV430A), CW 002, and CW 011

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#### **ABSTRACT**

**Background:** The ultra–short-acting neuromuscular blocker gantacurium is chemically degraded *in vitro* by rapid adduction of L-cysteine to its central olefinic double bond. Preliminary data have suggested that exogenous (intravenous) L-cysteine abolishes gantacurium blockade. Two new analogues of gantacurium (CW 002 and CW 011) have been synthesized to undergo slower L-cysteine adduction, yielding intermediate duration. L-Cysteine adduction to and antagonism of these novel agents is further defined herein.

**Methods:** Comparative reaction half-time for L-cysteine adduction *in vitro* of the three compounds was determined by high-performance liquid chromatography.  $ED_{95}$  for twitch inhibition in monkeys under isoflurane was calculated, and duration at  $\sim 4-5 \times ED_{95}$  was correlated with reaction half-time for adduction. Speed of L-cysteine antagonism was contrasted with anticholinesterase reversal. Potencies of CW 002 and its adduction product were compared to provide a basis for L-cysteine antagonism.

**Results:** Rate of L-cysteine adduction *in vitro* (reaction half-time) was 11.4 and 13.7 min for CW 002 and CW

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011 versus 0.2 min for gantacurium, and was inversely related to duration of block (P < 0.0001). CW 002 and CW 011 were  $3 \times$  longer acting than gantacurium (28.1 and 33.3 min vs. 10.4 min), but only half the duration of cisatracurium. The adduct of CW 002 was  $\sim 70 \times$  less potent than CW 002. L-Cysteine (10–50 mg/kg intravenously) given 1 min after approximately  $4-5 \times$  ED<sub>95</sub> doses of all the three compounds abolished block within 2-3 min

**Conclusions:** L-Cysteine adduction occurs at different rates by design in olefinic isoquinolinium diester neuromuscular blockers, yielding corresponding durations of action. Antagonism by exogenous L-cysteine is superior to anticholinesterases, inducing inactivation of the active molecules to restore function rapidly at any time.

#### What We Already Know about This Topic

The neuromuscular blocking drug gantacurium is rapidly antagonized by L-cysteine

#### What This Article Tells Us That Is New

- The rate of L-cysteine adduction to gantacurium and to two new analogues (CW 002 and CW 011) in vitro was inversely related to the duration of block in monkeys
- Intravenous administration of L-cysteine antagonized block from these drugs within 2–3 min ("immediate chemical reversal") when given 1 min after four to five times their ED<sub>95</sub> for neuromuscular blockade
- This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 9A.
- Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org).

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THE ultra–short-acting nondepolarizing neuromuscular blocking agent (NMBA) gantacurium (AV430A or GW 280430A) rapidly combines with L-cysteine in vitro to form a presumably less-active degradation product (adduct). Gantacurium itself and its adduct may undergo further breakdown by alkaline hydrolysis. All the above reactions are nonenzymatic. 1,2

Gantacurium (fig. 1A) is an asymmetrical isoquinolinium diester of chlorofumaric acid in which one of the central double-bonded (olefinic) carbons is activated (given increased electrophilic character) by a strongly electronegative chlorine substitution designed to accelerate the adduction reaction. We hypothesized that nonhalogenated olefinic isoquinolinium compounds analogous to gantacurium, such as symmetrical or asymmetrical maleates or fumarates, should also undergo the adduction reaction with L-cysteine but at slower rates. The absence of chlorine in such compounds should result in less activation of the olefinic carbons, which are then influenced only by the two relatively weakly electronegative adjacent  $\alpha$ -carboxyl (ester) groups located on both sides of the central olefinic double bond. We tested this hypothesis in two new nonhalogenated olefinic diester ana-

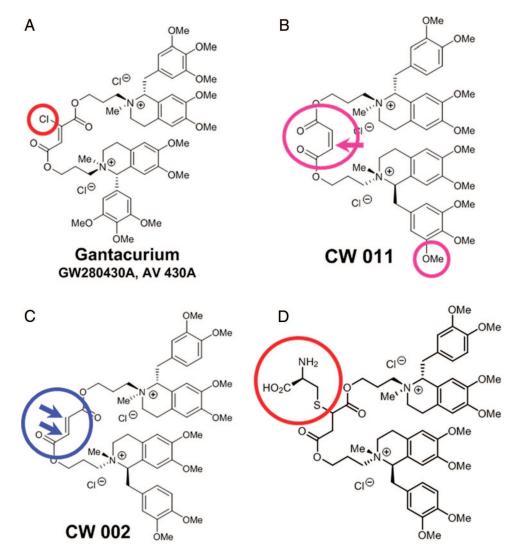


Fig. 1. The chemical formulae of gantacurium (A), CW 011 (B), and CW 002 (C). Chemical features are as follows: chlorine substitution (red circle) on the olefinic double bond of gantacurium, a chlorofumarate, is designed to accelerate the L-cysteine adduction reaction (see Supplemental Digital Content 1, appendix 3, http://links.lww.com/ALN/A590). The fumarate CW 002 is symmetrical with no halogen (chlorine) substitutions and undergoes ∟-cysteine adduction more slowly than gantacurium, at either olefinic carbon (blue arrows), enabled by the adjacent  $\alpha$ -carboxyl (ester) groups. The maleate CW 011 is asymmetrical in that one isoquinolinium group contains an extra methoxy substitution (magenta circle). This may reduce access of L-cysteine to the olefin (magenta arrow) and may decrease the rate of the adduction reaction (see table 3). The chemical formula of NB 1043-10, the L-cysteine adduct of CW 002, is also shown (D). The L-cysteine adduction is highlighted by the red circle.

NB 1043-10 L-Cysteine adduct of CW 002 logs of gantacurium: CW 002 (a symmetrical fumarate) and CW 011 (an asymmetrical maleate), which are members of two series of compounds which are under investigation. We compared the relative rates of L-cysteine adduction *in vitro* of gantacurium and the two new compounds. See figures 1A–D for structural details.

The chlorofumarate gantacurium is ultra–short-acting in monkeys and humans, most likely because of an accelerated adduction reaction with endogenous L-cysteine. The neuro-muscular blocking properties of both CW 002 and CW 011 were expected to be longer lasting than gantacurium because of predictably slower L-cysteine adduction reactions in these non-halogenated compounds. The corresponding L-cysteine adduct derivatives of the compounds were anticipated to be considerably less active than the parent compounds.

In view of the chemical–pharmacologic relationships proposed and described earlier, we hypothesized that the duration of the blocking effect *in vivo* of these and other related compounds should be governed principally by the rate of chemical degradation and inactivation by endogenous L-cysteine and should be inversely proportional to the rate of this adduction reaction *in vitro*. Also, intravenous administration of exogenous L-cysteine should induce faster recovery *in vivo* by accelerating the adduction reaction according to laws of mass action. We also anticipated that direct chemical antagonism (inactivation) by exogenous L-cysteine of these NMBAs *in vivo* should be faster and more complete than the indirect mechanism of competitive reversal by conventional anticholinesterases. A preliminary report has been published.<sup>4</sup>

The studies were designed to (1) correlate the rate of adduction *in vitro* of L-cysteine to the compounds *versus* duration of block in the anesthetized monkey; (2) evaluate the speed and completeness of chemical antagonism of block by exogenous (intravenous) L-cysteine in comparison with anticholinesterase reversal; (3) synthesize nondepolarizing NMBAs with pharmacologic properties in monkeys forecasting intermediate duration of effect in humans, as, for example, in cisatracurium, but offering the added unique feature of rapid chemical antagonism at any point by exogenous L-cysteine.

# **Materials and Methods**

# Synthesis of New Compounds

The chemical pathways of synthesis of gantacurium, CW 002 and CW 011, and NB 1043–10 (fig. 1D), an L-cysteine adduct of CW 002, are given in Supplemental Digital Content 1 (see appendices 1 and 2, http://links.lww.com/ALN/A590). Samples of the four compounds were assayed at 92–95% area peak purity by high-performance liquid chromatography. Gantacurium and CW 002 standards used for identification purposes have been structurally characterized by nuclear magnetic resonance, infrared spectrometry, mass spectrometry, ultraviolet spectrometry, and elemental analysis. CW 011 product peak identity was assigned by analogy to the gantacurium and CW 002 syntheses because the syntheses involved trivial variations of the chemical processes devel-

oped to prepare gantacurium and CW 002 from well-characterized isoquinoline alcohols (see Supplemental Digital Content 1, appendices 1 and 2, http://links.lww.com/ALN/A590).

#### Cisatracurium

Cisatracurium (Nimbex®; Abbott, Chicago, IL) was evaluated as a comparator for the study.

#### **Neostigmine and Edrophonium**

Commercial preparations (American Regent, Inc., Shirley, NY) were administered at standard concentrations.

# L-Cysteine

L-Cysteine hydrochloride monohydrate (Sigma-Aldrich, St. Louis, MO; 98% purity) was dissolved in 0.9% NaCl at a concentration of 200 mg/ml; pH was adjusted with NaOH and buffered to 4.5–5.5.

#### Studies in Anesthetized Rhesus Monkeys

Animal Preparation and Care. Experiments were approved by the Institutional Animal Care and Use Committees of the Weill Medical College of Cornell University (New York, New York) and of the Albany Medical College (Albany, New York) at which the studies were conducted. A colony of 10 adult male monkeys (Macaca mulatta) weighing 8-18 kg was studied at approximately 6-week intervals. Animals were housed and cared for in accordance with the Guide for Care and Use of Laboratory Animals (National Research Council, Washington, DC). They were fed a standard Old World monkey diet, enriched with fruits and vegetables, and other dietary novelties and were followed up throughout the study to verify normal health by physical examination, body weight, and clinical laboratory studies (complete blood count, blood urea nitrogen and creatinine, and liver function tests).

**Anesthesia and Experimental Set Up.** On the day of each study, monkeys received ketamine (7–10 mg/kg intramuscular) followed by tracheal intubation under topical anesthesia with 4% lidocaine. Ventilation was controlled at 10 ml/kg and 20 breaths/min with isoflurane (1.0–2.0%) and N<sub>2</sub>O/O<sub>2</sub> (2:1 mixture). Lactated Ringer's solution was administered at approximately 10 ml·kg<sup>-1</sup>·h<sup>-1</sup>. Arterial pressure was monitored from a femoral, superficial tibial, or radial (22-gauge) cannula. Heart rate was measured by tachograph from the arterial pulse wave. Core temperature was kept at 36.5°–38.0°C by warming blankets. Electrocardiogram and pulse oximetry were monitored continuously.

Needle electrodes (25 gauge) transmitting square-wave pulses of 0.2-ms duration at supramaximal voltage, which were generated by a Grass S-88 stimulator (Grass Instruments, Quincy, MA), were placed at the peroneal nerve at the knee to elicit twitch responses of the extensor digitorum of the foot at 0.15 Hz. A small slip (10–20%) of the tendon was dissected free under sterile technique and tied to a Grass FT 10 force transducer (Grass Instruments) at a baseline tension of 50 g. Train-of-four (TOF) stimulation (2 Hz for 2 s) was

interposed at appropriate points, especially 1-2 min before NMBA dosing and after recovery of twitch to 95% of baseline, at which TOF was subsequently evaluated every 1-2 min until normal (TOF > 100%).

Recordings of circulatory and neuromuscular data were made on a Grass 7B polygraph (Grass Instruments). A baseline period of 15-20 min was allowed for stabilization of recordings before dosing.2

At the end of each experiment, animals were awakened, analgesics were given per veterinary practice, and animals were returned to their domiciles and attended until standing.

# Determination of Neuromuscular Blocking Potency and Duration

Dose-response curves for twitch blockade by gantacurium, CW 002, CW 011, and cisatracurium were generated as follows. To ensure minimal cumulative or residual influence on these data, sequential dosing was performed in an escalating fashion. Successive doses were separated by at least three estimated half-lives beyond complete recovery of the previous dose to TOF of 110-120%, which is normal for these monkeys. Only the first one or two doses yielding 5-99% blockade were included from any single experiment for computation of dose-response data.

Comparative studies of spontaneous recovery versus antagonism or reversal were performed at least three estimated half-lives after dose-response studies.

ED<sub>50</sub> and ED<sub>95</sub> were computed from the regression of log dose *versus* the logit of percentage blockade of twitch.

NB 1043-10, the L-cysteine adduct of CW 002, was available in limited quantity and was administered in cumulative fashion to three animals to compare approximate potency and duration of effect *versus* its parent compound CW 002.

#### In Vitro Degradation by L-Cysteine

First, to evaluate stability at physiologic pH and temperature, CW 002, CW 011, or gantacurium was dissolved in phosphate buffer (pH 7.4) at a concentration of 1,000  $\mu$ g/ml. Stability at pH 7.4 and 37°C was monitored by highperformance liquid chromatography for at least 2 h to evaluate background alkaline hydrolysis of each compound (see Supplemental Digital Content 1, appendix 3 for details, http://links.lww.com/ALN/A590).

In a second experiment, to evaluate degradation in the presence of L-cysteine, buffered solutions (pH 7.4) of gantacurium, CW 002, or CW 011 were freshly prepared at 37°C to give experimental concentrations of 1000  $\mu$ g/ml (CW 002 and CW 011) and 200 μg/ml (gantacurium). And addition to and mixing with a 5% molar excess of L-cysteine at time = 0, the concentration of NMBA/parent compound remaining at specified time points after mixing was determined by high-performance liquid chromatography. The concentration of blocking agent at each time point was determined from a separately prepared reaction mixture, because of the rapid rate of L-cysteine adduction. The reaction rate constant for the second-order reaction of each NMBA with L-cysteine was derived by plotting the natural log of ([NMBA],[Lcysteine]<sub>t=0</sub>/[NMBA]<sub>t=0</sub>[L-cysteine]<sub>t</sub>) versus time.

Adduction reaction half-time ( $t^{1/2}$ ) was calculated at specific concentrations selected for each new compound reflecting their relative potencies in vivo, using the reaction rate constant. Concentrations selected were 200, 100, and 50  $\mu$ g/ml, which are approximately proportional to the relative potencies (ED<sub>95</sub>) of gantacurium, CW 002, and CW 011, respectively. A degradation (adduction) pathway in the presence of L-cysteine for each of the three compounds was proposed.

## Comparative Studies of Antagonism by L-Cysteine versus Anticholinesterase Reversal

Pilot experiments had shown that the 5-95% twitch recovery slopes (times) for all the three test compounds were markedly accelerated (shortened) by exogenous L-cysteine (5-50 mg/kg intravenous). Comparisons of spontaneous recovery after a control dose of each compound were made with accelerated recovery after antagonism or inactivation by L-cysteine or reversal by standard anticholinesterases of a second dose of the compound, given approximately 3 estimated half-lives after recovery of the control dose to TOF 110-120%. One test of reversal or antagonism was done per experiment, as a paired comparison with the previously given control dose.

Details are as follows. Control doses (first dose) of the compounds were 0.5 mg/kg gantacurium, 0.15 mg/kg CW 002, and 0.10 mg/kg CW 011. These doses had been shown to be approximately  $4-5 \times ED_{95}$ . They were given to ensure 100% twitch inhibition to simulate intubating dosage in clinical practice. Antagonism of each compound at these doses was tested in a first series of experiments by administration of L-cysteine or the anticholinesterase at 2% twitch height at the beginning of recovery from the second dose of the compound (NMBA) ("standard reversal").

Antagonism was then tested in a second series of experiments, at +1 min after injection of a second dose of the test compound (NMBA), to simulate various difficult clinical scenarios ("immediate reversal").

# Antagonism of Gantacurium by L-Cysteine or **Edrophonium**

Spontaneous recovery from a first dose of gantacurium at approximately 5× ED<sub>95</sub> (0.50 mg/kg) was compared with edrophonium reversal (1.0 mg/kg + atropine 0.05 mg/kg) or Lcysteine antagonism (10 mg/kg) of a second dose. The control dose was allowed to recover to TOF 110-120%; 20 min later, a second dose was followed by edrophonium or L-cysteine either at the beginning of recovery at 2% twitch height or at 1 min after blocking drug administration, at 100% blockade. Measurements were made of the total duration from gantacurium injection to recovery of twitch to 95% of baseline and of the slope of recovery (interval of recovery from 5 to 95% twitch height). These were compared during spontaneous recovery versus after L-cysteine or edrophonium antagonism.

Table 1. Potency and Duration of Effect of Gantacurium, CW 011, CW 002 and Its ∟-Cysteine Adduct (NB 1043-10) and Cisatracurium in Monkeys under Isoflurane\*

	ED <sub>50</sub>		ED <sub>95</sub>		Administered Dose		Duration
Compound	mg/kg $\pm$ SD/SEM†	n	mg/kg $\pm$ SD/SEM†	n	mg/kg‡	n§	(min ± SD/SEM)
Gantacurium	0.032 ± 0.018/0.0026	51	0.100 ± 0.0035/0.005	51	0.10 0.50	29 9	7.3 ± 2.1/0.4 10.4 ± 3.1/1.0#
CW 002	$0.022\pm0.008/0.0020$	18	$0.042\pm0.016/0.004$	18	0.05 0.15	8 50	20.5 ± 7.1/2.5 28.1 ± 7.1/1.0**
CW 011	$0.014\pm0.004/0.0009$	23	$0.025\pm0.008/0.002$	23	0.03 0.10	6 11	20.8 ± 6.3/2.6 33.3 + 7.2/2.2**
Cisatracurium	$0.017\pm0.003/0.0007$	22	$0.028\pm0.006/0.0024$	22	0.10 0.035 0.12	5	$35.5 \pm 8.2/3.7$
NB 1043-10†† (Cysteine adduct of CW 002)	0.750 ± 0.499/0.1384	13	2.76 ± 0.96/0.27	13	0.12 N/A	4 N/A	58.0 ± 18.6/9.3 ~30–35‡‡

<sup>\*</sup> Twitch of extensor digitorum elicited at 0.15 Hz. † Calculated from dose–response curve. ‡ Actual doses administered. § Number of observations.  $\parallel$  From injection to recovery of twitch to 95% of baseline. # P < 0.01 vs. CW 002 and CW 011. \*\* P < 0.01 vs. cisatracurium. †† Available in limited quantity. ‡‡ Spontaneous recovery (times approximate) following cumulative dosage. ED = effective dose; n = number of points used to construct the dose–response curve.

# Standard Antagonism of CW 002 by L-Cysteine versus Neostigmine

A control dose of CW 002 at approximately  $4\times$  ED $_{95}$  (0.15 mg/kg) was first given and allowed to recover spontaneously to TOF 110–120%. Sixty minutes later, a second dose of CW 002 was given, followed by either neostigmine (0.05 mg/kg + atropine 0.05 mg/kg) or L-cysteine (50 mg/kg) at the beginning of spontaneous recovery, at 2% twitch height. Total duration and 5–95% recovery intervals were compared.

# Immediate Antagonism of CW 002 by L-Cysteine versus Neostigmine: L-Cysteine Dose-Response

A control dose of 0.15 mg/kg CW 002 was allowed to recover spontaneously to TOF 110–120%. A second dose was given 60 min later, followed by either neostigmine (0.05 mg/kg + atropine 0.05 mg/kg) or L-cysteine (10, 20, 30, or 50 mg/kg) 1 min later. Total duration and 5–95% recovery intervals were compared during spontaneous recovery *versus* antagonism by L-cysteine or reversal by neostigmine.

# Immediate Antagonism by L-Cysteine of CW 011: Dose-Response Comparing Optimal Dosage of L-Cysteine in Antagonism of Escalating Dosage of CW 011

This series of paired comparisons was designed to generate dose–response data comparing spontaneous recovery of high doses of CW 011 (from 4 to  $64\times$  ED<sub>95</sub>) *versus* immediate antagonism at +1 min by an optimal dose of L-cysteine (50 mg/kg), previously determined during studies of immediate antagonism of CW 002. This experiment was done to explore the limits of chemical antagonism by L-cysteine of these olefinic diester neuromuscular blocking drugs, using CW 011 as a typical example with an intermediate duration of action. Five groups of animals first received a control dose of CW 011 at 4, 8, 16, 32, or  $64\times$  ED<sub>95</sub> (0.1–1.6 mg/kg). Sixty minutes after spontaneous recovery of the control dose to TOF 110–120%, the same dose was repeated, followed 1

min later by L-cysteine (50 mg/kg). Total duration of action and 5–95% recovery interval were compared during spontaneous recovery and after antagonism by L-cysteine.

#### Data Analysis

Microsoft Excel 2007 Analysis ToolPak (Microsoft Corporation, Redmond, WA) was used for all statistical analysis.

Dose–response curves were generated by linear regression of dose *versus* the logit of percentage blockade of twitch.  $ED_{95}$  and  $ED_{50}$  ( $\pm SD/SEM$ ) were calculated. Doses producing 0–5% or 100% twitch inhibition were not included in dose–response calculations.

Statistical comparisons were made by paired or unpaired two-tailed t test to compare differences in ED<sub>95</sub>, total duration, and 5–95% intervals.

Linear regression was used to examine the relationship of chemical inactivation, that is, adduction rate  $(t^{1/2})$  in vitro versus duration of action in vivo. Comparisons of speed of reversal versus L-cysteine dosage were performed by ANOVA to establish optimal L-cysteine dosage.

A value of P less than 0.05 was considered significant in all tests.

#### Results

#### Neuromuscular Blockade: Potency and Duration

Gantacurium, CW 002, and CW 011 all showed typical nondepolarizing block with fade of tetanus and TOF and antagonism by anticholinesterases in classic fashion. The duration of block at approximately  $4-5\times$  ED $_{95}$  doses of both CW 002 and CW 011 is about half that of cisatracurium (P<0.01) but approximately  $3\times$  longer than that of gantacurium (P<0.01, table 1). The 5–95% recovery intervals of both CW 002 and CW 011 after approximately  $4\times$  ED $_{95}$  doses were significantly shorter than the corresponding interval for cisatracurium (table 2, P<0.01).

Table 2. Standard Reversal of CW 002 or Cisatracurium: Spontaneous Recovery vs. L-Cysteine or Neostigmine Administered at 2% Twitch Height

Drug	Dose (mg/kg)	Type of Recovery	n	Total Duration (min $\pm$ SD/SE)*	5–95% Interval (min $\pm$ SD/SE)†
CW 002 Cisatracurium	0.15	Spontaneous recovery Neostigmine reversal‡ Spontaneous recovery L-Cysteine antagonism# Spontaneous recovery Neostigmine reversal‡ L-Cysteine antagonism#	7 7 4 4 8 4	$24.8 \pm 4.8/1.8$ $22.5 \pm 8.0/3.0$ § $29.4 \pm 5.3/2.6$ $19.9 \pm 5.3/2.6^{**}$ $36.9 \pm 8.3/2.9$ $24.8 \pm 3.8/1.9^{**}$ $40.0 \pm 6.0/3.5$ §	$\begin{array}{c} 10.8 \pm 1.7/0.7 \\ 8.6 \pm 3.7/1.4 \  \\ 12.3 \pm 2.6/1.2 \\ 2.1 \pm 0.6/0.3^{**} + 1 \\ 20.3 \pm 6.7/2.4 \\ 12.3 \pm 2.6/1.3^{**} \\ 23.3 \pm 5.0/2.9 \\ \end{array}$

<sup>\*</sup> Total duration (min ± SE) from injection of neuromuscular blockade to recovery of twitch to 95% of baseline. † Interval during recovery from 5% to 95% twitch height.  $\ddagger$  Dose 0.05 mg/kg + atropine 0.1 mg/kg.  $\S P > 0.05$  vs. spontaneous recovery. \*\* P < 0.01 vs. spontaneous recovery. †† P < 0.01 vs. 0.05 vs. cisatracurium reversal by neostigmine. # Dose 50 mg/kg. cisatracurium reversal by neostigmine.

CW 002 and CW 011 showed high potency; the calculated ED<sub>95</sub> values indicating equivalent potency were as follows: 0.025 mg/kg CW 011, 0.042 mg/kg CW 002, and 0.028 mg/kg cisatracurium. The ED<sub>95</sub> of gantacurium was 0.100 mg/kg (table 1). SDs and SEMs are given in table 1.

NB 1043–10, the L-cysteine adduct derivative of CW 002, showed nondepolarizing characteristics, for example, fade of tetanus and TOF, and an ED<sub>95</sub> of 2.76 mg/kg, a decrease in potency of approximately 70× versus CW 002 (SD and SEM in table 1). The duration of effect of the adduct is in the same range as the duration of the parent ( $\sim 30-35$  min).

# Degradation in Vitro

The nonhalogenated compounds CW 002 and CW 011 remained stable over 2 h as a 1,000 µg/ml buffered aqueous solution at 37°C and pH 7.4. The estimated  $t\frac{1}{2}$  values for (background) alkaline hydrolysis of CW 002 and CW 011 in the absence of L-cysteine were 495 and 389 min, respectively. When a 5% molar excess of L-cysteine was added, initial peaks were nearly fully replaced within 10-30 min by new peaks identified as L-cysteine adducts of the compounds. The calculated  $t^{1/2}$  values for the adduction reactions were 11.4 min at 100 μg/ml CW 002 and 13.7 min at 50 μg/ml CW 011 (a concentration ratio of  $\sim$ 2:1 reflecting relative potencies in vivo; see table 3).

The peaks identified as the initial L-cysteine adducts of CW 002 and CW 011 subsequently diminished rapidly ( $t^{1/2}$ ~60 min) with the evolution of new peaks corresponding to the alkaline hydrolysis products of the adducts. The breakdown of CW 002 and CW 011 is described in detail in the appendix.

The chromatographic changes observed in the case of gantacurium occurred much faster compared with CW 002 and CW 011 (see the appendix and table 3). Baseline chromatograms showed approximately 10% alkaline hydrolysis of gantacurium within the first 10 min, as evidenced by the decrease in peak area of gantacurium with a corresponding increase in the area of two new peaks of significance, the hydrolysis product AV-6 and one additional peak, presumably the other hydrolysis product. The estimated  $t\frac{1}{2}$  for alkaline hydrolysis of gantacurium was 56 min. At a gantacurium concentration of 200 µg/ml, introduction of L-cysteine yielded peaks suggestive of both adduction and alkaline hydrolysis that appeared rapidly and simultaneously, with L-cysteine adduction predominating (see the appendix and table 3).

The half time of L-cysteine adduction at a gantacurium concentration of 200 µg/ml was calculated to be 0.2 min. Table 3 summarizes the half times of basic background (alkaline) hydrolysis, the calculated adduction rate constants, and the half-times of L-cysteine adduction for CW 002, CW 011, and gantacurium at assumed concentrations of 100, 50, and 200 µg/ml, respectively, reflecting their approximate relative potencies in the monkey.

Table 3. Reaction Half-times (t1/2) in Vitro\*

	<i>t</i> 1/2, min					
Compound	Basic	Cysteine	Estimated			
	Aqueous	Adduction	Cysteine Adduct			
	Hydrolysis	Reaction	Hydrolysis			
Gantacurium	56	0.20	>300			
CW 002	495	11.4	~60			
CW 011	389	13.7	~60			

<sup>\*</sup> Reactions performed in phosphate buffer at pH 7.4 and 37°C. Initial concentrations of compounds in measurements of aqueous hydrolysis were 1,000  $\mu$ g/ml for all the three compounds. Standard aliquots of neuromuscular blocking agents at concentrations of 1,000  $\mu$ g/ml (CW 002 and CW 011) and 200  $\mu$ g/ml (gantacurium) were combined with a 5% > stoichiometric concentration of L-cysteine to measure L-cysteine adduction. L-Cysteine adduction t1/2 were calculated at assumed concentrations of gantacurium (200  $\mu$ g/ml), CW 002 (100  $\mu$ g/ml), and CW 011 (50μg/ml) that reflect their relative potency (ED<sub>95</sub>) in the monkey (ratios 4:2:1 reflecting ED<sub>95</sub> values of 0.100, 0.042, and 0.025 mg/kg, respectively). Cysteine adduct hydrolysis was estimated after the adduction reaction under the same conditions. See Supplemental Digital Content 1, appendix 3 for further details, http://links.lww.com/ALN/A590

n = number of observations.

#### Correlation of L-Cysteine Adduction In Vitro vs. Duration in Monkeys at ~ 4-5x ED 95

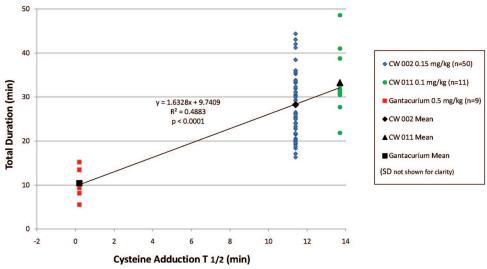


Fig. 2. Correlation of L-cysteine adduction reaction rate ( $t^{1/2}$ ) in vitro with total duration of action (recovery of twitch to 95% of control height) of gantacurium, CW 002, and CW 011 at approximately  $4-5 \times ED_{95}$  in anesthetized monkeys.  $R^2 = 0.4883$  (P < 0.0001). Twitch of the extensor digitorum was elicited at 0.15 Hz.

# Rate of $\iota$ -Cysteine Adduction in Vitro versus Duration of Block in Vivo

The rate of L-cysteine adduction *in vitro* (t/2) was inversely related to the durations of action of gantacurium, CW 002, and CW 011 at approximately 4–5× ED<sub>95</sub> in the monkey (r<sup>2</sup> = 0.4883, P < 0.0001). The regression is shown in figure 2.

# Comparative Studies of L-Cysteine Antagonism versus Reversal by Anticholinesterases

**Antagonism of Gantacurium by L-cysteine** *versus* **Reversal by Edrophonium.** Edrophonium (1.0 mg/kg + atropine 0.05 mg/kg) given at 2% twitch height during early recovery from gantacurium (0.5 mg/kg) shortened the total duration and 5–95% recovery interval. L-Cysteine (10 mg/kg) given at this point caused a greater acceleration of recovery than did edrophonium (fig. 3). However, the difference was not statistically significant in this small sample.

On the other hand, L-cysteine (10 mg/kg) given at 1 min after gantacurium rapidly abolished neuromuscular blockade, shortening the 5-95% recovery interval to  $1.3 \pm 0.8$ min (SD) from 3.8  $\pm$  1.4 min (P < 0.001) and reducing the total duration from  $10.4 \pm 3.1$  min to  $3.0 \pm 1.0$  min versus spontaneous recovery (P < 0.001). L-Cysteine antagonism of gantacurium at +1 min was significantly faster than edrophonium reversal at  $+1 \min (P < 0.01; \text{ fig. 3}); \text{TOF reached}$ a ratio of more than 100% nearly simultaneously with increase of twitch to 95% of baseline (see fig. 4). The speed of L-cysteine antagonism, when the 5–95% recovery intervals were compared following L-cysteine administration at +1 min or at 2% twitch height, did not differ significantly (P > 0.05; compare figs. 3A and B). Figure 4 shows the immediate antagonistic effect of L-cysteine (10 mg/kg) at +1 min after approximately  $5 \times ED_{95}$  of gantacurium (0.5 mg/kg).

**Standard Reversal of CW 002 and Cisatracurium by Neostigmine.** The blocking effects of CW 002 (n = 7) and cisatracurium (n = 4) were reversed as expected at 2% twitch height by neostigmine (0.05 mg/kg + atropine 0.05 mg/kg). Total duration of action and 5–95% recovery interval were shortened significantly (P < 0.05, table 2). CW 002 reversal by neostigmine was significantly faster than cisatracurium reversal by neostigmine (P < 0.05) when 5–95% recovery intervals during reversal were compared (table 2).

Standard Antagonism of CW 002 by L-Cysteine: Comparison with Reversal by Neostigmine. Antagonism of CW 002 (0.15 mg/kg) at 2% twitch height by L-cysteine at optimal dosage, 50 mg/kg, (see also *Immediate Antagonism* below) shortened the 5–95% recovery interval from  $10.8 \pm 1.7$  min (SD) during spontaneous recovery to  $2.1 \pm 0.6$  min (P < 0.01). Comparative neostigmine reversal of CW 002 at 2% twitch height was significantly slower, showing a 5–95% interval of  $8.6 \pm 3.7$  min (P < 0.01 vs. L-cysteine antagonism).

**Immediate Antagonism of CW 002 by L-Cysteine: Dose Response.** Neostigmine was not effective in accelerating recovery of block by CW 002 when given at 1 min after a dose of CW 002 of 0.15 mg/kg (fig. 5A).

Four comparisons of spontaneous recovery after 0.15 mg/kg CW 002 were made *versus* L-cysteine antagonism at 1 min after CW 002 (*Immediate Antagonism*), by increasing L-cysteine dosage of 10, 20, 30, or 50 mg/kg. Immediate antagonism of CW 002 block by L-cysteine was highly effective at all doses, shortening both the total duration of action and the 5–95% recovery interval (P < 0.001 in all comparisons, fig. 5B). The rapidity of L-cysteine antagonism peaked at 30–50 mg/kg, such that 50 mg/kg seemed an optimal or maximal dose (fig. 5B). A dose of 50 mg/kg L-cysteine restored neuromuscular function to 95% of baseline twitch height within 2.2  $\pm$  0.3 min (SD); TOF was restored to a ratio of more than 100% within 1–2 min later (fig.

#### Antagonism of Gantacurium by Edrophonium or L-cysteine

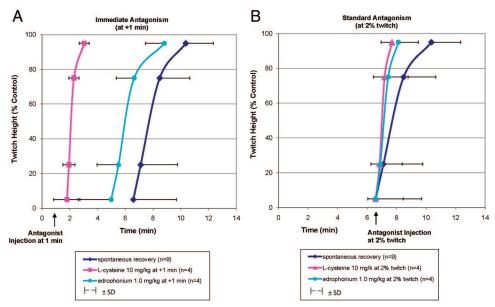


Fig. 3. Comparative antagonism of gantacurium (0.5 mg/kg or  $\sim$ 5  $\times$  ED<sub>95</sub>) by edrophonium (1.0 mg/kg with atropine 0.05 mg/kg) or L-cysteine (10 mg/kg). Gantacurium was injected at t = 0. Antagonist was given at the beginning of recovery at 2% twitch height (standard antagonism, B); or at 1 min after gantacurium administration (immediate antagonism, A). Antagonism by L-cysteine is faster than edrophonium at 2% twitch and is equally rapid at either 2% twitch or at 1 min after gantacurium (P < 0.05). Antagonism by L-cysteine at 1 min is significantly faster (P < 0.001) than edrophonium, which shifts the dose-duration curve to the left by only ~2 min. Curves show summarized data from experiments in anesthetized rhesus monkeys in which twitch of the extensor digitorum was elicited at 0.15 Hz (see also fig. 4).

6). The 5-95% interval after L-cysteine antagonism at 1 min after 0.15 mg/kg CW 002 was similar to the same interval after L-cysteine antagonism during the beginning of recovery at 2% twitch height:  $1.7 \pm 1.1 \text{ min (SD)}$  versus  $2.1 \pm 0.6 \text{ min, respec-}$ tively (P > 0.05).

L-Cysteine Does Not Antagonize Cisatracurium-induced **Block.** L-Cysteine (50 mg/kg) given at 2% twitch height at initiation of spontaneous recovery had no effect on recovery from cisatracurium; there was no change in the total duration or the 5–95% recovery interval (P > 0.05, see table 3).

Immediate L-Cysteine Antagonism at +1 min of Escalating Dosage of CW 011 (4-64 $\times$  ED<sub>95</sub>). L-Cysteine (50 mg/kg) caused highly significant acceleration (P < 0.001

in all cases) of recovery from CW 011 at all CW 011 doses tested from 0.1 to 1.6 mg/kg (4× to 64× ED<sub>95</sub>). Both total duration and 5-95% recovery interval were shortened significantly (fig. 7).

#### **Discussion**

### Relation of L-Cysteine Degradation in Vitro to Duration in Vivo

Because the durations of action of gantacurium, CW 002, and CW 011 were inversely related to the respective rates of degradation of the compounds by L-cysteine adduction in vitro (fig. 2), the adduction reaction is most likely the rate-

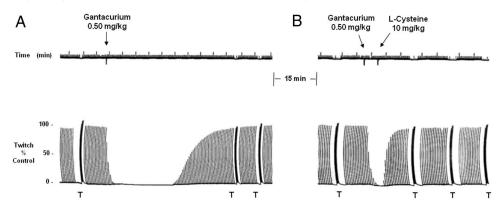


Fig. 4. An example of immediate antagonism of gantacurium by L-cysteine. Twitch was elicited at 0.15 Hz in an anesthetized rhesus monkey. Train-of-four (TOF) stimulation was interposed at T. (A) A control dose of gantacurium, 0.5 mg/kg ( $\sim$ 5 × ED<sub>95</sub>), was injected at marker. (B) One minute after a second dose, L-cysteine (10 mg/kg) restored twitch and TOF to normal within 2 min.

# Immediate Antagonism of ~4x ED 95 CW 002 (0.15 mg/kg) by L-cysteine or Neostigmine at + 1 min

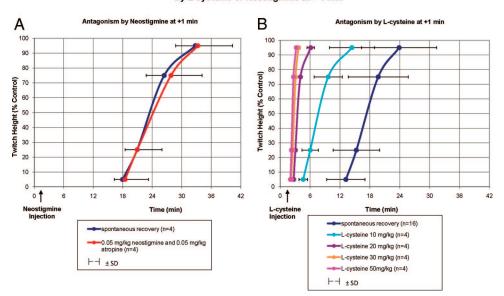
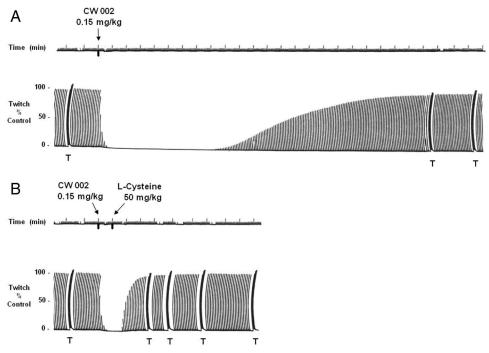


Fig. 5. Immediate antagonism of CW 002 blockade 1 min after CW 002 dosage of 0.15 mg/kg, or  $\sim$ 4  $\times$  ED<sub>95</sub>, injected at t=0. Neostigmine (0.05 mg/kg + atropine 0.05 mg/kg) or L-cysteine (10, 20, 30, or 50 mg/kg) was given at +1 min. Neostigmine did not shorten recovery (A), whereas L-cysteine produced a dose-related acceleration of recovery (B), peaking at 50 mg/kg. Data were taken from anesthetized rhesus monkeys. Twitch of the extensor digitorum was elicited at 0.15 Hz (see also fig. 6).

limiting step governing the kinetics of neuromuscular block *in vivo*. The adduction products seem far less potent than the parent compounds (compare CW 002 and NB 1043–10); the adducts subsequently undergo alkaline hydrolysis to less potent fragments within a few hours (the potency of these fragments is in the range of 15–25 mg/kg for neuromuscular

blockade in monkeys; unpublished laboratory records, John J. Savarese, M.D., Weill Medical College of Cornell University, New York, New York, 2009). All fragments are positively charged monoquaternary substances (see figs. 8–10 in the appendix) and quite soluble in water; therefore, they are likely excretable in urine and possibly bile (not as yet proven



**Fig. 6.** An example of immediate antagonism of CW 002 by  $_{\rm L}$ -cysteine. Twitch of the extensor digitorum was elicited at 0.15 Hz in a rhesus monkey under isoflurane. (A) A control dose of CW 002 (0.15 mg/kg,  $_{\rm L}$ 4  $\times$  ED $_{\rm 95}$ ) was injected at marker and allowed to recover spontaneously. (B) Sixty minutes later, the same dose was given at marker, followed 1 min later by  $_{\rm L}$ -cysteine (50 mg/kg). Twitch and train-of-four (TOF) (T) were restored to baseline within 2 and 3 min, respectively.

#### Comparison of Spontaneous Recoveries from Escalating Doses of CW 011 vs. L-Cysteine Antagonism at + 1 min

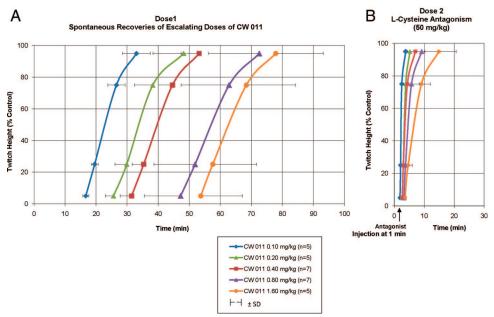


Fig. 7. Immediate L-cysteine (50 mg/kg) antagonism of escalating dosage of CW 011 (4-64  $\times$  ED<sub>95</sub>): comparison of spontaneous recoveries (A) vs. recovery accelerated by L-cysteine given at +1 min after CW 011 (B). CW 011 was injected at t=0. All paired comparisons P<0.001. Data were from groups of anesthetized monkeys; twitch of extensor digitorum was elicited at 0.15 Hz. Dosage pairs are color coded.

experimentally). Facile antagonism of blockade by administration of exogenous L-cysteine at +1 min, or at 2% twitch height, after approximately  $4-5 \times ED_{95}$  doses indicates that the adduction reaction can be accelerated in vivo at any time according to laws of mass action.

## L-Cysteine Antagonism: Comparisons versus Anticholinesterases

L-Cysteine antagonism was significantly faster than reversal by anticholinesterases. Evaluations at 1 min after approximately  $4-5 \times ED_{95}$  doses of the new compounds especially showed a marked superiority of chemical antagonism or inactivation by L-cysteine over the competitive effect of anticholinesterases in terms of speed and completeness of antagonism.

Comparisons of L-cysteine antagonism of gantacurium with reversal by edrophonium are appropriate. The rapidly acting edrophonium would theoretically be considered a better antagonist of the ultra-short-acting gantacurium than neostigmine in view of the more slowly developing antagonistic effect of neostigmine.<sup>5,6</sup>

The requirement for higher dosage of L-cysteine to induce rapid antagonism of the intermediate-duration compounds CW 002 and CW 011 is consistent with the slower rate of adduction in vitro by L-cysteine to these compounds compared with gantacurium and the longer duration in monkeys. CW 002 and CW 011 are about two and four times more potent than gantacurium, respectively. This means that concentrations in vivo needed for neuromuscular blockade will be approximately two and four times lower than gantacurium concentration. Plasma L-cysteine levels remain more or less constant throughout life in humans.<sup>7</sup> Therefore, according to mass action mechanisms, the adduction reaction will be slower in the presence of the same L-cysteine concentrations in plasma in the case of potent NMBAs in which plasma levels of the NMBA will be lower. This necessitates higher concentrations of L-cysteine (higher dosage of exogenous L-cysteine) during chemical antagonism in vivo to bring the reaction kinetics up to rates comparable with the reaction with the less-potent gantacurium, thereby inducing antagonism in a similar time-frame of about 2-3 min. Higher concentrations (dosage) of L-cysteine must also be needed for similarly rapid antagonism of the nonhalogenated compounds to increase the kinetics of the adduction reaction to compensate for the lack of the accelerating effect of the chlorine substitution.

# Comparative Antagonism of Gantacurium, CW 002, and CW 011 by L-Cysteine

Comparative data in tables 1, 2, and 3, and figures 3, 5, and 7 show that L-cysteine dosage for rapid (~2–3 min) antagonism of  $4-5 \times ED_{95}$  doses of gantacurium at +1 min after injection is much lower (10 mg/kg) than the dosage requirement for similarly rapid antagonism of CW 002 or CW 011 (50 mg/kg). This difference can be explained by the differences in molecular design of the compounds in which chlorine substitution in gantacurium is a powerful accelerator of L-cysteine adduction to the olefin, resulting in a short (0.2) min)  $t^{1/2}$  for the adduction reaction (fig. 2 and table 2). This is the likely mechanism explaining both the ultra-short duration of gantacurium and its rapid antagonism by low doses of L-cysteine in the monkey.

Similarly, in CW 002 and CW 011, the lack of chlorine results in a much slower  $t\frac{1}{2}$  for L-cysteine adduction in vitro and comparatively longer duration of action in vivo, as well as a higher L-cysteine dosage requirement (50 mg/kg) to presumably accelerate the adduction reaction to a rate compatible with antagonism in the same sort of 2–3 min time frame. It also is apparent that the similar range of duration of effect of approximately 30 min in monkeys of CW 002 and CW 011 at approximately  $4 \times ED_{95}$  and the similar  $t^{1/2}$  of the adduction reaction in vitro of these two compounds would result in similar antagonism by L-cysteine in terms of dosage requirement and rate of antagonism. This is indeed the case: table 2 shows a  $t^{1/2}$  of 11.4 and 13.7 min for L-cysteine adduction to CW 002 and CW 011, respectively, and figures 5 and 7 show complete antagonism within approximately 2–3 min of  $4 \times ED_{95}$  doses of the two compounds by L-cysteine dosage of 50 mg/kg. On the other hand, 10 mg/kg L-cysteine, although inducing antagonism of gantacurium blockade in 2 min (figs. 3 and 4), required approximately 13 min for antagonism of CW 002 (fig. 5B).

# Potential Clinical Advantages of L-Cysteine Chemical Antagonism

L-Cysteine antagonism gives the capacity to rapidly and completely antagonize nearly any depth of blockade after the administration of fully paralyzing doses of these new NMBAs, such as intubating dosage or inadvertent overdose: all three compounds show rapid ( $\sim\!2\text{--}3$  min) antagonism by L-cysteine at 1 min after approximately  $4\text{--}5\times$  ED $_{95}$  doses. L-Cysteine antagonism uniquely shows similarly rapid effectiveness at any point during blockade with no change in dosage requirement.

The above features of L-cysteine chemical antagonism may help reduce the key safety risk of residual weakness during clinical reversal or spontaneous recovery from neuromuscular blockade: increased reversal or recovery speed should shorten any interval of risk of potential residual weakness. This risk correlates with a TOF less than 0.7 and may be manifested as subnormal oropharyngeal function, or upper airway collapse. P-14 A TOF of 0.9 is now accepted as a more conservative standard of safety in clinical recovery from neuromuscular blockade, Safety in clinical recovery from neuromuscular blockade, but this is difficult to achieve in 10–15 min when anticholinesterases are given for reversal of deep blockade.

The incidence of postoperative pulmonary complications is related to age, intraabdominal or intrathoracic surgery, and longer duration of action of the NMBA, <sup>21</sup> implying that faster and more complete recovery may reduce the risk of such complications. L-Cysteine-induced rapid degradation of these new NMBAs resulting in clearance or removal of active NMBA molecules within minutes may reduce such risk in comparison with neostigmine reversal in which the clearance of NMBA is unaffected. Of course, clinical studies are needed to test this hypothesis rigorously, but the absence of

NMBA guaranteed by L-cysteine degradation in a chemical reaction is obviously superior to a neostigmine-induced limited shift of the dose–response curve to the right, and no observed change in clearance of NMBA following neostigmine.

#### L-Cysteine Pharmacology and Toxicology

The amino acid L-cysteine is a normal building block of protein and a conditionally essential amino acid in infants. L-Cysteine is commonly administered in human therapeutics. For instance, it may be added to total parenteral nutrition regimens for babies in doses of approximately  $80~{\rm mg}\cdot{\rm kg}^{-1}\cdot{\rm day}^{-1}$ . Total amounts may reach 1–2 g/kg or more during the course of 2–4 weeks.  $^{22}$  Because L-cysteine hydrochloride is acidic, the pH of total parenteral nutrition solutions may be alkalinized to help prevent metabolic acidosis, hence L-cysteine pH was adjusted to 4.5–5.5 in the studies described here.  $^{23}$ 

Some concern has been expressed regarding previous toxicological studies that reported potential developmental defects in the central nervous system after large doses of L-cysteine (1–1.5 g/kg) that are close to LD<sub>50</sub> (1,140 mg/kg, L-cysteine data sheets, pg. 4; Sigma-Aldrich) were given to neonatal mice and rats.<sup>24</sup> Such studies may provide safety guidelines in terms of maximal tolerated dosage of L-cysteine in total parenteral nutrition solutions.<sup>25,26</sup>

On the other hand, when given in pharmacologic dosage (a single bolus of 10-50 mg/kg for antagonism of neuromuscular block as reported here or 80 mg  $\cdot$  kg $^{-1}$  · day $^{-1}$  for total parenteral nutrition supplementation), <sup>22</sup> L-cysteine has no known toxicity. The derivative *N*-acetyl L-cysteine may be given as a precursor to L-cysteine to aid replenishment of the antioxidant activity of glutathione in the treatment of acetaminophen (Tylenol®; McNeil PPC, Inc., Fort Washington, PA) toxicity and related (or unrelated) acute liver failure; suggested dosage is 300 mg/kg intravenously during a 20-h period, or 1.3 g/kg orally during a 72-h interval. <sup>27–29</sup>

We have performed appropriate studies supporting the safety of L-cysteine in two species based on the U.S. Food and Drug Administration guidelines; no evidence of toxicity for L-cysteine alone or during reversal of CW 002 was found in the rat or dog, supporting safety to enable initiation of human phase I studies. Administration of intravenous bolus doses of up to 500 mg/kg of L-cysteine to conscious rats and 400 mg/kg to conscious dogs showed no evidence of any histologic, biochemical, or hematologic pathology. L-Cysteine (200 mg/kg) given for immediate reversal of CW 002 (0.40 or 0.60 mg/kg) resulted in no subsequent evidence of toxicity in anesthetized dogs. An investigational new drug application has been submitted to the U.S. Food and Drug Administration for phase I studies of the combination.

The neuromuscular and hemodynamic pharmacology of CW 002 and L-cysteine during antagonism of CW 002 blockade in the dog has been reported. A wide safety ratio for neuromuscular blockade *versus* circulatory effect of CW 002 was found. <sup>30</sup> The hemodynamic effects of L-cysteine are also minimal in the dog at doses (50 mg/kg) that completely

antagonize full paralysis within 5 min when given 1 min after approximately  $10 \times ED_{95}$  doses of CW 002.<sup>31</sup>

# A Few Structure–Activity Relations Governing the Chemistry of L-Cysteine Adduction

A number of chemical modifiers of the rate of the adduction reaction have been identified, three examples of which are illustrated in gantacurium, CW 002, and CW 011. The central double bond (olefin) is essential; analogous succinates are not affected by L-cysteine (unpublished laboratory records from monkeys, John J. Savarese, M.D., Weill Medical College of Cornell University, 2008-2009). Halogen (chlorine) substitution on the central olefin yields the fastest adduction reaction, similar to that in gantacurium.<sup>2</sup> In nonhalogenated symmetrical fumarates such as CW 002, the reaction is slower in vitro, corresponding with durations of action in monkeys that are roughly 3× longer than the action of gantacurium. In nonhalogenated asymmetrical maleates such as CW 011, access of L-cysteine to the central olefin can be further modulated by substitution of quaternary isoquinolinium groups of various sizes. Larger groups with additional methoxy substitutions on one side of the olefin tend to slow adduction, similar to that in CW 011.

Another key factor modulating the rate of L-cysteine adduction *in vivo* is neuromuscular blocking potency: more potent compounds such as CW 011 will achieve lower molar peak plasma concentrations, slowing down the rate of L-cysteine adduction by a reduced mass action effect and enabling the duration of action to lengthen.

#### Chemical Antagonism of Neuromuscular Blockade

L-Cysteine antagonizes neuromuscular blockade in chemical fashion by inactivation of the NMBA in an organic reaction requiring no enzymatic catalyst and taking place under physiologic conditions of pH 7.4 and 37°C. This differs from the action of sugammadex, which is basically a complexing (chelating, encapsulating) agent that specifically binds rocuronium and vecuronium. 32-35 L-Cysteine converts the active NMBA ultimately to essentially inactive fragments in a series of reactions: the first step in this cascade is adduction; the final fragments are smaller monoquaternary charged molecules that seem to be much less active than the adducts themselves (ED<sub>95</sub> for neuromuscular blockade by the fragments in the monkey is in the range of 15-25 mg/kg; unpublished laboratory records, John J. Savarese, M.D., Weill Medical College of Cornell University, 2009–2010). The L-cysteine breakdown pathway is a one-way reaction in which the active NMBA cannot reform. This feature is clinically relevant because the absence of blocking drug assured by this degradation and reversal mechanism suggests that residual weakness may not occur at all after L-cysteine chemical reversal. Clinical studies will be needed to asses this idea.

#### Conclusions

Gantacurium, CW 002, and CW 011 are examples of ole-finic (double-bonded) isoquinolinium diester NMBAs that

are degraded chemically to inactive derivatives (adducts) by condensation (adduction) of endogenous L-cysteine to the double bond followed by nonenzymatic alkaline hydrolysis to fragments that are less active than the adducts. The rate of degradation *in vitro* correlates inversely with the duration of action *in vivo*. Design of compounds to yield neuromuscular blockers of ultra-short to intermediate duration can be done in accordance with structure—activity relationships.

The blocking effects of these compounds are rapidly abolished simply by supplying more (exogenous) L-cysteine intravenously to accelerate the adduction reaction *in vivo*. Chemical antagonism such as this essentially causes immediate clearance (in kinetic terms) of the active drug. Therefore, L-cysteine provides a novel method of facile chemical antagonism of neuromuscular blockade, superior to anticholinesterase reversal in that L-cysteine antagonism inactivates the NMBA and occurs much more rapidly and is in contrast effective and complete at any point during block, at any depth of blockade.

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Dedicated to the late William B. "Bill" Wastila, Ph.D. (Senior Research Scientist, Burroughs Wellcome Co., Research Triangle Park, North Carolina), mentor, scientist, coworker, and friend.

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# Appendix: Chemical Pathways of Degradation of CW 002, CW 011, and Gantacurium (Figs. 8-10)

Fig. 8. Degradation of CW 002. The molecule is relatively stable at pH 7.4 and 37°C (A) undergoing relatively slow alkaline hydrolysis in the absence of L-cysteine. Addition of L-cysteine (B) results in rapid conversion to the adduct (Int. I) with reaction half-time ( $t\frac{1}{2}$ ) = 11.4 min. The adduct itself then undergoes alkaline hydrolysis with  $t\frac{1}{2} \sim 60$  min (C) to the fragments Intermediate (Int.) II and CU-11.

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Fig. 9. Degradation of CW 011. The molecule is relatively stable at pH 7.4 and 37°C (A). Because the molecule is asymmetrical, conversion to two adducts results on addition of L-cysteine (B), and both adducts then undergo alkaline hydrolysis with reaction half-time ( $t^{1/2}$ )  $\sim$  60 min (C).

**Adduct Hydrolysis Product** 

#### **Hydrolysis Products**

**Fig. 10.** Degradation of gantacurium. The molecule is relatively unstable at pH 7.4 and 37°C and undergoes relatively rapid alkaline hydrolysis with reaction half-time ( $t^{1/2}$ ) = 56 min (A). With addition of L-cysteine, there is rapid conversion to the adduct (B), followed by much slower alkaline hydrolysis of the adduct itself (C).