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Neuromuscular blockers and their reversal: have we finally found the on-off switches?

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Abstract

Background: A nondepolarizing neuromuscular blocking agent (NMBA) with a succinylcholine-like quick onset and offset has been the holy grail of the science of neuromuscular blockade. Although this drug is still elusive, the advent of promising new drug combinations like rocuronium–sugammadex and gantacurium–L-cysteine may achieve the same end result. The type of NMBA; the type, timing, and dose of their reversal drugs; the means of monitoring NMB; and the site of monitoring are potentially on the verge of a paradigm shift.

Main text: A comprehensive search using PubMed and Google Scholar and Medline search was made by using keywords gantacurium, L-cysteine, calabadion, and newer neuromuscular blocking agents for peer-reviewed English language manuscripts published before December 2019. Out of the 97 articles screened, 16 were found to be eligible (original articles) and included in this review.

Conclusion: Quantitative, objective neuromuscular monitoring should be included in the minimum monitoring standards. Gantacurium is a new promising nondepolarizing NMBA with desirable succinylcholine-like onset and duration of action without its side effects. A broad-spectrum reversal agent (calabadion) can be used for both depolarizing and nondepolarizing NMB as well as general anesthetics (etomidate and ketamine). A novel drug (WP [6]) can block the side effects of succinylcholine; all are staring at us from the horizon.

Keywords: Calabadion, Gantacurium, L-cysteine, Neostigmine, Sugammadex, WP [6]

Summary

This is a review article about neuromuscular blocking agents (NMBAs) and the broad-spectrum reversal agents.

A non-depolarizing neuromuscular blocking agent (NMBA) with a succinylcholine-like quick onset and offset has for long been the holy grail of the science of neuromuscular blockade. Although this drug is still elusive, the advent of promising new drug combinations like rocuronium–sugammadex and gantacurium–L-cysteine may achieve same end result. The type of NMBA; the type, timing, and dose of their reversal drugs; the means of monitoring NMB; and the site of monitoring are potentially on the verge of a paradigm shift. A broad-spectrum reversal agent (calabadion) for both depolarizing and non-depolarizing NMBAs as well as general anesthetics (etomidate and ketamine) and a novel drug (WP [6]) to block the side effects of succinylcholine are staring at us from the horizon. A comprehensive search using PubMed and Google Scholar and Medline search was made via using keywords gantacurium, L-cysteine, calabadion, and newer neuromuscular blocking agents for peer-reviewed English language manuscripts published before December 2019. Out of the 97 articles screened, 16 were found to be eligible (original articles) and included in this review.

Quantitative, objective neuromuscular monitoring should be included in the minimum monitoring standards. Gantacurium is a new promising nondepolarizing NMBA with desirable succinylcholine-like onset and duration of action minus the side effects. The new broad-spectrum reversible agent calabadion-2 stands out prominently since it does not only reverse any depth of

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NMB caused by any NMBA, but can also reverse general anaesthetic induction agents and local anaesthetic toxicity. Human clinical trials should be undertaken on a priority basis to explore these exciting realms.

Background

Evolution of neuromuscular blocking agents (NMBAs) commenced with d-tubocurarine (1942) inspired by Amazon-Indian poison arrows (Raghavendra 2002). Notorious for its histamine release, it paved the way for gallamine, the first synthetic NMBA used clinically (1947), but this was highly nephrotoxic. Succinylcholine (1951) was the first leptocurare used clinically but caused fasciculations or momentary muscle excitation preceding muscle relaxation just like decamethonium the only other member of the depolarizing NMBA group (not reversed by anticholinesterase) (Raghavendra 2002). The then existing nondepolarizing NMBAs were replaced by pancuronium the first aminosteroidal NMBA introduced in 1964 and its congener vecuronium (1984). Neither releases histamine, both have a slow onset and unpredictable duration of action in patients with hepatic/renal impairment, but vecuronium is cardio-stable unlike pancuronium which causes tachycardia attributable to vagolytic action. Atracurium and cisatracurium were introduced in 1981 and 1999 respectively, both with and without histamine release, respectively, are belonging to benzylisoquinolium NMBAs. They are eliminated by Hoffmann degradation at physiological temperature and pH. Unfortunately, all these nondepolarizing NMBAs had a slow onset. When Bowman et al. (1988) demonstrated an inverse relationship between NMBA potency and block onset (Bowman et al. 1988), quick/rapid onset (within a minute) NMBAs mivacurium (1992), rocuronium (1994), and rapacuronium (1999) were developed. Gantacurium and its congeners comprise the most modern additions.

Pharmacological reversal of NMBAs begins with the carbamate group, acetylcholinesterase inhibitor "neostigmine" for all practical purposes and since time immemorial (first clinical use 1931; FDA approval 1939), despite drawbacks. Indirect in action, neostigmine cannot reverse profound NMB. It may induce muscle weakness if injected in large doses subsequent to recovery from NMBA (post-operative recurarization) resulting in postoperative respiratory complications (Murphy et al. 2018; Brull and Kopman 2017). Bradycardia, arrhythmias, salivation, flushing, hypotension, and bronchospasm (cholinergic stimulation) may result if not co-administered with anticholinergics (atropine, glycopyrrolate) (Murphy et al. 2018; Brull and Kopman 2017). The only two other clinically available anticholinesterases are pyridostigmine and edrophonium (Colovic et al. 2013).

The ideal NMBA has rapid-onset, quick-offset, non-cumulative, nondepolarizing action, reversible by an antagonist and devoid of clinically relevant adverse effects (Savarese and Kitz 1975; Raghavendra 2002). Rapid onset assumes greatest importance during difficult face-masking with inadequate ventilation/oxygenation, inability to maintain/protect the airway, and anticipation of deteriorating clinical status of the patient besides emergency and obstetric surgery. Finding an NMBA with neuromuscular properties identical to succinylcholine minus its side effect profile is the holy grail of NMB science. Rocuronium has a comparable onset-time but at the cost of a prolonged duration of action (Lien 2013). Reversal is possible with neostigmine only after roughly 30 min of NMBA administration depending upon the train-of-four ratio (TOFr). A specific reversal agent, sugammadex, can reverse profound degrees of rocuronium- and vecuronium-induced NMB (unlike neostigmine) but has its limitations (Haerter and Simons 2015). Moreover, its lipophilic cavity is not roomy enough to envelope benzylisoquinoliums. A broad-spectrum reversal agent, universal for all NMBA, capable of reversing any depth of NMB, is undergoing human clinical trials only now. Supramolecular chemists have developed a brand-new container, calabadion, with a much larger cavity than cyclodextrins (sugammadex) that can envelope and inactivate benzylisoquinoliums as well (Hoffmann et al. 2013).

We may soon witness a paradigm shift from neostigmine to this promising new agent calabadion-2 that can reverse NMB caused by both benzylisoquinolium and aminosteroid NMBA.

This review comprises novel, quick-onset NMBAs and reversal agents facilitating their quick-offset: the on-off switches for neuromuscular block. A comprehensive PubMed, MEDLINE, and Google Scholar search using keywords gantacurium, L-cysteine, calabadion, and newer neuromuscular blocking agents was made for peer-reviewed English language manuscripts published before December 2019, and reference crawling was done. Out of the 97 articles screened, 16 were found to be eligible (original articles) and included in this review.

Despite wide varieties of available NMBAs (Fig. 1), quest for the ideal NMBA is still going on. Quick-onset NMBAs will be briefly discussed focussing on mivacurium, gantacurium, and analogs.

Main text

Mivacurium (Mivacron; Abbott Laboratories Inc.)

Mivacurium comprises a choline-like bis-benzyl-tetrahydroisoquinolinium diesteric nondepolarizing NMBA. Spatial orientation of the methylated phenolic moiety results in three (*cis-trans*, *trans-trans*, *cis-cis*) stereoisomers (Lien 2013). It undergoes butyrylcholinesterase metabolism albeit slower than succinylcholine. Although mivacurium is the shortest acting nondepolarizing NMBA available, its

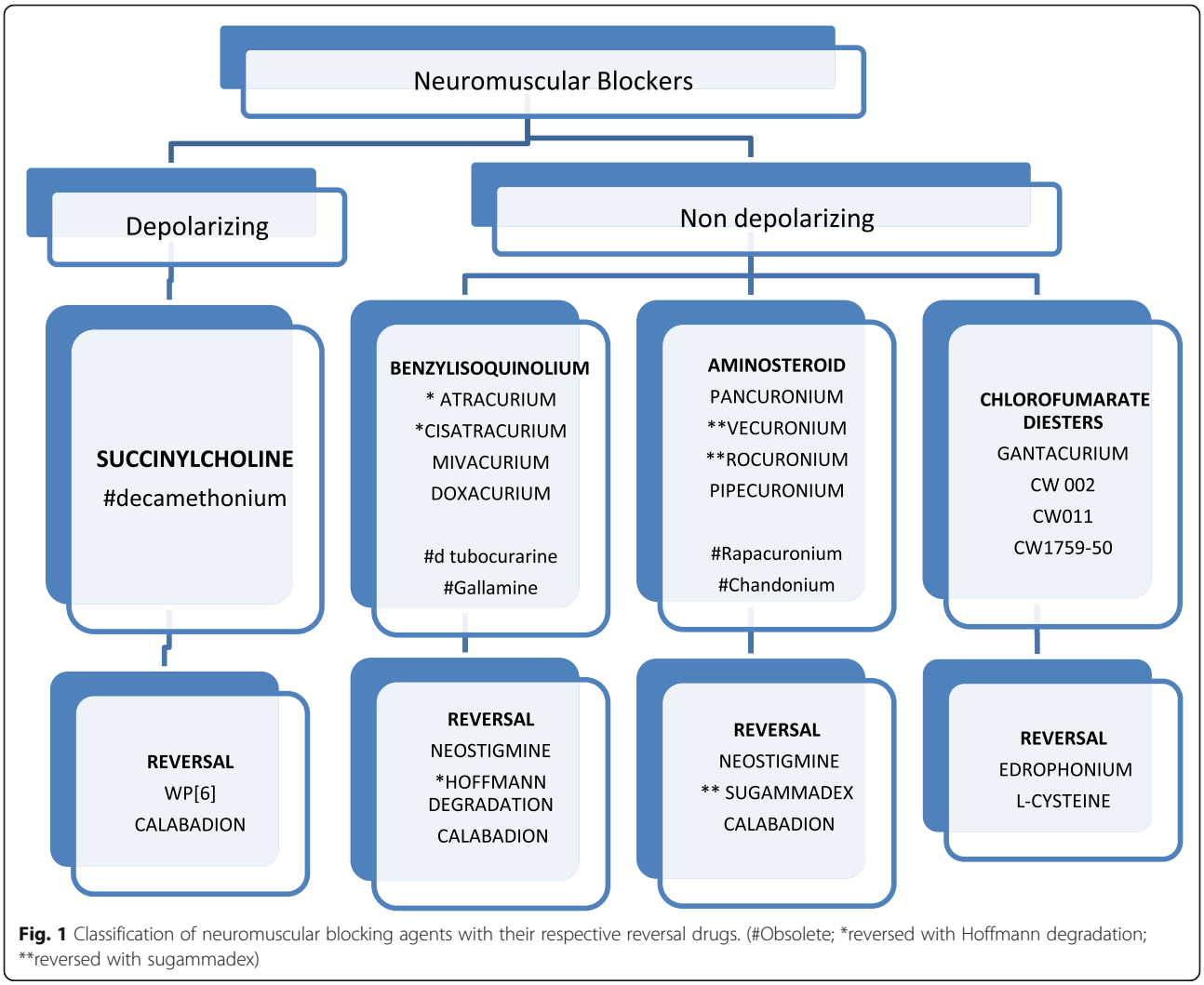

Fig. 1 Classification of neuromuscular blocking agents with their respective reversal drugs. (#Obsolete; *reversed with Hoffmann degradation; **reversed with sugammadex)

duration of action is slightly longer than that of succinylcholine. Despite producing 100% block at laryngeal adductors within 2.5 min and the recovery index being 6 min versus 15 min for atracurium and 30 min for vecuronium infusions, mivacurium is still not popular (Diefenbach et al. 1995). No tachyphylaxis or phase-2 block is seen after prolonged infusion. A major drawback of mivacurium is possible inadequate intubating conditions after a 2 × ED^{95} dose since mivacurium metabolism begins while a block is still developing. FDA approval was obtained in 1992, but Abbott ceased marketing mivacurium in the USA in 2006 due to loss of a chemical intermediary supplier. Since this was not due to safety or efficacy concerns, FDA has placed mivacron (2 mg equivalent/ml) under "discontinued drug product list" section of the orange book (United States Food and Drug Administration n.d.).

Gantacurium (AV430A; GW280430A)

This olefinic compound signifies the birth of a new generation of NMBAs. Chemically, gantacurium is an asymmetric, enantiomeric, isoquinoliniumchlorofumaric acid diester. Gantacurium is a single isomer just like cisatracurium (unlike mixed-isomers atracurium and mivacurium) (Savarese et al. 2010; Boer and Carlos 2018; Heerdt et al. 2015; Lien et al. 2009) and needs reconstitution before administration (Heerdt et al. 2015).

Gantacurium (Table 1) is a rapid onset, nondepolarizing NMBA currently undergoing clinical trials. Intravenous L-cysteine can reverse gantacurium blockade of any depth akin to sugammadex reversal of rocuronium. Compared with the depolarizing muscle relaxant succinylcholine, gantacurium (2–3 × ED^{95}) causes a 100% neuromuscular block at the laryngeal adductors within 60 s, whereas succinylcholine (3 × ED^{95}) reaches its maximal effect in 45 s (Boer and Carlos 2018). Spontaneous recovery after gantacurium (2 × ED^{95})-induced neuromuscular block mimics that of succinylcholine-induced neuromuscular block without unwanted succinylcholine side effects. However, gantacurium is not yet available in clinical practice.

Table 1 Analysis of in vivo studies involving novel, quick-onset NMBAs and reversal agents facilitating their quick-offset: the on-off switches

Name (year)	Study type, study subjects	Methods	Result	Remark/conclusion
Gantacurium; CW002; CW011				
Savarese et al. (2004)	Preclinical Gantacurium versus mivacurium Anaesthetized rhesus monkeys, (n = 8), adult male cats (n = 8)	Each monkey studied 8 times 3 weeks apart over 6 months Each monkey given successive doses of gantacurium (0.03, 0.05, 0.08, 0.20, 0.40, 0.80, 1.60, and 3.20 mg/kg) 15 min after TOFr normalization after previous doses Similarly, each cat given doses of 0.02–6.4 mg/kg successively Gantacurium infusion at rates of 17–38 μg/kg/min for 60 min in 4 monkeys	Gantacurium and mivacurium are equipotent (ED^{95} 0.06 mg/kg for both) At 0.2 mg/kg (3 × ED^{95}), time to 95% twitch recovery was 8.5 ± 0.5 min (gantacurium) versus 22.0 ± 2.6 min (mivacurium)	Duration of action of gantacurium is short (½ to 1/3 that of mivacurium)
			As gantacurium dose was doubled, the total duration of effect lengthened by only 1.5–3 min	Elimination half-life of gantacurium is 1.5–3 min
			Slopes of recovery remained parallel after all doses ≤ 50 × ED^{95} (3.2 mg/kg) Histamine release occurred at 3.2 mg/kg for gantacurium and 0.8 mg/kg for mivacurium	No cumulative effect Speed of recovery from gantacurium block little affected by up to 60 min infusion
Belmont et al. (2004)	Open-label ascending-dose study Healthy human male volunteers aged 18–59 years (n = 31)	Part 1: to determine an ED^{95}dose in 11 subjects. Subject 1 given an initial dose of 0.02 mg/kg followed by an estimated dose that would produce 50% block (ED^{50}). A lack of response from the initial 0.02 mg/kg dose would lead to a doubling of next dose until twitch suppression. The ED^{50}dose was based on a log-probit analysis with a historic slope estimate of 7.0. Subject 2: three doses of estimated ED^{25}, ED^{50}, and ED^{75}. Subject 3: doses of ED^{50}, ED^{75}, ED^{90}. Subjects 4–11: doses of estimated ED^{25}, ED^{50}, ED^{75}, ED^{90} Part 2: Safety and pharmacodynamics of ascending multiples of ED^{95}doses in 20 volunteers	ED^{95} was 0.19 ± 0.014 mg/kg Onset time of maximum block at the adductor pollicis ranged from 2.6 ± 0.3 to 1.5 ± 0.3 min for doses 0.18 mg/kg (ED^{95}) to 0.72 mg/kg (4 × ED^{95}). Time to 90% block was 2.1 ± 0.7 min and 1.3 ± 0.2 min for the above doses Clinical and total durations were 4.7–10.1 min and 9.9–16.1 min, respectively, for doses of 0.18–0.72 mg/kg 5–95% recovery rate was 7 min And 25–75% recovery rate was 3 min for all doses of GW280430A	Onset time of gantacurium block is quick but dose dependent Time to recovery from NMB is short and does not increase with increasing dose of gantacurium
Heerdt et al. (2004)	Dose escalation study for cardiopulmonary side effects of GW280430A Adult male beagle dogs (n = 10)	Grp1: Potency of GW280430A was assessed by incremental bolus doses starting at 0.01 mg/kg until 100% block Grp-2: GW280430A was first administered as a bolus of 2 × ED^{95} determined in group 1. At 90% twitch recovery, an infusion of 0.010 mg/kg/min was initiated. The infusion rate was then titrated to establish a stable 90–95% block of twitch and discontinued after 60–90 min. After a 30-min stabilization period, a normal response to TOF was verified, and cardiopulmonary side effects of GW280430A were determined by injecting incrementally larger boluses at 12-min intervals, starting with 0.2 mg/kg. An adverse response was a ≥ 10% change in the observed cardiopulmonary variables. ABG samples were obtained for histamine analysis before and 1 min after each dose,	Infusion dose required to produce 90–95% NMB was 0.010 mg/kg/min ED^{95} ranged from 0.049 to 0.082 mg/kg (mean 0.064 mg/kg). At ED^{95}, onset of NMB ranged from 90 to 128 s (mean, 107 s), with a duration of 3.2–6.2 min (mean, 5.2 min) At 3 × ED^{95}, onset ranged from 44 to 74 s (mean, 58 s), with a duration of 4.7–8.5 min (mean, 7 min). Infusion rates required to produce 90–95% NMB ranged from 0.009 to 0.015 mg/kg/min (mean, 0.012 mg/kg/min) In the two dogs that received a 60-min infusion, single-twitch height returned to baseline after 5.1 and 3.9 min, respectively. In dogs receiving a 90-min infusion, single-twitch height returned to baseline in 3.2 ± 0.3 min. No changes in peak inspiratory	Gantacurium has no hemodynamic effect until a dose ≥ 25 times the ED^{95} is administered as a rapid intravenous bolus. This effect is transient, may be the result of histamine release with secondary systemic vasodilation, and is not accompanied by changes in peak inspiratory pressure or pulmonary compliance.

Table 1 Analysis of in vivo studies involving novel, quick-onset NMBAs and reversal agents facilitating their quick-offset: the on-off switches *(Continued)*

Name (year)	Study type, study subjects	Methods	Result	Remark/conclusion
		regardless of evidence of hemodynamic effects	pressure or pulmonary compliance occurred during the infusion. A modest increase in heart rate from 138 ± 6 to 157 ± 6 beats/min occurred	
Sunaga et al. (2010a)	In vivo study Urethane anesthetized male Hartley guinea pigs ($n = 6 \times 5 = 30$)	Guinea pigs were tracheostomized and ventilated with continuous digital recordings of pulmonary inflation pressure (PIP) and HR. The ED^{95} for NMBAs was defined. Transient and reproducible changes in PIP and HR were recorded after vagal stimulation or IV acetylcholine before and after pretreatment with escalating doses of gantacurium, CW002, cisatracurium/single dose of rapacuronium.	ED^{95} for gantacurium, CW002, cisatracurium, and rapacuronium was 0.064, 0.012, 0.10, and 0.31 mg/kg, respectively. Gantacurium, CW002, and cisatracurium had no effects on baseline pulmonary inflation pressures and were devoid of significant interactions with M2 and M3 muscarinic receptors in vivo	Gantacurium and CW002 are devoid of airway muscarinic receptor (M3; bronchial smooth musculature) effects at doses several times higher than ED^{95}
Heerdt et al. (2016)	Dose escalation clinical trial in healthy human volunteers ($n = 34$)	Each group received a fixed CW002 dose (0.02, 0.04, 0.06, 0.08, 0.10, and 0.14 mg/kg) BP, HR, and airway dynamic compliance monitored; NMB assessed with mechanomyography at adductor pollicis ABG before and after CW002 injection for plasma histamine Potency estimated from a baseline sigmoid Emax model.	ED50 was 0.036 mg/kg ED^{95} was 0.077 mg/kg (95% CI, 0.044 to 0.114 mg/kg). At 0.14 mg/kg ($1.8 \times ED^{95}$), 80% twitch depression occurred in 94 s with complete block in 200 ± 87 s Clinical recovery (25% of maximum twitch) occurred in 34 ± 3.4 min, with a 5 to 95% recovery interval of 35.0 ± 2.7 min. Time to TOFr > 0.9 was 59 to 86 min. No histamine release < 10% change in blood pressure, heart rate, and dynamic airway compliance.	In healthy subjects on sevoflurane/N_2O, CW002 ($1.8 \times ED^{95}$) produces a clinical duration of action < 40 min, no histamine release, and minimal hemodynamic and airway compliance changes
Savarese et al. (2018)	High-performance liquid chromatography and mass spectrometry Rhesus monkeys ($n = 17$)	Adduction of CW 1759-50 with L-cysteine was studied in monkeys. ED95 for NMB was established. Spontaneous recovery was compared to reversal by L-cysteine in paired studies of boluses or infusions. Changes in mean arterial pressure and heart rate after very large doses of 15 to 60 × ED^{95} were compared.	The half-time of adduction of L-cysteine to CW 1759-50 in vitro was 2.3 min. The ED^{95} of CW 1759-50 was 0.069 mg/kg; ED^{95} of gantacurium was 0.081 mg/kg Duration of action: CW 1759-50, 8.2 ± 1.5 min; and gantacurium, 7.4 ± 1.9 min; L-cysteine (30 mg/kg) shortened recovery (i.e., induced reversal) from CW 1759-50 after boluses/infusions. Recovery intervals (5 to 95% twitch) ranged from 6.1 to 6.7 min after boluses of 0.10 to 0.50 mg/kg, as well as control infusions Dose ratios comparing changes of 30% in MAP or HR to ED^{95} for NMB (ED 30% **Δ** [MAP or HR]/ED^{95}) were higher for CW 1759-50 than for gantacurium.	CW 1759-50, similar to gantacurium, is an ultra-short acting neuromuscular blocking agent, antagonized by L-cysteine The circulatory effects are much reduced in comparison with gantacurium, warranting a trial in humans.
Kaullen et al. (2018)	Ascending dose study in healthy human volunteers under propofol–sevoflurane anaesthesia ($n = 34$)	Population pharmacokinetic/pharmacodynamic models developed using plasma drug concentration data from a previously published dose–response study. Subjects were from five different dose cohorts (receiving 0.04, 0.06, 0.08, 0.10, and 0.14 mg/kg, respectively). Serial arterial plasma concentrations and	A four-compartment model was fit to the concentration–time data; a transit compartment (sigmoid E_{max} model) was fit to the pharmacokinetic/pharmacodynamic data. The population pharmacokinetics of CW002 was linear with very low inter-individual variability in clearance (10.8%). The time to 80% block was 1.5, 0.8, and 0.7	CW002 has predictable pharmacokinetics and is likely to have a rapid onset with an intermediate duration of action at $3 \times ED^{95}$

Table 1 Analysis of in vivo studies involving novel, quick-onset NMBAs and reversal agents facilitating their quick-offset: the on-off switches *(Continued)*

Name (year)	Study type, study subjects	Methods	Result	Remark/conclusion
		muscle twitch heights were recorded.	min for 2×, 3×, 4 × ED^{95} doses, respectively. The simulated 25 to 75% recovery index was dose independent	
L-Cysteine				
Savarese et al. (2010)	In vitro: high-performance liquid chromatography In vivo: monkeys under isoflurane	Comparative reaction half-time for L-cysteine adduction for gantacurium, CW 002, CW011 ED^{95} for twitch inhibition in monkeys was calculated Duration at 4–5 ED^{95} was correlated with reaction half-time for adduction. Speed of L-cysteine antagonism was compared with neostigmine reversal. Potencies of CW 002 and its adduction product were compared to provide a basis for L-cysteine antagonism.	Rate of L-cysteine adduction in vitro (reaction half-time) was 0.2 min, 11.4 min, and 13.7 min for gantacurium, CW002, and CW011 and was inversely related to duration of block. CW002 and CW011 were longer-acting than gantacurium (28.1 and 33.3 min vs. 10.4 min), but only half the duration of cisatracurium. Cysteine adduct of CW002 was 70 times less potent than CW002. IV L-Cysteine (10–50 mg/kg) given 1 min after 4–5 ED^{95} doses of gantacurium, CW002, and CW011 abolished NMB within 2–3 min.	L-Cysteine adduction occurs at different rates in olefinic isoquinolinium diester NMBs, with corresponding durations of action. Exogenous L-cysteine is superior to anticholinesterases, inactivating active molecules to rapidly reverse NMB at any time
Sunaga et al. (2010b)	In vivo: dogs (dose: response) ($n = 6 \times 4 = 24$), dogs (toxicology) ($n = 16$)	Six anesthetized dogs were each studied four times recording muscle twitch, HR, and IBP; after CW002 (0.08 mg/kg or 9 × ED^{95}), time to spontaneous muscle recovery was determined. CW002 was then injected again followed 1 min later by 10, 20, 50, or 100 mg/kg L-cysteine. After twitch recovery, CW002 was given a third time to determine whether residual L-cysteine influenced duration. Additional group of dogs received CW002 followed by vehicle/200 mg/kg L-cysteine. Dogs were awakened and observed for 2–14 days before sacrificing for analyses	L-cysteine at all doses accelerated recovery from CW002, with both 50 and 100 mg/kg decreasing median duration from more than 70 min to less than 5 min. After reversal, duration of a subsequent CW002 dose was reduced in a dose-dependent manner. L-cysteine had less than 10% effect on blood pressure and heart rate. Animals receiving a single 200-mg/kg dose of L-cysteine showed no clinical, anatomic, biochemical, or histologic evidence of organ toxicity.	The optimal L-cysteine dose for rapidly reversing the neuromuscular blockade produced by a large dose of CW002 in dogs is approximately 50 mg/kg, which has no concomitant hemodynamic effect. A dose of 200 mg/kg had no evident organ toxicity.
Calabadion-1; calabadion-2				
Ma et al. (2012a)	NMR spectra and direct and competitive UV/Vis binding assays in vitro Adult male Sprague–Dawley rats ($n = 8$) in vivo	Complete NMB (2X ED90) was induced with rocuronium (3.5 mg/kg). Mechanical ventilation maintained until recovery of spontaneous ventilation. 30 s after onset of complete NMB either placebo or calabadion (30, 60, or 90 mg/kg), administered at maximum twitch depression (T1 = 0)	Determination of binding constants of the two cucurbit[n]urils with NMBs (pancuronium, atracurium, cisatracurium, rocuronium, vecuronium) in vitro resulted in K_a values ranging from 2.4×10^4/M to 8.4×10^6/M Calabadion reverses NMB in rat model	Two acyclic cucurbit[n]uril molecular containers with SO_3^- bind NMBA in vitro Calabadion reverses NMB in vivo
Ma et al. (2012b)	Job plots constructed from ^{1}H NMR experiments	Binding constants determined for the interaction between calabadion-2 and by UV–vis and ^{1}H NMR competition experiments	The K_a values for complexes between calabadion and seven local anaesthetics fall in the range of 10^3 to $10^8 M^{-1}$	Calabadion may reverse local anesthetic toxicity
Hoffmann et al. (2013)	In vivo study: rats ($n = 60$) Calabadion-1 elimination determined by ^{1}H NMR assay.	Rats were anesthetized, tracheotomized, IV, arterial lines placed. After rocuronium (3.5 mg/kg) or cisatracurium (0.6 mg/kg), NMBA was quantified by acceleromyography. Calabadion-1 at 30, 60, and 90 mg/kg (for rocuronium) or 90, 120, and 150 mg/kg	After the administration of rocuronium, resumption of spontaneous breathing and recovery of TOFr to 0.9 were accelerated from 12.3 and 16.2 min with placebo to 4.6 min with neostigmine/glycopyrrolate to 15 and 84 s with calabadion-1 (90	Calabadion-1 causes rapid and complete reversal of the effects of steroidal and benzylisoquinoline NMBA. In healthy rats, calabadion-1 produced a dose-dependent reversal of NMB from cisatracurium and rocuronium without affecting heart rate, blood pressure or

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Name (year)	Study type, study subjects	Methods	Result	Remark/conclusion
		(for cisatracurium), or neostigmine/glycopyrrolate at 0.06/0.012 mg/kg injected at maximum twitch depression. HR, IBP, ABG noted	mg/kg), respectively. After the administration of cisatracurium, recovery of breathing and TOFr of 0.9 were accelerated from 8.7 and 9.9 min with placebo to 2.8 and 7.6 min with neostigmine/glycopyrrolate to 47 and 87 s with calabadion-1 (150 mg/kg), respectively. Calabadion-1 did not affect HR, MAP, pH, carbon dioxide pressure, and oxygen tension. More than 90% of the IV administered calabadion-1 appeared in the urine within 1 h.	arterial blood, gas tensions or pH.
(Sparr et al. 2001; Sagir et al 2014; Haerter and Simons (2015)	In vitro (competition binding assays and urine analysis) Ex vivo (n = 34; phrenic nerve hemidiaphragm preparation) In vivo (n = 108; quadriceps femoris muscle of the rat).	The dose–response relationship of drugs to reverse vecuronium-, rocuronium-, and cisatracurium-induced NMB studied Cumulative dose–response curves of calabadions, neostigmine, or sugammadex were created ex vivo at a steady-state deep NMB. In living rats, the dose–response relationship of the test drugs to reverse deep block was studied Amount of calabadion-2 excreted in urine measured	Calabadion-2 binds rocuronium with 89 times the affinity of sugammadex ($K_a = 3.4 \times 10^9 M^{-1}$ and $K_a = 3.8 \times 10^7 M^{-1}$) Sugammadex and calabadion-2 have 1:1 binding ratio for rocuronium. The molar potency of calabadion-2 to reverse vecuronium and rocuronium was higher compared with that of sugammadex. Renal elimination of calabadion-2 No effect on blood pressure /heart rate	Calabadion-2 reverses benzylisoquinolines and steroidal NMBAs in rats faster than sugammadex. Calabadion-2 is renally eliminated and well tolerated in rats.
Ganapati et al. (2016)	Binding constants determined by direct or competitive UV/Vis assays or direct ^1H NMR titrations Simulation of in vivo equilibria using modeling software Gepasi.	Binding constants determined for the interaction between calabadion-2 and 27 commonly used drugs, drug dosages in the rat model, estimated plasma concentrations, and binding constants toward calabadion-1	Weak-binders ($K_a < 105\ M^{-1}$): neutral/anionic drugs (diclofenac, acetaminophen, chloramphenicol, aminophylline), highly hydrophilic drugs (tetracycline, kanamycin, doxycycline, vancomycin) and zwitterionic drugs (amoxicillin, cefepime) Stronger-binders ($K_a > 105\ M^{-1}$): hydrophobic polycyclic cations (morphine, naloxone, atropine) and aromatic ammonium ions (dibucaine, propranolol, imipramine) Strongest binders: procaine, succinylcholine	Neither the binding affinity nor the standard dosages of the drugs were high enough to displace NMBA from its calabadion-2 container.
Diaz-gil et al. (2016)	Sprague-Dawley rats (n = 60) Swiss Webster mice (n = 35)	Initial bolus etomidate over 40 s till BSR of 70%, then infusion rate of 0.1–0.3 mg kg^{-1} min^{-1} Either a stepwise increasing calabadion-2 infusion of 40, 60, 80, and 100 mg kg^{-1} min^{-1}over 5 min each (n = 10) or a 20-min saline infusion of equivalent total fluid volume (n = 3) 4 mg/kg etomidate bolus/30 mg/kg ketamine followed by calabadion 80 mg/kg	Dose-dependent reversal of effects of ketamine and etomidate on electroencephalographic predictors of depth of anesthesia, drug-induced hypotension, time to recovery of righting reflex, and functional mobility. Therapeutic index, 16:1 and 3:1 for ketamine and etomidate	Calabadion-2 reverses etomidate and ketamine anesthesia at non-toxic doses It does not reverse propofol, isoflurane
WP[6]				
Zhang et al. (2019)	Female Balb/c mice (8–10 weeks old) 7 groups (n = 6 each) WP[6] vs SC[4]A vs CB[7] vs saline placebo (control group)	Firstly, IV Sch (0.75 mg/kg) Then, IV WP[6] (doses of 10, 20, and 50 mg/kg) or SC[4]A (dose of 20 and 50 mg/kg) or CB[7] (100 mg/kg) or IV saline (100 ml/20 g) immediately.	In rats treated with WP[6] (20 mg/kg or 50 mg/kg) at 30 s after IV Sch, the mean serum potassium level in these rats kept steady 2-fold increase in creatine kinase (serum biomarker of muscular	Supramolecular therapeutics to treat the side effects of Sch

Table 1 Analysis of in vivo studies involving novel, quick-onset NMBAs and reversal agents facilitating their quick-offset: the on-off switches *(Continued)*

Name (year)	Study type, study subjects	Methods	Result	Remark/conclusion
	Rats	24 h survival recorded. Sacrificing mice after 2 weeks noting hematological parameters and organ damage i.m. (via the right anterior tibia muscle) WP[6] 30 s after i.m. Sch	damage) in control group 15 min post SCh but no rhabdomyolysis in WP[6] Grp	

Akin to cisatracurium and atracurium inactivation by Hofmann elimination, gantacurium is metabolized by spontaneous cysteine adduction (fast process) and pH-sensitive hydrolysis (slow process). Former is independent of the liver, kidneys, pH, or temperature. Endogenous L-cysteine replaces the chlorine moiety of gantacurium producing a heterocyclic ring which does not interact with the post-junctional acetylcholine receptors (Lien et al. 2009).

Exogenous L-cystine enantiomer is an essential constituent of parental nutrition. A bolus dose of 10–50 mg/kg for reversal of gantacurium-induced NMB has no known toxicity. In preclinical trials, L-cysteine administered just 1 min after 8 × ED^{95} of gantacurium reduced the recovery to a TOFr ≥ 0.90 by 6 min without any signs of residual NMB or recurarization. These results need clinical validation. Metabolites of gantacurium lack neuromuscular properties with no hepatorenal elimination (Savarese et al. 2010; Boer and Carlos 2018; Heerdt et al. 2015; Lien et al. 2009).

Cholinesterase inhibitor edrophonium has a peak effect of less than 2 min (Savarese et al. 2010). In humans, edrophonium reduces the reversal time of a gantacurium-induced NMB at 10% recovery of twitch 1 to a TOFr ≥ 0.90 to 3. 8 min. It took 7.5 min for spontaneous reversal of the same NMB. Peak effect being 7–11 min, neostigmine is not suitable for reversal of a gantacurium-induced neuromuscular block (Savarese et al. 2010).

Tables 2 and 3 provides a comparative analysis of early onset NMBAs at different doses expressed as multiples of ED^{95} (effective dose of NMBA required to reduce twitch height by 95%). Intubating dose is roughly twice the ED^{95}. Block onset time is inversely proportional to NMBA potency. Histamine release by chemical (anaphylactoid) or immunologic (anaphylactic) mechanisms is clinically indistinguishable. NMBAs are the most frequently implicated class of drugs with succinylcholine being the commonest culprit in intraoperative anaphylaxis (Ezzat et al. 2011; Naguib et al. 1995; Spoerl et al. 2017).

CW002

CW002 (Table 1) is a quick-onset intermediate-acting, tetrahydroisoquinolinium nondepolarizing NMBA with minimal cardiopulmonary effects currently undergoing clinical trials (Savarese et al. 2010; Boer and Carlos 2018; Heerdt et al. 2016). It differs from gantacurium in lacking a chlorine-moiety at the fumarate double bond. CW002 undergoes pH-dependent L-cysteine adduction and ester hydrolysis and can be reversed at any depth by exogenous cysteine injection (Boer and Carlos 2018; Heerdt et al. 2016). To date, no human study on exogenous L-cysteine reversal of CW002 exists, but in Rhesus monkeys L-cysteine 50 mg/kg resulted in a reversal of neuromuscular block within 2–3 min when administered 60 s after 4–5× ED^{95}

CW011

This is a non-halogenated olefinic diester congener of gantacurium with a similar onset but intermediate duration of action (Savarese et al. 2010; Boer and Carlos 2018). L-cysteine dose required is 50 mg/kg for antagonism of CW011 as against 10 mg/kg for gantacurium antagonism since chlorine (halogen) substitution in gantacurium is a powerful accelerator of L-cysteine adduction (short t1/2 of 0.2 min) as against t1/2 of 11.4 and 13.7 min in CW002 and CW011 respectively. This explains both the ultrashort duration of onset and offset of gantacurium (Savarese et al. 2010; Boer and Carlos 2018).

CW1759-50

This rapid-onset, ultrashort-acting NMBA claims a superior clinical profile to gantacurium and entails reduced hemodynamic perturbations (Table 1). The half-time of adduction of L-cysteine to CW 1759-50 in vitro is 2.3 min. The ED^{95} of CW 1759-50 is 0.069 mg/kg which is similar to that of gantacurium (0.081 mg/kg) (Savarese et al. 2018). Human clinical trials on this promising agent are recommended.

Pinnatoxins and 20-methylspirolide-G (20-meSPX-G)

Derived from marine planktons and dinoflagellates, pinnatoxins (macrocyclic imines) and 20-meSPX-G (cyclic amine) are nAchr competitive antagonists, targeting embryonic (α1)2βγδ and adult (α1)2βεδ skeletal muscle neuromuscular junction receptors (Delcourt et al. 2019; Couesnon et al. 2016). 20-meSPX-G is 75 times more potent than d-tubocurarine (Couesnon et al. 2016). In mouse bioassays, the action of 20-meSPX-G is fully

Table 2 Comparative analysis of early onset NMBAs at different doses expressed as multiples of ED^{95} (effective dose 95)

Drug (ED^{95})	Dose	Onset time	Histamine (Savarese et al. 2004; Belmont et al. 2004)	Duration of action (recovery to TOFr ≥ 0.90) reversal/recovery
Succinylcholine (Heerdt et al. 2004) (0.3 mg/kg)	0.45 mg/kg	65 s	Yes	5 min (Spont. recovery)
	0.6 mg/kg	55 s	Yes	6.4 min (Spont. recovery)
	3–4 ED^{95}, 1 mg/kg	45 s	Yes	12.5 min, hyperkalemia, bradycardia, masseter muscle spasm, fasciculations, postoperative myalgia, ↑IOP
Mivacurium (Lien 2013; Diefenbach et al. 1995) 0.06–0.08 mg/kg	2–3 ED^{95}	2.5 min	Yes	10–12 min (withdrawn from the US market 2006), erythema, hypotension, bronchospasm
	3 ED^{95}	NA	Yes	22 min (Rh)
Rapacuronium (ORG-9487) (Blobner et al. 2000; Sunaga et al. 2010a; Heerdt et al. 2016) 1 mg/kg	1.5 ED^{95}	60–90 s	No	< 20 min, withdrawn from market (2001): fatal bronchospasm
	1.5–2.5 ED^{95}	50–60 s	Yes	20 min, tachycardia, hypotension, and bronchospasm
Cisatracurium (Savarese et al. 2018) 0.05 mg/kg	3–4ED^{95}	5–6 min	No	30–45 min (Hoffman degradation) Not affected by hepatic/renal disease
Rocuronium (De May et al 1994; Sunaga et al. 2010b; Chavan et al 2016, Kaullen et al. 2018) 0.3 mg/kg	1–1.5× ED^{95}	60–180 s	No	18–20 min (Spont) Duration prolonged in liver disease
	2× ED^{95}	60–90 s	No	3.75 times SCH; 3.75 × 6 min (Spont)
	3–4× ED^{95}	50–60 s	No	7.5 times SCH; RSI dose; reversible by high dose sugammadex, as early as 3 min post-administration
Gantacurium (Savarese et al. 2010; Boer and Carlos 2018; Heerdt et al. 2015; Lien et al. 2009) (0.19 mg/kg) Not available for clinical use	2× ED^{95}	70 s	No	15 min (Spont.)
	2.5× ED^{95}	60 s	No	8 min (Spont. recovery identical to Sch) (Lien et al. 2009)
	4–5× ED^{95} (rhesus)	60 s	No	10.4 min (Spont.) 2–3 min(L-cysteine 50 mg/kg 1 min after gantacurium)
	4–5× ED^{95}	90 s	Yes	15 min (Spont) study on human volunteers (Ma et al. 2012a)
CW002 (Savarese et al. 2010; Boer and Carlos 2018; Ma et al. 2012b) (0.077 mg/kg) Not available for clinical use	1.8 x ED^{95}	90 s	No	33.8 min (clinical duration of action), 73 min (spont. reversal; TOFr 0.9), minimal cardiopulmonary effects
	4–5×ED^{95} (rhesus)	NA	Yes	2–3 min with L-cysteine (50 mg/kg) given 60 s after CW002
CW011 (Savarese et al. 2010; Boer and Carlos 2018) (0.025 mg/kg); not clinically used	4–5×ED^{95} (rhesus)	NA	Yes	20.8 min (clinical duration of action) 2–3 min with L-cysteine(50 mg/kg) reversal

IV intravenous, *MG* Myasthenia gravis, *S/E* side effects, *TOF* train of four, *TOFR* train of four ratio

reversible producing muscle paralysis but no lethality (Couesnon et al. 2016). A new class of nondepolarizing NMBAs may emerge from here.

Structure activity relationships

The active site (anionic binding region) of postsynaptic nicotinic acetylcholine receptors (nAchR) is similar to that of acetylcholinesterase, and both require a quaternary amine to bind with it. A succinylcholine molecule comprises two acetylcholine molecules linked together because two anionic binding sites of each nAchR need to be simultaneously occupied by two acetylcholine molecules for channel opening (Fig. 2) Succinylcholine and many nondepolarizing NMBA (pancuronium, atracurium) are bis-quaternary ammonium compounds with two quaternary ammonium nitrogen (one for each anionic binding site of nAchR) bridged by 10–12 carbon atoms (for maximal potency). Monoquarternary aminosteroid NMBAs are less potent but have faster onset. Neostigmine is also a quaternary ammonium compound, and hence, it binds acetylcholinesterase. Acetylcholine is hydrolyzed within 100 μs whereas neostigmine acts as a

Table 3 Classification, dose, and side effects of reversal agents

Class of reversal agent	Name of drug Duration of action	Dose	Side effects	Remarks
Carbamate group AntiAch esterase (Ganapati et al. 2016)	Neostigmine 0.5–2 h	TOF count < 1: do NOT give neostigmine TOF count 2–4 (tactile/visual fade; TOFR < 0.4): 0.05–0.07 mg/kg TOF count = 4 (no tactile/visual fade; TOFR = 0.4–0.9): 0.02–0.03 mg/kg TOFR is ≥0.9: do NOT give neostigmine	Residual block, muscarinic S/E, (bradycardia, arrhythmias, salivation, bronchospasm, increased airway secretions, nausea, vomiting, diarrhea, micturition)	Does not cross blood-brain barrier MG treatment Paralytic ileus Urinary retention
	Pyridostigmine 3–6 h			MG treatment
Alcohol group AntiAch esterase	Edrophonium 5-15 min			MG diagnosis
Gamma Cyclodextrin	Sugammadex Renal excretion after 24 h	TOF count = T_2: 2mg/kg Post-tetanic count = 1–2: 4 mg/kg 3 min post-IV rocuronium (1.2 mg/kg): 16 mg/kg	Marked bradycardia and cardiac arrest, coagulopathy, anaphylaxis, contraceptive failure	Approved in Europe (2008), Japan (2010), Australia, Middle East, US (2015)
Non-essential amino acid	L-Cysteine Adduct-hydrolysis 300 min: gantacurium 60 min: CW002 60 min: CW011	10–50 mg/kg 1 min after 8× ED^{95} gantacurium		
Cucurbit[n]urils	Calabadion-1 90% renal; 1 h	60 mg/kg (rocuronium rat) 120 mg/kg (cisatra; rat)	Not significant	
	Calabadion-2 69% and 42% renal excretion after 1 h	5–10 mg/kg (rat) 40–80 mg/kg (rat)	Not significant	Not yet available for clinical use

IV intravenous, *MG* Myasthenia gravis, *S/E* side effects, *TOF* train of four, *TOFR* train of four ratio

competitive inhibitor of acetylcholine and is hydrolyzed in minutes (40×10^6 times slower). Sugammadex and calabadion are encapsulating reversal agents (Fig. 3).

Five important questions need evidence-based answers before we discuss newer reversal agents.

1. Should a peripheral nerve stimulator (PNS) be used as guide in all patients given NMBA?
2. Should we reverse of NMBA at the end of surgery?
3. What is the optimal timing of reversal agent?
4. What is the optimal dose of reversal agent?
5. Which kind of reversal agent should we use?

There is a convincing evidence that if the anesthesiologists do not reverse NMBA with a reversal agent, it will be translated into a high incidence of post-operative residual NMB. In one study on 568 consecutive patients, 42% of patients in whom vecuronium-induced NMB was not reversed with an anticholinesterase displayed TOFr< 0.7 on postoperative ward arrival (Baillard et al. 2000). Similarly, 57% of patients who received cisatracurium ($2 \times ED^{95}$) and 44% of those receiving rocuronium ($2 \times ED^{95}$) and were not reversed had TOFr < 0.9 on reaching SICU (Maybauer et al. 2007).

Alarmingly, 89% of elderly patients displayed postoperative residual NMB after intraoperative rocuronium administration in one study (Pietraszewski and Gaszyński 2013). Even reversal with sugammadex, if lacking peripheral nerve stimulation (PNS) guidance, does not guarantee protection from residual NMB (Kotake et al. 2013). Even 2 h after a single bolus dose of intermediate-acting NMBA (vecuronium, rocuronium, atracurium), 45% out of 526 consecutive patients showed TOFr< 0.9 when a reversal agent was avoided (Debaene et al. 2003). NMBA residual weakness of the jaw and tongue may cause retention of secretions, aspiration, and pneumonia (Grayling and Sweeney 2007).

Five-second head raise, tongue-protrusion, eye-opening, coughing, and adequacy of tidal volume are frequently used qualitative predictors of recovery from

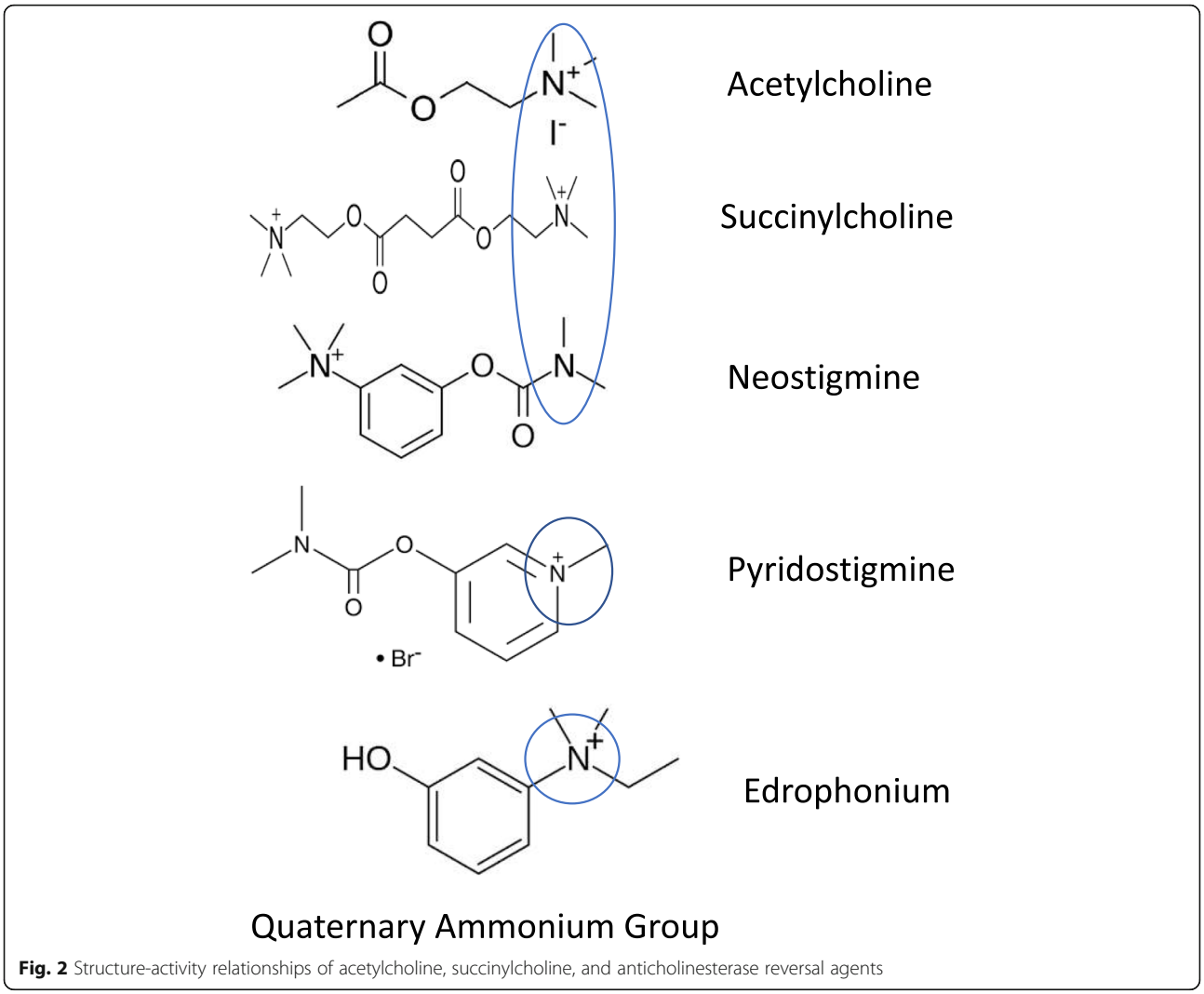

Fig. 2 Structure-activity relationships of acetylcholine, succinylcholine, and anticholinesterase reversal agents

NMBA but cannot exclude clinically significant residual curarization (TOFr 0.5–0.9) (Hemmerling and Le 2007; Hunter 2017). Although most specific, a sustained tongue depressor test has poor sensitivity (18%) for predicting TOFr< 0.9 (Rodney et al. 2015). Measuring grip strength of the dominant hand using electronic hand dynamometer gives a strong correlation (0.89) with TOFr without being distressing to the awake patient (Pei et al. 2019).

Hence, services of a neuromuscular monitor (peripheral nerve stimulator provides only a qualitative assessment) are imperative for quantitative assessment of depth of NMB towards the end of surgery (Brull and Kopman 2017; Gelb et al. 2018). Electromyography is the gold standard, followed by mechanomyography. Nevertheless, acceleromyography and kinemyography (both piezoelectric crystal based) command better clinical utility (Brull and Kopman 2017). Orbicularis oculi and corrugator supercilii, being more centrally located than adductor pollicis, are now being recommended as the preferred site of monitoring NMB onset, for their more faithful representation of NMB onset in the airway musculature (Lien 2013).

Now, tackling the second question, if TOFr is 0.9, then a reversal agent is not required at all. Moreover, if neostigmine or other anticholinesterases are administered at this juncture, it is proved to be not just useless but also counterproductive. Neostigmine may potentially cause reduced genioglossus muscle activity causing increased upper-airway collapsibility in response to negative pharyngeal pressure, recurarization, and upper-airway muscle weakness if given at TOFr 0.9–1, probably by depolarizing or open-channel neuromuscular block and Ach-receptor desensitization (Herbstreit et al. 2010; Eikermann et al. 2008).

Neostigmine dose exceeding 0.07 mg/kg has a ceiling effect. Moreover, excessive neostigmine may precipitate cholinergic crisis with attendant muscle weakness. A decade back, it was realized that neostigmine should be administered after appearance of at least two TOF

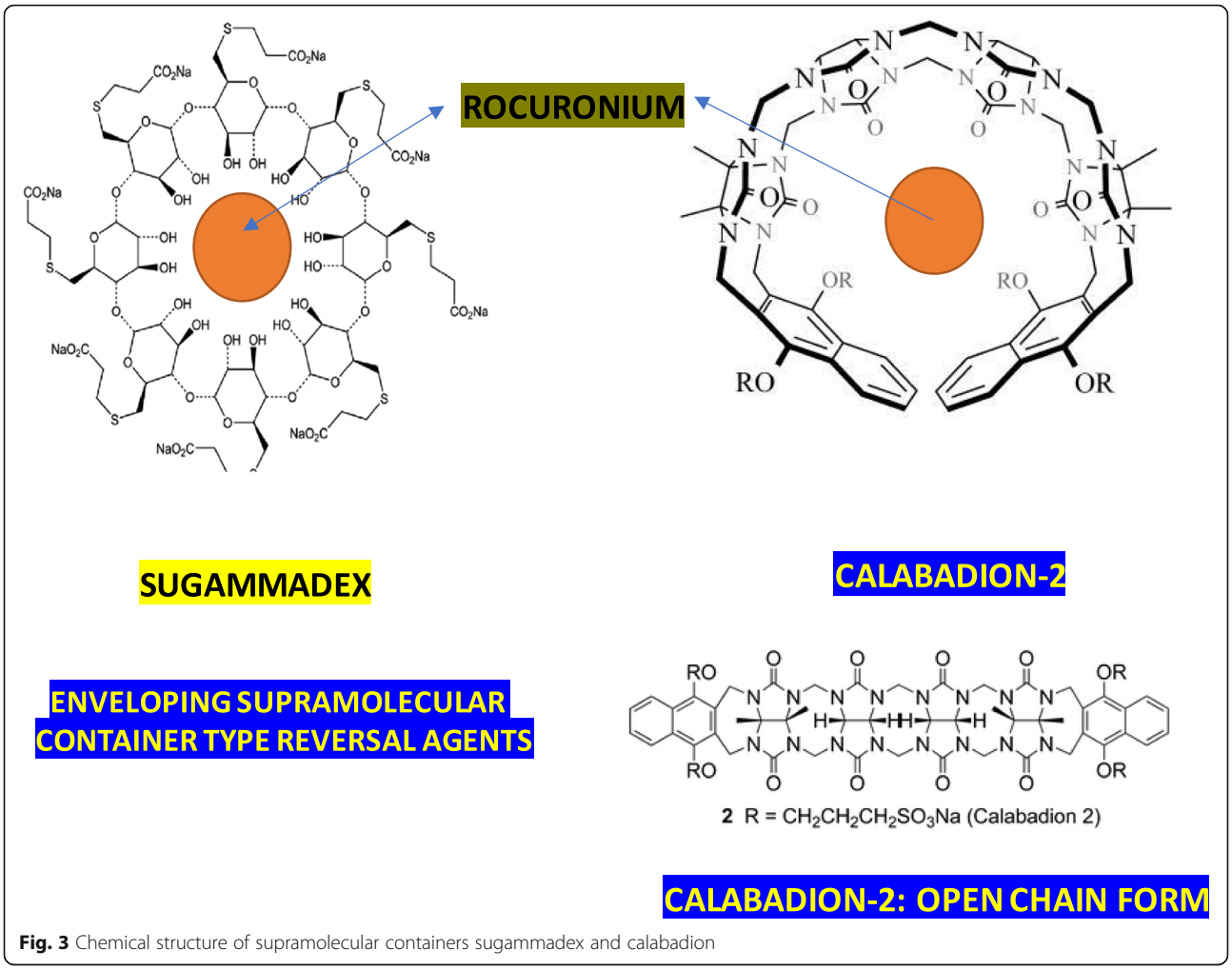

Fig. 3 Chemical structure of supramolecular containers sugammadex and calabadion

twitches to reduce postoperative residual paralysis (Brull and Murphy 2010). It is showed that if neostigmine is given at a TOFr of 0.4, then it will assure TOFr 0.9 within 10 min, but not if it is administered earlier (Song et al. 2015). Hence, ideally, neostigmine should be administered only in the window period of TOFr 0.1–0.8, neither before appearance of all four TOF twitches, nor after TOFr 0.9 is achieved. Without PNS availability, making such a fine distinction is difficult. Moreover, time-pressure, anesthesia time, and monetary and human resource factors may prohibit keeping the OT table occupied indefinitely after end of surgery waiting for a spontaneous reversal of NMB. Also, a deeper plane of NMB is required for endoscopic surgery, foreign body removal, and minimally invasive surgery (laparoscopic/robot assisted) although the keyhole port incisions ensure a speedy closure at the end of surgery giving very little time for spontaneous reversal of NMB. A deeper block improves the surgeon satisfaction score (Blobner et al. 2015) by allowing better anatomical exposure at reduced insufflation pressures and avoiding catastrophic patient movement with robotic arms docked. Here comes the role of reversal agents that are direct-acting supramolecular containers: sugammadex and calabadions that can quickly reverse any depth of NMB. The trident of speed, reliability, and safety summarizes the goal for reversal agents.

Sugammadex (gamma cyclodextrin; Bridion; Merck)

Unlike neostigmine, which indirectly reverses NMBA block by increasing acetylcholine concentration at the neuromuscular junction, sugammadex directly inactivates steroidal nondepolarizing NMBAs by effective encapsulation. Sugammadex (2 mg/kg) provides faster reversal (2.7 min versus 17.9 min) of vecuronium-induced neuromuscular blockade compared with neostigmine (50 μg/kg) (Khuenl-Brady et al. 2010). Sugammadex is equally effective in reversing rocuronium-induced block regardless of propofol or sevoflurane anesthesia (Vanacker et al. 2007).

Time to recovery (TOFr0.9) after 2 mg/kg sugammadex administered on appearance of second twitch is 1.9 min and 2.9 min for rocuronium and vecuronium respectively. Similarly, time to recovery after 16 mg/kg sugammadex

administered 3 min after 1.2 mg/kg rocuronium is just 1.7 mins (Herring et al. 2017).

Time to spontaneous recovery of first twitch after a single bolus dose of rocuronium was 17 min versus 24 min after rocuronium infusion in most patients. Some patients took 70 min after discontinuing infusion for spontaneous recovery to a TOFr of 75% highlighting the importance of a reversal agent in all cases (Jellish et al. 2000). The additional cost of using sugammadex was estimated at $77/person when compared to neostigmine/glycopyrrolate combination in one study (Money et al. 2019).

Sugammadex 1.0 mg/kg, but not 0.5 mg/kg, adequately reversed a vecuronium-induced NMB at threshold TOF-count of four but without preventing recurarization (Asztalos et al. 2017). Under-dosing of sugammadex as a potential cost-saving strategy in reversal of deep NMB is not recommended as transient success can transcend into disaster like post-operative residual curarization with attendant respiratory complications. Although in use for a decade in Europe and Japan, sugammadex was rejected thrice by the US-FDA on grounds of allergic (Miyazaki et al. 2018; Menéndez-Ozcoidi et al. 2011) and hemorrhagic complications before being accepted in December 2015, despite a relatively high anaphylaxis rate of 1/2580 patients (0.39%) (Miyazaki et al. 2018). Sugammadex prolongs activated partial thromboplastin time and prothrombin time and may cause oral-contraceptive failure (Rahe-Meyer et al. 2014). Potential litigation over side effects is a concern. Although chronic dexamethasone administration induces resistance to NMBAs by augmenting surface and junctional nAchR density, it does not augment sugammadex reversal of rocuronium (Oh et al. 2019).

L-Cysteine

Gantacurium undergoes chemical degradation involving nonessential amino acid, "cysteine" adduction to its central fumarate double bond. In this Michael-type addition reaction, cysteine replaces chlorine to form a heterocyclic ring between the two quaternary heads of gantacurium forming a cysteine adduct with minimal neuromuscular blocking effect (Lien et al. 2009).

Exogenously administered L-cysteine (Sigma-Aldrich, St. Louis, MO; 98% purity; 10–50 mg/kg) (Savarese et al. 2010) can reverse any depth of gantacurium, CW002 and CW011 blockade. L-Cysteine adduction half-life calculated at gantacurium (200 g/ml), CW002 (100 g/ml), and CW011 (50 g/ml) (4:2:1 relative potency ratio) was 0.2 min, 11.4 min, and 13.7 mins respectively, in rhesus monkeys (Heerdt et al. 2015). Cysteine-adduct hydrolysis time was estimated to be 300 min for gantacurium-cysteine adduct and 60 min each for CW002and CW011 respectively.

Calabadions (cucurbit[n]urils *n* = 5, 6, 7, 8, 10)

Professor Lyle Isaacs established Calabash Biosciences after developing a novel group of molecular containers called calabadions to satisfy the market demand of US anesthesiologists.

Calabadion-1

This is an acyclic, glycoluril, tetrameric, cucurbituril container. Hoffman et al. (2013) administered Calabadion-1 at 30, 60, and 90 mg/kg (for rocuronium; 3.5 mg/kg) and 90, 120, and 150 mg/kg (for cisatracurium 0.6 mg/kg), or neostigmine/glycopyrrolate (0.06/0.012 mg/kg) in rats. The recovery time to TOFr 0.9 was 16.2 min for placebo, 4.6 min for neostigmine, and 84 s for calabadion-1. Cardiopulmonary parameters and blood pH were unaltered. Ninety percent of calabadion-1 was renally excreted within an hour. Calabadion-1-rocuronium complex (K_a = 8.4 ± 0.9 × 10^6/M) has a comparable binding-constant (affinity) with that of sugammadex-rocuronium complex (K_a = 1.1 ± 0.2 × 10^7/M), but the binding-constant for calabadion-1-cisatracurium complex is 10-times lesser. Calabadion-1-acetylcholine complex has a binding-constant 350 times smaller than that for calabadion-1-rocuronium. Akin to its predecessor cucurbituril (Haerter and Simons 2015), calabadion-1, forms stable host–guest complexes with local anesthetics in vitro (Ma et al. 2012b).

Calabadion-2 (Calabash Bioscience, Inc. College Park, Maryland)

Haerter et al. (2015) demonstrated through in vitro studies that calabadion-2 (K_a = 3.4 × 10^9 M^{-1}) binds rocuronium with 89 times the affinity of sugammadex (K_a = 3.8 × 10^7 M^{-1}). The results of proton nuclear magnetic resonance urinalysis, competition binding assays, and ex vivo study (n = 34; phrenic nerve hemidiaphragm preparation) obtained in the absence of metabolic deactivation displayed a 1:1 binding ratio of sugammadex and calabadion-2 toward rocuronium. In live rat models (n = 108; quadriceps femoris muscle), calabadion-2 rapidly reversed 2 × ED90 vecuronium-, rocuronium-, and cisatracurium-induced neuromuscular block in a dose-dependent manner much faster than sugammadex. Calabadion-2 exhibited a higher molar potency to reverse vecuronium and rocuronium, versus sugammadex. Calabadion-2 was eliminated via kidneys, was well tolerated, and had no hemodynamic perturbations. One-hour post-intravenous calabadion-2 (40–80 mg/kg), 49% of the drug was detectable in urine while at lower dosage (5–10 mg/kg), 62% of calabadion-2 appeared.

The enhanced target-binding affinity of calabadion-2 is attributable to its larger hydrophobic cavity shaped by two naphthalene walls versus two benzene walls of calabadion-1 (Zhang et al. 2014). Selectivity of

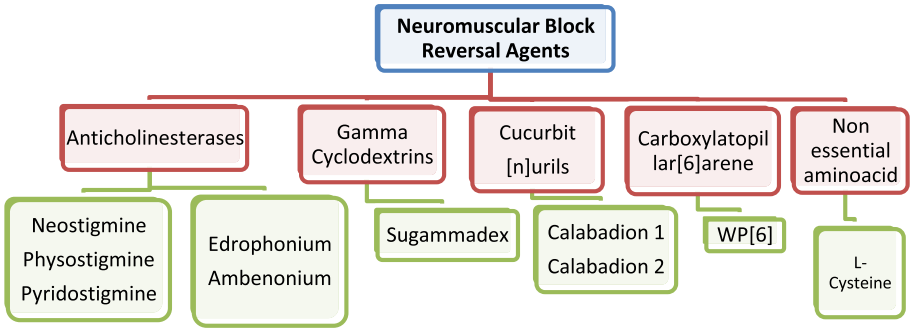

Fig. 4 Classification of neuromuscular block reversal agents

calabadion-2 for rocuronium is 18,900 times that of acetylcholine while that of calabadion-1 is just 350 times that of acetylcholine (same as that of sugammadex).

Ganapati et al. (2016) studied the effect of 27 common drugs (Table 1) on the calabadion-2-NMBA (cisatracurium/rocuronium/vecuronium) complex. Neither the binding affinity nor the standard dosages of these drugs were high enough to displace NMBA from its calabadion-2 container.

Additional benefits of calabadion: a new concept of reversal of general anesthesia

Reversal of general anesthetic induction and maintenance agents and not just NMBAs (Fig. 4) is possible with calabadion-2 potentially translating into time and monetary benefits by slashing operation theater time, reducing postoperative complications, and reversing toxic overdose in hospital and recreational settings.

Experiments on rats (Diaz-Gil et al. 2016) demonstrated that calabadion-2 reverses etomidate and ketamine anesthesia by chemical encapsulation at non-toxic plasma concentrations. Electroencephalographic predictors of depth of anesthesia, drug-induced hypotension, recovery of righting-reflex, and functional mobility were studied. Calabadion-2 neither inhibited the human ether-à-go-go-related channel nor was it mutagenic (Ames test). Based on maximum tolerable dose and acceleration of righting reflex recovery, the therapeutic index of calabadion-2 was 16:1 and 3:1 for ketamine and etomidate reversal respectively.

Calabadions seem potentially useful in additional domains like local anesthetic (including cocaine) toxicity (Grabitz et al. 2015; Isaacs et al. 2018).

Water-soluble carboxylatopillar [6] arene (WP [6])

Zhang et al. (2019) studied the antidotal properties of a supramolecular synthetic receptor WP [6] for succinylcholine-induced hyperkalemia, cardiac arrhythmias, rhabdomyolysis, and paralysis in succinylcholine-overdosed mouse models. They reported a reduced incidence of cardiac arrhythmias, hyperkalemia, and muscular damage when WP [6] was injected immediately after succinylcholine explained by reversal of succinylcholine-induced depolarization and diminished efflux of intracellular potassium. It remains to be seen whether and after how much time WP [6] can reverse succinylcholine-induced paralysis if injected simultaneously with succinylcholine in humans.

Conclusion

Quantitative, objective neuromuscular monitoring should be included in the minimum monitoring standards. Gantacurium is a new promising nondepolarizing NMBA with desirable succinylcholine-like onset and duration of action without its side effects. The broad-spectrum reversal agent calabadion-2 stands out prominently since it does not only reverse any depth of NMB caused by any NMBA, but can also reverse general anesthetic induction agents and local anesthetic toxicity. Human clinical trials should be undertaken on a priority basis to explore these exciting realms.

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Authors' contributions

SS organized the concept; wrote, reviewed, and supervised the manuscript; and designed the figures and tables. CR reviewed and shared author's concepts and supervised the final manuscript. PA reviewed and shared author's concepts. AE reviewed and shared author's concepts and supervised final manuscript. All authors have read and approved the manuscript.

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Competing interests

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