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Review Article

Drug-specific cyclodextrins: the future of rapid neuromuscular block reversal?

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Abstract

Chemical modification of γ-cyclodextrin afforded Org-25969 that has a cavity dimension capable of forming a binary host-guest complex with the steroidal neuromuscular blocker rocuronium bromide with high affinity. In this complex, rocuronium is encapsulated inside the cavity of Org-25969. As a consequence, the neuromuscular blocking activity of rocuronium can be reversed by Org-25969. The reversal produced by Org-25969 is more efficacious than the standard combination of acetylcholinesterase inhibitor and muscarinic receptor antagonist, *e.g.*, neostigmine + atropine. Unlike neostigmine + atropine, Org-25969 does not interfere with the acetylcholine homeostasis. At the effective reversal dose (0.5 μmol/kg i.v.), Org-25969 produced negligible changes in hemodynamic parameters in anesthetized guinea pigs, cats and monkeys. Org-25969 is also effective in reversing profound block induced by 3 times the ED_{90} of rocuronium in guinea pigs at a rate at least 3 times faster than neostigmine + atropine. Therefore, Org-25969 is also potentially useful for early or escape reversal of rocuronium, for instance, in a "cannot intubate, cannot ventilate" situation.

Introduction

The introduction of neuromuscular blockers (NMBs, also known as skeletal muscle relaxants) into anesthetic practice in the 1940s is widely regarded as a major advance in patient management under surgery. Intravenous administration of NMBs causes skeletal muscle relaxation, thus facilitating endotracheal intubation and allowing surgical access to body cavities, in particular the abdomen and thorax, without hindrance from voluntary or reflex muscle movement (1). In recent years, NMBs are often used in day care procedures, such as laparoscopic surgery, microsurgery, open eye surgery, some ear-nose-throat procedures and pediatric procedures (2). In cases where sedation and analgesia alone have proved inadequate, NMBs are also used in the care of critically ill patients undergoing intensive therapy, to facilitate compliance with mechanical ventilation (3-5).

Based on their mechanisms of action, NMBs are divided into two categories: depolarizing and nondepolarizing. Most of the clinically used NMBs are nondepolarizing. These include cisatracurium, mivacurium, pancuronium, vecuronium and rocuronium. They act as competitive antagonists of the nicotinic acetylcholine receptor (nAChR) at the neuromuscular junction. By blocking the acetylcholine-induced activation of the ion channel, nondepolarizing NMBs prevent cell membrane depolarization, and as a result, the muscle becomes flaccid. Depolarizing NMBs act as agonists of the nAChR. They stimulate an initial opening of the ion channel, producing contractions known as fasciculation. However, since these drugs are broken down relatively slowly by cholinesterase enzymes, compared to the very rapid hydrolysis of acetylcholine by acetylcholinesterase (AChE), they bind to the receptor longer than acetylcholine. This causes persistent depolarization and desensitization of the end-plate. Succinylcholine is the only depolarizing NMB that is still in clinical use because of its fast onset and extra short duration of action.

The main reason for the clinical preference of nondepolarizing to depolarizing NMBs is because of the side effects of depolarizing NMBs. Succinylcholine, for instance, has been associated with malignant hyperthermia syndrome, masseter muscle rigidity and bradycardia

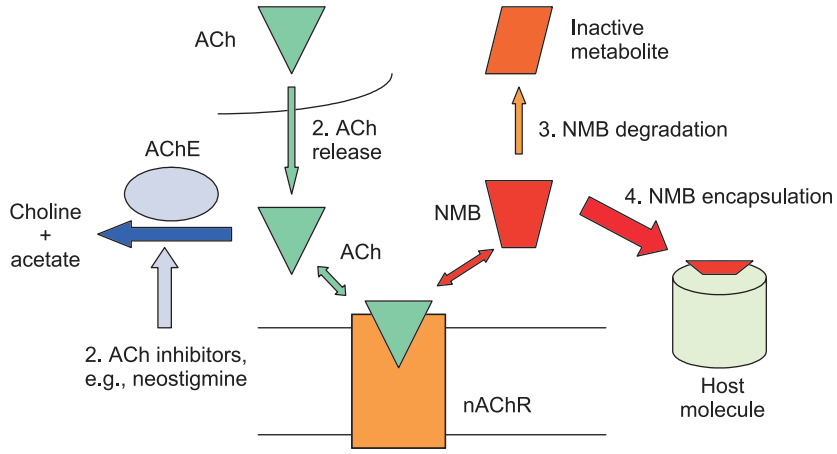

Fig. 1. Molecular mechanisms of NMB reversal. 1) Inhibition of acetylcholinesterase (AChE) increases the acetylcholine (ACh) levels at the neuromuscular junction and gives competitive advantage for ACh to occupy the nicotinic acetylcholine receptor (aAChR) and therefore the recovery of muscle function. 2) Certain potassium channel blockers, *e.g.*, 4-aminopyridine, increase the release of ACh and hence cause NMB reversal. 3) Metabolism or degradation of NMBs to inactive metabolites is partly responsible for spontaneous recovery. 4) Chemical encapsulation of NMB removes NMB from its site of action, leading to the recovery of muscle function.

(6). There have now been moves to limit the use of this drug in children. This is not to say that nondepolarizing NMBs are free of side effects. On the contrary, some nondepolarizing NMBs can cause cardiovascular side effects possibly through their vagolytic and calcium channel blocking activities or nonimmunological histamine release (6).

Therefore, an ideal NMB should have fast onset of block, adequate duration of block with fast recovery, minimum side effects, reasonable cost and capable of reversing profound block.

Since NMBs are only used to facilitate surgical procedures, their action should be reversed at the end of surgery or a period of intensive care to avoid unnecessary exposure, side effects or postoperative complications. Residual paralysis, *i.e.*, impaired laryngeal and pharyngeal muscle function, alterations in hypoxic ventilatory control, reduced margin of safety, *etc.*, has been observed frequently in postoperative patients (7). With the establishment of more rigorous criteria for defining adequacy of neuromuscular recovery (a train-of-four ratio of 0.9 instead of the previous standard of 0.7), residual paralysis becomes a more frequent adverse side effect (8) and is not always easy to recognize clinically. Therefore, the action of neuromuscular blockers should always be reversed at the end of surgery unless there is unequivocal evidence of adequate neuromuscular function.

Molecular mechanisms of neuromuscular block reversal

To reverse the effects of an NMB is to dissociate its binding to the nAChR and recover the transmission by ACh (Fig. 1). Since the receptor occupancy by NMB is competitive in nature, its action can be reversed by either increasing concentration of the competing ACh (only suitable for nondepolarizing NMBs) or decreasing the concentration of the NMB itself (9).

Increase of acetylcholine in neuromuscular junction

All clinically used reversal agents are AChE inhibitors, such as neostigmine and edrophonium, which inhibit the breakdown of acetylcholine so as to increase the level of acetylcholine at the neuromuscular junction and to gain competitive advantage for acetylcholine to bind to the nAChR.

These agents are only suitable for the reversal of nondepolarizing NMBs and should not be used for the reversal of depolarizing NMBs such as succinylcholine. This is because succinylcholine itself is an agonist of nAChR and it is inactivated by cholinesterase. Any attempt to reverse succinylcholine with an AChE inhibitor will cause overstimulation of cholinergic systems and could be potentially life threatening.

The use of AChE inhibitors as NMB reversal agents has two other major drawbacks. First, AChE inhibition causes nonselective potentiation of neurotransmission to all synapses (both somatic and autonomic) involving acetylcholine, especially those in the heart, and leads to many side effects including bradycardia, hypotension, *etc*. Therefore, in practice, these agents are often used in combination with a muscarinic acetylcholine receptor (mAChR) antagonist such as atropine or glycopyrrolate to antagonize the muscarinic effects of acetylcholine in the autonomic parasympathetic neuroeffector junctions (*e.g.*, the heart). Unfortunately, mAChR antagonists themselves cause a number of side effects such as tachycardia, dry mouth, blurred vision, *etc.*, and furthermore may

affect cardiac conduction. Attempts to design "clean" AChE inhibitors without intrinsic mAChR agonistic activity such as neostigmine or with mAChR antagonistic activity have been reported (10-12).

Secondly, AChE inhibitors can only be used when residual neuromuscular activity (>10% twitch activity) is present. Occasionally, either due to hypersensitivity of the patient or accidental overdose, administration of NMBs can cause complete blockade of neuromuscular function (also known as profound block). At present, there is no reliable treatment to reverse such a profound block. Attempts to overcome a profound block with high doses of AChE inhibitors has the risk of inducing a life-threatening "cholinergic crisis", resulting in a broad range of symptoms related to overenhanced stimulation of nAChR and mAChR.

Another mechanism to increase synaptic ACh levels is to inhibit potassium conductance, which prolongs the nerve action potential and produces greater calcium influx resulting in increased release of ACh. 4-Aminopyridine is thought to act in this mechanism and has been studied for possible use as an NMB reversal agent (13). However, side effects such as those in the central nervous system have prevented the introduction of this class of compounds into clinical practice.

Decrease of NMBs in neuromuscular junction

After intravenous administration, NMBs undergo tissue distribution, metabolism and elimination. Eventually, the neuromuscular blocking effects of the NMB will reduce and muscle function recovers spontaneously. Chemical and/or enzymatic degradation to inactive metabolites are important factors for the spontaneous recovery of several NMBs. Atracurium and its pure steric isomer cisatracurium undergo Hofmann elimination to inactive mono-quinoliniums. Succinylcholine and mivacurium are inactivated by plasma cholinesterase. Therefore, it is not recommended to reverse mivacurium by AChE inhibitors although it is a nondepolarizing NMB.

In patients with cholinesterase deficiency, administration of exogenous human serum cholinesterase has been shown to reverse a profound block by succinylcholine or mivacurium (14, 15). However, immunogenic concern and high cost may limit the use of such a reversal enzyme.

Chemical encapsulation of NMB by a synthetic host molecule (or synthetic receptor) offers a completely novel mechanism for NMB reversal (16, 17). Since this mechanism of action does not involve direct interaction with cholinergic systems, it should circumvent the undesired side effects attendant with AChE inhibitors and furthermore might be used for the reversal of profound block. The discovery of an NMB-binding cyclodextrin (Org-25969) and its animal pharmacology will be the main subject of this review and are described below.

Miscellaneous

Several other molecules with less defined mechanisms of action have also been reported to reverse NMBs (9). These include germine monoacetate, suramine and heparin. None of these agents have the desired chemical and pharmacological profiles suitable for introduction into clinical practice.

An ideal NMB reversal agent should have a fast onset of action, long enough duration of action to prevent recurarization, capable of reversing profound block, minimum side effects and reasonable cost.

Cyclodextrins as reversal agents of neuromuscular block

Discovery of Org-25969, a rocuronium-reversing cyclodextrin

Inspired by the advance in supramolecular chemistry and literature reports on host-guest complex formation between charged cyclophanes and steroids, Organon scientists in Scotland designed and synthesized a group of negatively charged cyclophanes in an attempt to chelate positively charged steroidal NMBs such as pancuronium (16). Due to the desire for high water solubility and biological compatibility, the attention was later turned to a well-known group of host molecules, cyclodextrins (CDs) and their ability to reverse the effects of steroidal NMBs such as vecuronium and rocuronium (17). From the early *in vitro* studies (mouse hemidiaphragm and NMR titration), it was observed that the cavity diameter of a cyclodextrin is very important to its reversal activity. For example, to reverse rocuronium it was necessary to have a cavity diameter of γ-cyclodextrin (γ-CD) of 7.5-8.3 Å (17).

Two chemical strategies were used to increase the binding affinity of γ-CD to rocuronium. The first was to extend the cavity depth of γ-CD in order to achieve full encapsulation of all four steroidal rings in the lipophilic cavity. The second was to introduce anionic functional groups, *e.g.*, carboxyls, at the rim of the cavity. It was envisaged that an extended cavity achieved by perfacial substitution of γ-CD with lipophilic groups would not only increase van der Waals interaction between the CD and rocuronium, but also the total hydrophobic area inside the cavity and therefore the improved hydrophobic interaction with rocuronium. The negatively charged groups at the periphery of the CD ring would provide electrostatic interaction with the positively charged nitrogens of rocuronium, and also very importantly to maintain the high water solubility of the resulting host molecule (17).

As illustrated in Figure 2, three key structural modifications led to the discovery of Org-25969, a synthetic cyclodextrin with high affinity to rocuronium ($K_a = 10^7\ M^{-1}$) (18).

A) Identification of propionic acid side chain: Org-25169 (a statistic mixture with a degree of substitution of 3.8 per molecule) was found to reverse rocuronium block in monkeys with efficacy slightly better than that of neostigmine/atropine combination (18).

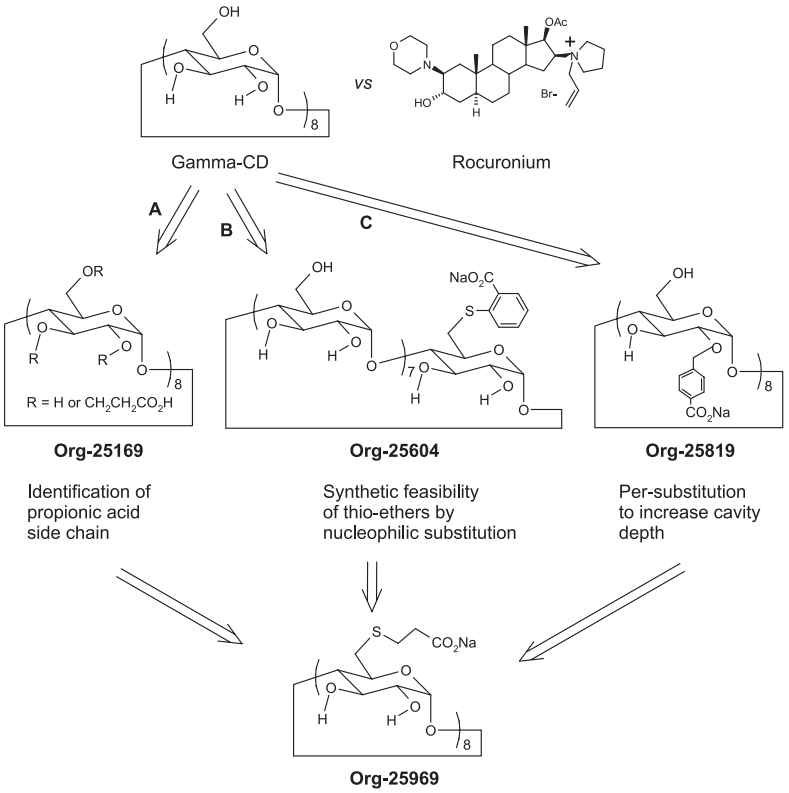

Fig. 2. Discovery of Org-25969. A) Identification of propionic acid side chain such as in Org-25169. B) Synthetic feasibility of 6-thio-ethers such as Org-25604. C) Per-substitution to create an extension of the guest binding cavity such as in Org-25819. Org-25969 is a per-6-substituted γ-cyclodextrin with propionic acid side chains linked via thiol-ethers to the γ-CD ring.

B) Synthetic feasibility of 6-thio-ethers: Org-25604 showed better *in vivo* potency in guinea pigs (ED_{50} = 0.9 μmol/kg i.v., maximum reversal 102% at 8 μmol/kg) than the lead γ-CD (ED_{50} = 4.0 μmol/kg i.v., maximum reversal 104% at 47 μmol/kg) (19).

C) Per-substitution to increase cavity depth: Org-25819 showed much more potent *in vitro/in vivo* activity than γ-CD and mono-substituted analogue in guinea pigs (ED_{50} = 0.21 μmol/kg i.v., maximum reversal 97% at 0.8 μmol/kg) (20).

Since thiols are much better nucleophiles than alcohol, we decided to synthesize the per-6-mercapto equivalent of Org-25169, resulting in Org-25969, which like Org-25819 has an extended cavity from perfacial substitution. Therefore, the discovery of Org-25969 was a result of combining rational design with synthetic feasibility.

Chemistry and structure-activity relationship

Org-25969 [6-per-(2-carboxyethylthio)-6-per-deoxy-γ-cyclodextrin sodium salt] can be synthesized by directly reacting 3-mercaptopropionic acid with the readily prepared per-6-bromo-(or iodo-)-γ-CD or by reacting 3-mercaptopropionic ester with per-6-bromo-(or iodo-)-γ-CD, followed by hydrolysis of the ester (19) (Fig. 3).

X-ray crystallographic data showed that the cavity dimension of Org-25969 had a high degree of structural complementarity with rocuronium (Fig. 4) (17). The cavity depth of ~11Å is optimal for encapsulating the four hydrophobic steroidal rings of rocuronium, leaving the hydrophilic substituents 3-OH and 2-morpholine at ring-A protruding outside the cavity and exposed to water. Further extending or shortening the cavity depth resulted in reduced binding affinity (19, 21).

The ring-D quaternary ammonium group of rocuronium is surrounded by the negatively charged carboxyl groups of Org-25969, which contribute to the binding affinity by forming electrostatic interaction with the positively charged quaternary nitrogen of rocuronium. Isothermal titration calorimetry studies indicated that this electrostatic interaction contributed approximately –12.7 kJ mol^{-1} towards the enthalpy gain. Org-25969 has a 90-fold higher affinity than its neutral hydroxyl analogue (K_a = 1.8 x 10^7 M^{-1} *vs.* 2.0 x 10^5 M^{-1}) for complexing with rocuronium (19).

In vitro *and* in vivo *reversal potency*

When tested in organ bath against rocuronium-induced neuromuscular block (99.4 ± 0.5% block, rocuro-

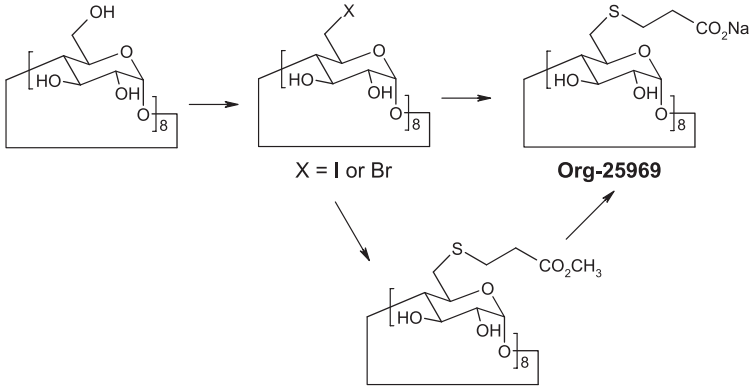

Fig. 3. Chemical synthesis of Org-25969 (19).

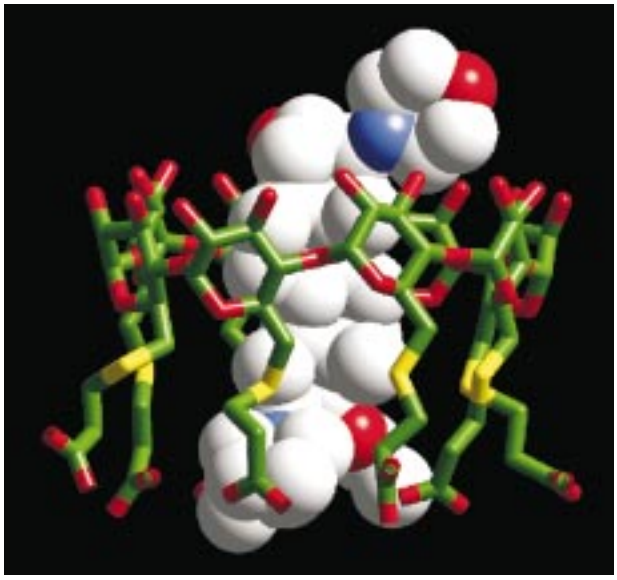

Fig. 4. X-ray crystal structure of Org-25969/rocuronium complex. The hydrophobic steroidal skeleton of rocuronium is encapsulated inside the lipophilic cavity of Org-25969 and the hydrophilic substituents (3-OH and 2-morpholine) of rocuronium protrude outside the ring and are exposed to water. The quaternary nitrogen of rocuronium is surrounded by the negatively charged carboxyls of Org-25969 through electrostatic interaction.

nium 3.6 μM) in isolated mouse hemidiaphragm, Org-25969 showed an EC_{50} (concentration to recover 50% of muscle contraction) of 1.2 ± 0.8 μM. A maximum reversal of 95.1 ± 2.3% was achieved at the concentration of 3.6 μM, equivalent to the rocuronium molar concentration (19).

In anesthetized guinea pigs, cumulative intravenous dosing of Org-25969 gave 50% reversal (ED_{50}) at 0.03 μmol/kg against a steady 84.6 ± 2.0% block of m. gastrocnemius contractions induced by continuous infusion of rocuronium (infusion rate 15.1 ± 2.2 nmol/kg/min). A maximum reversal of 92.5 ± 5.3% was achieved at 0.3 μmol/kg (19). Similarly, an ED_{50} of 0.05 ± 0.04 μmol/kg was obtained for the reversal of rocuronium-induced block in anesthetized cats (93 ± 2% block of m. tibialis contractions, rocuronium infusion 10.24 ± 1.31 nmol/kg/min). The maximum reversal of 117% was achieved at 0.16 ± 0.01 μmol/kg (19).

Org-25969 has also been tested in anesthetized rhesus monkeys (17). At 1.0 mg/kg (or 0.46 μmol/kg i.v.), Org-25969 reversed the rocuronium-induced block (block depth 90%) in a rapid and efficacious fashion to reach 90% recovery of muscle contraction within 3 min. By contrast, the standard treatment with neostigmine (40 μg/kg) and atropine (15 μg/kg) only produced the same level of recovery in >6 min.

Reversal of profound block

Experimental profound block was defined as 100% neuromuscular block induced by 3 times the concentration of rocuronium needed to obtain 90% block, *i.e.*, block induced by 10.8 μM (or 3 x 3.6 μM) of rocuronium (22). As shown in Figure 5, at concentrations equimolar to that of the blocker, Org-25969 achieved efficient reversal against both 3.6 μM (EC_{90}) and 10.8 μM of rocuronium-induced block in isolated mouse hemidiaphragm. Neostigmine (7.0 μM) was effective against the block induced by the EC_{90} but was not able to reverse the effects of 3 times the EC_{90} concentration, even at higher concentrations up to 9.0 μM (22).

The capability of Org-25969 to reverse profound block is a further proof for its chemical encapsulation mechanism of action.

Cardiovascular side effects

When administered by intravenous infusion (0.05 μmol/kg/min for 30 min) to guinea pigs, Org-25969 alone did not cause any significant hemodynamic changes, indicating its lack of intrinsic activity (19).

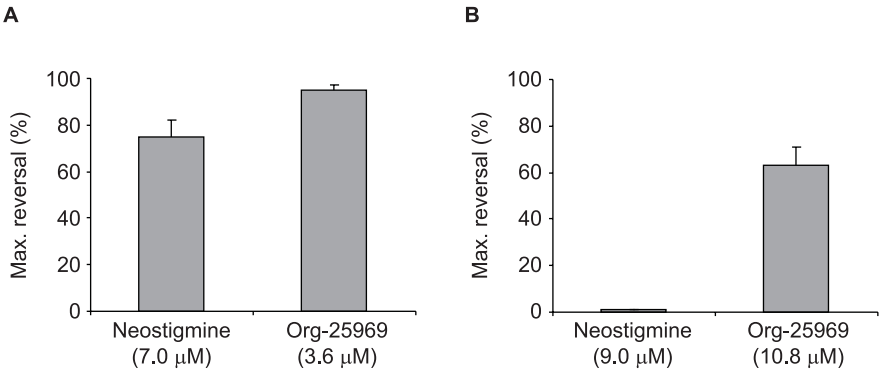

Fig. 5. Reversal of 3.6 μM (A) and 10.8 μM (B) of rocuronium-induced block in mouse hemidiaphgram by Org-25969 and neostigmine. While both Org-25969 and neostigmine are effective in reversing the 90% block induced by 3.6 μM rocuronium, only Org-25969 was able to reverse the profound block induced by 3 times the EC_{90} of rocuronium.

Cumulative dosing of Org-25969 in anesthetized cats up to 1.8 μmol/kg (almost 4 times the effective reversal dose) did not produce significant changes in any of the hemodynamic parameters measured, *e.g.*, mean blood pressure, heart rate, left ventricular pressure, left ventricular contractility, bradycardia induced by vagal stimulation and nictitating membrane contractions induced by sympathetic stimulation (19). In contrast, the clinical standard combination of neostigmine (24.3 μg/kg) and atropine (15 μg/kg) exerted a profound effect on vagal stimulation (19).

Similarly, no significant changes in hemodynamic parameters, for example, blood pressure, heart rate, *etc.*, were observed in anesthetized rhesus monkeys during and after treatment with Org-25969 up to 10 mg/kg i.v. (10 times the effective reversal dose) (17). All animals recovered completely without any complications including abdominal discomfort as seen after neostigmine. No signs of recurarization were observed.

Selectivity

Org-25969 has been tested for reversal of different NMBs (22). As shown in Table I, Org-25969 selectively reverses steroidal NMBs in mouse diaphragm *in vitro*. A similar selectivity profile has been found *in vivo* in guinea pigs, *i.e.*, Org-25969 reverses steroidal NMBs more readily than nonsteroidal NMBs (23).

This selectivity of Org-25969 for steroidal NMBs over nonsteroidal NMBs originates from the size of Org-25969's cavity and its structural complementarity with the rigid hydrophobic steroid skeleton.

Org-25969 has also been tested for *in vitro* selectivity against a panel of more than 40 drugs that may be used during anesthesia, using isothermal titration calorimetry. These included hypnotics (propofol, thiopenthane, *etc.*), analgesics (fentanyl, remifentanil, *etc.*), antibiotics (vancomycin, gentamycin, *etc.*), bronchodilators (salbutamol, aminophylline, *etc.*), cardiovascular drugs (digoxin, ephedrine, atropine, *etc.*) as well as other steroidal drugs (cortisone, hydrocortisone, dexamethasone, aldosterone, *etc.*). Org-25969 showed remarkable selectivity for rocuronium and other steroidal NMBs (*i.e.*, vecuronium and pancuronium) over all other drugs tested.

Among the non-NMB drugs, cortisone, hydrocortisone and aldosterone showed clear complex formation with Org-25969 (K_a = 81,400, 40,000 and 22,000 M^{-1}, respectively), but their affinities to Org-25969 are at least >120-fold less than that of rocuronium. This is because although these drugs share the same steroid backbone with rocuronium, they are neutral and do not contain positively charged groups that rocuronium uses to form

Table I: In vitro selectivity of Org-25969 against different NMBs (22).

NMB	90% block conc. (μM)	50% reversal by Org-25969 at μM	Maximum reversal (%)	Conc. of Org-25959 at maximum reversal (μM)
Rocuronium	3.60	1.2 ± 0.8	95.1 ± 2.3	3.6
Vecuronium	0.85	0.8 ± 0.1	90.6 ± 5.1	1.4
Rapacuronium	28.00	12.5 ± 1.8	64.2 ± 32.4	28.0
Pancuronium	0.90	1.2 ± 0.3	60.4 ± 8.7	1.8
Mivacurium	8.00	-	0.0	160.0
Atracurium	45.00	-	4.7 ± 4.7	1400.0
Succinylcholine	45.00	-	24.5 ± 8.8	900.0

electrostatic interaction with the negatively charged carboxyls at the rim of Org-25969 cavity.

Atropine, verapamil, phentolamine, naloxone and ketamine are the nonsteroid drugs forming complexes with Org-25969 but their affinity to Org-25969 are at least 400- to 700-fold lower than rocuronium.

Org-25969 itself is biologically inactive. At 36 μM, which is 10 times the maximum reversal concentration *in vitro*, Org-25969 did not modify mouse vas deferens contractions induced by field stimulation, mouse hemidiaphragm contractions induced by phrenic nerve stimulation and rat aortic ring contractions induced by (–)-noradrenaline, adrenaline, dopamine and 5-HT (19). Pretreatment of mouse diaphragm with 36 μM Org-25969 did not show any change in force of contraction, but gave 100% protection against 3.6 μM rocuronium, the concentration that produces 90% block in the untreated mouse diaphragm.

Pharmacokinetics and toxicity

In guinea pigs *in vivo*, it was found that following steady-state infusion, Org-25969 increased urine levels of rocuronium from 15 ng/ml to 30,172 ng/ml and plasma levels from 4,159 ng/ml to 5,919 ng/ml. In comparison, γ-CD increased urine and plasma levels of rocuronium from 15 ng/ml to 24,094 ng/ml and from 2,495 ng/ml to 2,735 ng/ml, respectively (25). It therefore appears that Org-25969 increases the clearance of rocuronium via the kidneys, thus contributing to its reversal efficacy as well as minimizing the possibility of recurarization.

In a pilot Ames test, Org-25969 did not show any signs of killing bacterial cells or induce concentration-related increase in the number of revertants per plate in both of the *Salmonella typhimurium* strains in the presence or absence of S9-mix. These results indicate that Org-25969 has no mutagenic activity.

Conclusions

As discussed above, the cyclodextrin-derived host molecule Org-25969 is a very effective reversal agent for rocuronium-induced neuromuscular block in all animal models tested. Its chemical encapsulation mechanism of action is unique and highly novel.

Org-25969 has a very rapid onset of action. In monkeys, it produced 90% reversal within 3 min whereas neostigmine/atropine required 6 min to produce the same level of reversal (17). It appears to increase the renal clearance of rocuronium and therefore recurarization is very unlikely to occur. It is cardiovascularly clean. At the effective reversal dose (0.46 μmol/kg), Org-25969 produces <10% changes in all hemodynamic parameters measured in guinea pigs, cats and monkeys.

Org-25969 is effective in reversing experimental profound block induced by 3 times the EC_{90} of rocuronium. It should be noted that it is clinically impossible to use neostigmine/atropine to reverse profound block because of the potential cholinergic crisis. The ability of Org-25969 to reverse rocuronium-induced profound block has an important clinical perspective. Rocuronium, due to its rapid onset of action, especially at higher doses (*e.g.*, >1 mg/kg) is the only suitable alternative to succinylcholine for rapid-sequence intubation in elective and in emergency cases (24). The main argument in favor of succinylcholine is its shorter duration in the situation of unanticipated difficult intubation, whereas the main argument in favor of rocuronium is the avoidance of the many adverse effects of succinylcholine. Since the action of rocuronium can be reversed almost at will by its specific host Org-25969, the use of rocuronium in combination with Org-25969 in rapid-sequence intubation may offer both the benefit of rapid intubation and a high level of patient safety.

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