Calabadion

A New Agent to Reverse the Effects of Benzylisoquinoline and Steroidal Neuromuscular-blocking Agents

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ABSTRACT

Introduction: To evaluate whether calabadion 1, an acyclic member of the Cucurbit[n]uril family of molecular containers, reverses benzylisoquinoline and steroidal neuromuscular-blocking agent effects.

Methods: A total of 60 rats were anesthetized, tracheotomized, and instrumented with IV and arterial catheters. Rocuronium (3.5 mg/kg) or cisatracurium (0.6 mg/kg) was administered and neuromuscular transmission quantified by acceleromyography. Calabadion 1 at 30, 60, and 90 mg/kg (for rocuronium) or 90, 120, and 150 mg/kg (for cisatracurium), or neostigmine/glycopyrrolate at 0.06/0.012 mg/kg were administered at maximum twitch depression, and renal calabadion 1 elimination was determined by using a ^{1}H NMR assay. The authors also measured heart rate, arterial blood gas parameters, and arterial blood pressure.

Results: After the administration of rocuronium, resumption of spontaneous breathing and recovery of train-of-four ratio to 0.9 were accelerated from 12.3 ± 1.1 and 16.2 ± 3.3 min with placebo to 4.6 ± 1.8 min with neostigmine/glycopyrrolate to 15 ± 8 and 84 ± 33 s with calabadion 1 (90 mg/kg), respectively. After the administration of cisatracurium, recovery of breathing and train-of-four ratio of 0.9 were accelerated from 8.7 ± 2.8 and 9.9 ± 1.7 min with placebo to 2.8 ± 0.8 and 7.6 ± 2.1 min with neostigmine/glycopyrrolate to 47 ± 13 and 87 ± 16 s with calabadion 1 (150 mg/kg), respectively. Calabadion 1 did not affect heart rate, mean arterial blood pressure, pH, carbon dioxide pressure, and oxygen tension. More than 90% of the IV administered calabadion 1 appeared in the urine within 1 h.

Conclusion: Calabadion 1 is a new drug for rapid and complete reversal of the effects of steroidal and benzylisoquinoline neuromuscular-blocking agents.

What We Already Know about This Topic

- Reversal of neuromuscular blockade with current agents may cause side effects or be incomplete
- Calabadion 1 is an experimental agent that binds to steroidal and benzylisoquinoline neuromuscular blockers, but its *in vivo* disposition and efficacy are unknown

What This Article Tells Us That Is New

- In healthy rats, calabadion 1 produced a dose-dependent reversal of neuromuscular blockade from cisatracurium and rocuronium without affecting heart rate, blood pressure, or arterial blood gas tensions or pH

MORE than 400 million patients receive curare-type neuromuscular-blocking agents (NMBAs) annually

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(IMS Health, Danbury, CT; MIDAS; September 2010) to facilitate tracheal intubation, optimize surgical exposure, or in the intensive care unit to control spontaneous breathing during mechanical ventilation in patients with patient-ventilator asynchrony.[1] Although NMBAs have undeniable importance in routine surgery and intensive care medicine, their use has been associated with postoperative respiratory dysfunction[2] and an increased incidence of severe postoperative respiratory complications compared with anesthetic techniques that do not use NMBAs.[2,3] This is mainly due to postoperative residual neuromuscular blockade, occurring in 20–50% of patients at the end of the case,[4,5] which can result in airway obstruction, hypoxia, and increased postanesthesia care unit length of stay.[6–9]

Reversal of neuromuscular blockade at the end of surgery is an important strategy for accelerating the recovery from neuromuscular transmission block.[10,11] To date, acetylcholinesterase inhibitors are most frequently used for reversal of NMBA,[10,12] but due to their indirect mode of action their ability to reverse profound residual neuromuscular blockade induced by a high concentration of muscle relaxants is limited[13,14] and they have undesirable muscarinergic side effects.[15,16]

Sugammadex[17] reverses any degree of vecuronium- or rocuronium-induced block[18,19] by encapsulating the drug and thereby inactivating it, but the drug has not yet been approved by the Food and Drug Administration because concerns related to allergic reactions and hemorrhagic side effects need to be addressed before resubmission. In addition, despite its ability to reverse all levels of steroidal neuromuscular blockade, sugammadex does not reverse residual neuromuscular blockade induced by benzylisoquinolines.

Apart from these current options for reversal of neuromuscular blockade, direct chemical antagonism for newly designed short- and intermediate-acting muscle relaxants like gantacurium and L-cysteine[20] might open up new possibilities in the future.

In the current study, we introduced calabadion 1, a new reversal agent for rapid and complete reversal of steroidal (rocuronium) as well as benzylisoquinoline (cisatracurium) NMBAs. Calabadion 1 is an acyclic member of the cucurbit[n]uril (CB[n], n = 5, 6, 7, 8, or 10) family of molecular containers that, similar to the mode of action of cyclodextrins, forms host–guest complexes with specific targets and thereby modifies the properties of drugs bound within its interior. The structure of calabadion 1 is composed of a central methylene-bridged glycoluril tetramer that is capped by two *o*-xylylene rings. The substituents of the bridging *o*-xylylene units (four sulfonate groups) are all point away from the cavity, which preorganizes the oligomer into the C-shaped configuration required for binding.

Calabadion 1 is acyclic and can able to flex its glycoluril oligomer backbone, expand its cavity, and thereby accommodates guests with a range of sizes (*e.g.,* from hexanediammonium to dimethyladamantaneammonium). Its structural features also enhance the solubility and binding affinity of

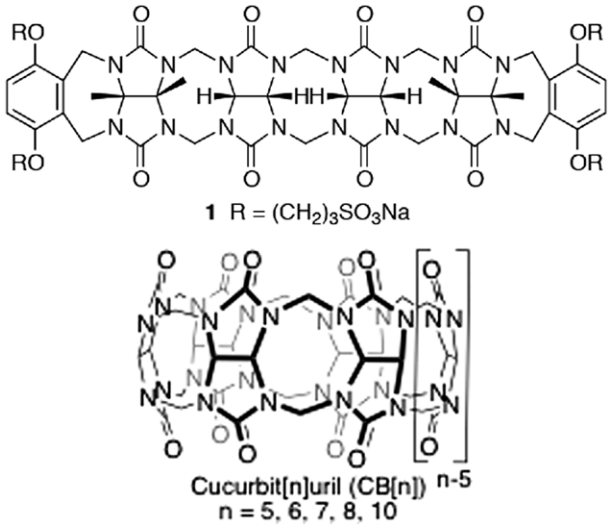

Fig. 1. Chemical structure of calabadion 1 and CB[n].

calabadion 1 toward cations due to additional electrostatic interactions (fig. 1). The development of calabadion 1 has been described in detail later in the article.[21] We have tested calabadion 1 to reverse neuromuscular blockade induced by a steroidal (rocuronium) as well as a benzylisoquinoline (cisatracurium) NMBA in rats. We report in this article (1) the first systematic estimate of the efficacy of calabadion 1 and its potency to reverse the muscle block caused by rocuronium or cisatracurium in live laboratory animals, (2) preliminary data on its cardiovascular and respiratory effects, and (3) the main route of elimination within the first hour after the administration.

Materials and Methods

A total of 60 adult male Sprague–Dawley rats weighing 280–350 g were used.

Institutional guidelines for animal care and usage for research purposes were strictly followed. All procedures involving animals were approved by the Institutional Animal Care and Use Committees at Harvard Medical School, and experiments were conducted at the research laboratories of the Massachusetts General Hospital, Boston, Massachusetts.

General Surgical Procedures

For all surgical procedures, rats were anesthetized (isoflurane: 5% induction and 1.5% maintenance, in 70% N_2O–30% O_2) and tracheotomized. Spontaneous breathing was maintained during surgery and after recovery from neuromuscular blockade. Rats were positioned supine with the head supported in a neutral midline position.

The left femoral vein and artery were cannulated for drug infusion and blood sampling. Arterial blood gases and pH were measured repetitively (Corning 178; Corning, NY), and continuous measurement of blood pressure (PowerLab; ADInstruments, Colorado Springs, CO) and heart rate were performed. When mechanical ventilation was

required (from injection of NMBAs to recovery), animal's lungs were ventilated *via* a tracheostomy tube (SAR-830; CWE, Ardmore, PA). Rectal temperature was kept stable or steady at 37.0° ± 0.1°C using a thermostatic heating pad (FHC, Bowdoinham, ME). A constant level of anesthesia was maintained throughout the experiment to eliminate cardiovascular response to tail pinch. In all treatment groups, systemic physiological parameters were kept well within normal range. Total urine volume was collected at the end of the case by a single puncture in the bladder, after which the animal was euthanized.

Assessment of Neuromuscular Transmission

The right leg was shaved and the femoral nerve was stimulated supramaximally with subcutaneous needle electrodes, and the evoked response of the quadriceps femoris muscle was measured by accelerometry, with the TOF-Watch SX Monitor (Organon Ireland Ltd., a part of Schering-Plough Corporation, Dublin, Ireland), as described previously.[22,23] The transducer was fixed to the skin ventromedially at the proximal end of the thigh, next to the tibial tuberosity (insertion point of the patellar ligament).

After determination of the supramaximal stimulation current and calibration of the TOF-Watch SX monitor (cal 1 mode), we stimulated the femoral nerve continuously at 1 Hz (10 mA ± 2) for at least 10 min until twitch height reached a stable plateau. We then re-calibrated the TOF-Watch SX monitor, took a baseline train-of-four (TOF) at 2 Hz, and continued to stimulate the femoral nerve at 1 Hz with the single twitch mode. We then injected the NMBAs in a volume of 0.5 cc over 5 s. When maximum twitch depression (T1 = 0) was achieved after 30 s, we administered placebo, neostigmine/glycopyrrolate, or calabadion 1. At the time of recovery of the single twitch height to 50% of baseline,[22] we changed the single twitch stimulation mode to TOF stimulation with a 12-s interval, in order to use a stimulation pattern that provides a higher resolution for shallow levels of neuromuscular blockade. Complete recovery of neuromuscular function was determined to be a TOF-ratio of more than 90%. TOF monitoring was continued for 20 min after complete recovery followed by another 40–60 min of monitoring of the physiological parameters.

Pilot Study

To obtain an estimate of the efficacy of calabadion 1 to reverse the neuromuscular block caused by rocuronium 3.5 mg/kg (2-fold ED90) or cisatracurium 0.6 mg/kg (2-fold ED90), we explored the dose–response relationship of calabadion in a pilot study (n = 10), by repetitively injecting calabadion 1 at doses of 10 mg/kg every 45 s until full reversal of neuromuscular block. On the basis of these pilot experiments, in order to study optimal calabadion 1-induced speed of recovery, we choose to administer calabadion 1 at doses of 0, 30, 60, and 90 mg/kg to generate a dose–response curve for rocuronium reversal and doses of 0, 90, 120, and 150 mg/kg for cisatracurium reversal.

Experimental Protocol

Rats were prepared as described aforementioned. Complete neuromuscular blockade (estimated 2-fold ED90) was induced with rocuronium (3.5 mg/kg; n = 30) or cisatracurium (0.6 mg/kg; n = 30) respectively, and then mechanical ventilation was started and maintained until recovery of spontaneous breathing. Thirty seconds after the onset of rocuronium-induced complete neuromuscular block, placebo, neostigmine/glycopyrrolate 0.06/0.012 mg/kg or calabadion 1 at dosages of 30, 60, or 90 mg/kg was administered for rocuronium reversal at maximum twitch depression (T1 = 0) in a random manner. Thirty seconds after the onset of cisatracurium-induced complete neuromuscular block, placebo or neostigmine/glycopyrrolate 0.06/0.012 mg/kg or calabadion 1 at dosages of 90, 120, or 150 mg/kg were administered at maximum twitch depression (T1 = 0) in a random manner.

Endpoints included time to recovery of spontaneous breathing which is defined as regular-frequent spontaneous breathing efforts on the ventilator, twitch height, and TOF-ratio. Arterial pressure and heart rate were measured continuously, and arterial blood gas was measured directly before administration of calabadion 1, 10 and 30 min later. At 60 min after the injection of calabadion 1, urine was collected from the rat and stored at −80°C until analysis.

Urine Analysis

For urine analysis of calabadion, we have taken 0.1 ml from each urine sample and dried them under high vacuum. The residue was dissolved in 0.5 ml deuterium oxide (D_2O) and 0.1 ml of 60 mM solution of 1,3,5-benzene tricarboxylic acid as an internal standard of known concentration was added. ^1H NMR spectra (600 MHz) were taken and used to determine the concentration of calabadion 1 in urine using the ratio of the integrals of the resonance for the internal standard (8.3 ppm, 3H) and the resonance for CH_3-groups of calabadion 1 (1.9-1.5 ppm, 12H).

Drugs

Calabadion 1 was synthesized in the Isaacs laboratory according to the published procedures.[24] Calabadion 1 is a powder that is readily dissolved in water. Isoflurane (Flurane; Baxter Healthcare Corporation, Deerfield, IL), cisatracurium (Abbott Laboratories, Abbott Park, IL)), rocuronium (Zemuron, Organon, NJ), and neostigmine/glycopyrrolate were obtained from clinical supplies.

Statistical Analysis

The primary outcome was efficacy expressed as speed of recovery of spontaneous breathing and TOF-ratio from rocuronium- and cisatracurium-induced neuromuscular block. We tested the primary hypothesis that calabadion

1 compared with placebo accelerates the time to recovery of spontaneous breathing and TOF-ratio from complete neuromuscular block in a dose-dependent manner. We tested the secondary hypothesis based on *in vitro* binding studies[21] that the potency of calabadion **1** for rocuronium reversal compared with cisatracurium reversal is higher, expressed as shorter reversal time to recovery of spontaneous breathing and TOF-ratio. As for power analysis, we expected, based on preliminary data, 30-s differences between placebo and calabadion 1 with an SD of 10 s. Based on these assumptions, we calculated that a sample size of five rats per group would provide a 90% power to detect a significant difference between groups (α-error: 0.05). Recovery times were defined as time from start of calabadion 1 injection to full recovery of the target endpoint (TOF-ratio, breathing). We used a mixed linear model (compound symmetry type) to test the primary and secondary hypotheses. The independent variables were: compound (cisatracurium/rocuronium), muscle function type (recovery of spontaneous breathing *vs.* extremity TOF-ratio), and calabadion 1 dose (probit-transformed data). We tested for a drug–dose interaction to demonstrate a difference in recovery slopes. For each specific NMBA used (cisatracurium and rocuronium), we tested the dose–response relationship of calabadion 1 to accelerate recovery of TOF-ratio to 80, 90, and 100%, after complete neuromuscular block, by using a repeated measures linear regression model (Scheffe *post hoc* test). This model was also applied to test the effects of calabadion 1 on safety-related variables, that is, heart rate, arterial blood pressure, pH, pO_2, and pCO_2. All data were expressed as mean ± SD. A two-tailed P value less than 0.05 was considered as an indication of a significant difference. SPSS Version 17.0 (SPSS Inc., Chicago, IL) was used for the statistical analysis.

Results

Calabadion 1 at the highest doses caused a rapid and complete reversal of cisatracurium- and rocuronium-induced neuromuscular block, and the time to recovery was dependent on the calabadion 1 dose and compounds (rocuronium *vs.* cisatracurium): intercept: F = 273.2; $P < 0.001$ and drug–dose interaction effect: F = 26.2; $P < 0.0001$; figure 2. Speed of recovery of spontaneous breathing was significantly faster compared with TOF-recovery (F = 39.2; $P < 0.0001$).

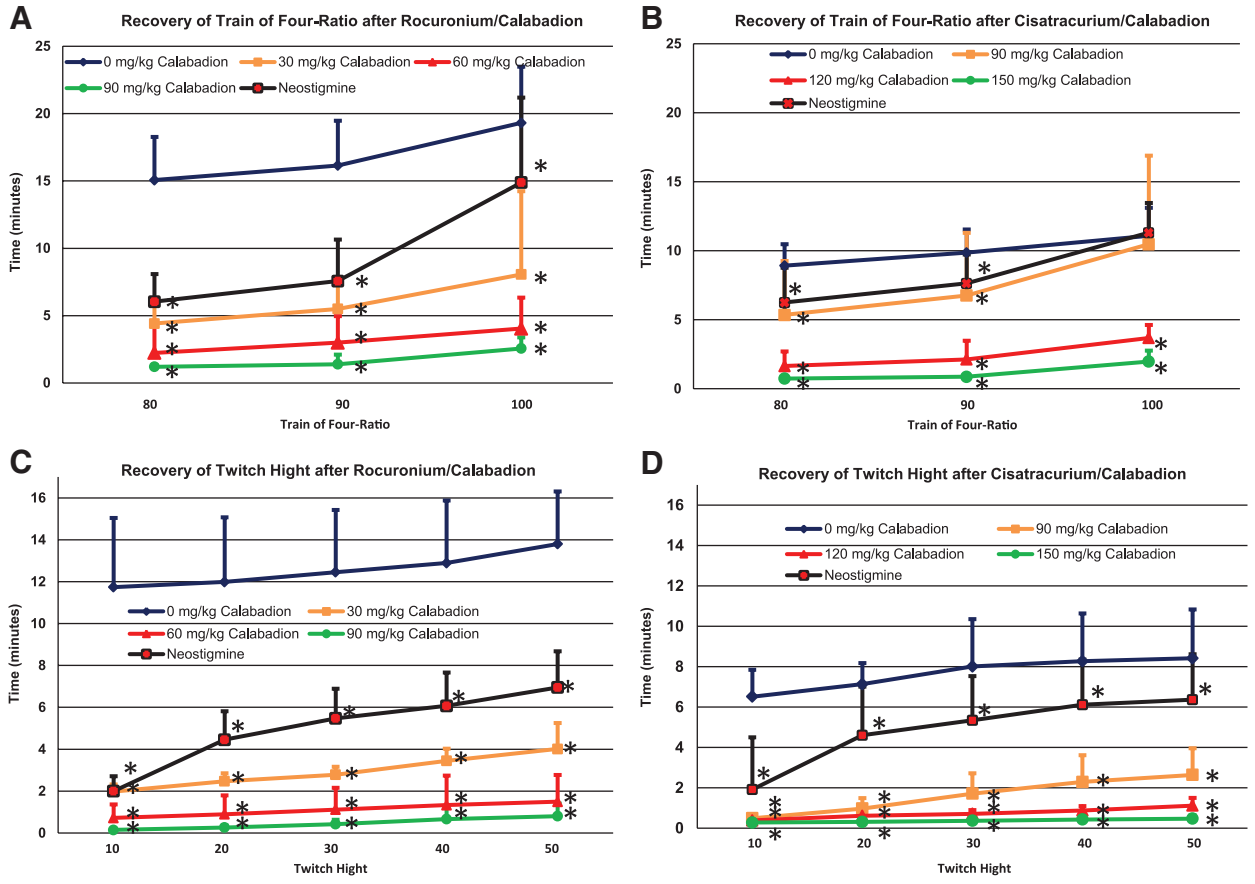

Fig. 2. Comparison of recovery of single twitch height and train-of-four ratio in the rocuronium (*A* and *C*) and cisatracurium (*B* and *D*) groups. Calabadion 1 significantly decreased time to recovery in both the groups. * Indicates $P < 0.01$ for recovery of single twitch height and train-of-four ratio compared with placebo. Data shown as mean ± SD.

Rocuronium Reversal

As for *rocuronium* reversal, time to recovery of the TOF-ratio of 0.9 amounted to 16.2 ± 3.3 min in the placebo group and 4.6 ± 1.8 min in the neostigmine/glycopyrrolate group. The recovery time was significantly shorter ($P < 0.001$) with all three doses of calabadion 1 as shown in figure 2A. Time to recovery of twitch height was also significantly shorter ($P < 0.0001$) with all three doses of calabadion 1 compared with placebo and neostigmine (fig. 2C). Of note, at the highest dose, recovery of spontaneous breathing occurred within ~10 s (0.15 ± 0.08 min; fig. 3), suggesting a reversal as fast as it approximately takes for the venous blood to arrive at the muscular capillary blood vessels in rats.[25,26] Time to recovery of spontaneous breathing after placebo and neosyigmine/glycopyrolate amounted to 12.3 ± 1.1 and 5.2 ± 2.2 min, respectively.

Cisatracurium Reversal

Similar to the reversal of rocuronium-induced neuromuscular block, calabadion 1 fully and rapidly reversed muscle paralysis caused by *cisatracurium* in a dose-dependent manner ($P < 0.001$). After the administration of cisatracurium, the time to recovery of TOF-ratio of 0.9 was reduced significantly for all the administered doses of calabadion 1 ($P < 0.001$), with a decrease from 9.9 ± 1.7 min with placebo to 7.6 ± 2.1 min with neostigmine/glycopyrrolate to 87 ± 16 s with the highest dose calabadion 1 administered (fig. 2C). Similarly, the recovery of twitch height (fig. 2D) was accelerated significantly by calabadion 1 compared with placebo and neostigmine coadministered with glycopyrrolate. After administration of the highest dose of calabadion 1, the time to recovery of spontaneous breathing amounted to 47 ± 13 s compared with 8.7 ± 2.8 min in the placebo group (fig. 3) and 2.8 ± 0.8 min in the neostigmine/glycopyrrolate group.

As shown in figure 3, the dose–response relationship for the effect of calabadion 1 on both outcome criteria was significantly shifted to the right for cisatracurium.

Side Effects

Calabadion 1 did not induce a significant change in heart rate, blood pressure, or arterial blood gas parameters as shown in figure 4 and table 1. Signs of residual blockade or re-paralysis (decrease in TOF-ratio or respiratory depression) were not observed within the monitoring period of at least 60 min after administration of calabadion 1.

In order to evaluate potential binding of calabadion 1 to cholesterol, we constructed a phase-solubility diagram for mixtures of calabadion 1 and cholesterol.[24] For this purpose, we stirred a known concentration of calabadion 1 (5, 15, 30, and 60 mM) with an excess of solid cholesterol until equilibrium was reached. After filtration, the absence of cholesterol in the supernatant was determined by ^{1}H NMR spectroscopy. Accordingly, we concluded that calabadion 1 does not bind to cholesterol.

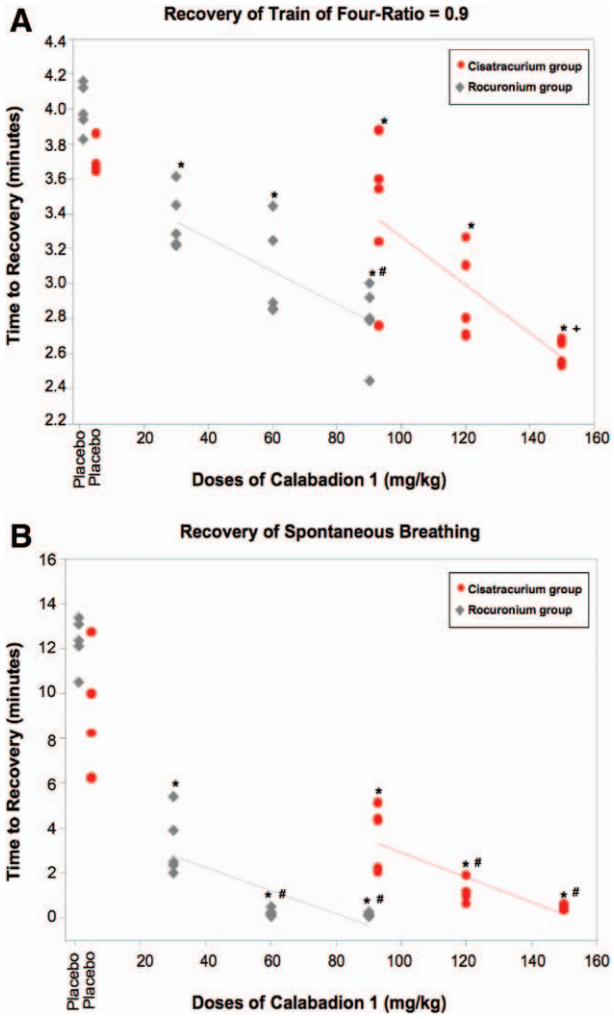

Fig. 3. Does–response relationship of calabadion reversal of cisatracurium- and rocuronium-induced neuromuscular blockade. (*A*) calabadion 1 dose *versus* recovery of train-of-four ratio of 0.9. (*B*) Calabadion 1 dose *versus* recovery of breathing. Calabadion 1 significantly decreased time to recovery of spontaneous breathing and train-of-four ratio to 0.9 for all doses administered in both groups. *$P < 0.001$ for recovery of spontaneous breathing and train-of-four ratio of 0.9 compared with placebo, +$P < 0.05$ *versus* 120 mg/kg group, #$P < 0.05$ *versus* 30 mg/kg.

Urinary Excretion of Calabadion 1

Calabadion 1 is being eliminated predominantly by the kidney. After the administration of a single dose of 30, 60, or 90 mg/kg of calabadion 1, almost 90–100% of the dose was recovered in urine within 1 h of IV dosing (fig. 5).

Discussion

Calabadion 1 caused a rapid, dose-dependent reversal of complete *rocuronium*- and *cisatracurium*-induced neuromuscular block without apparent effects on heart rate, blood pressure, pH, and pulmonary gas exchange. We did not observe signs or symptoms of re-paralysis, and a

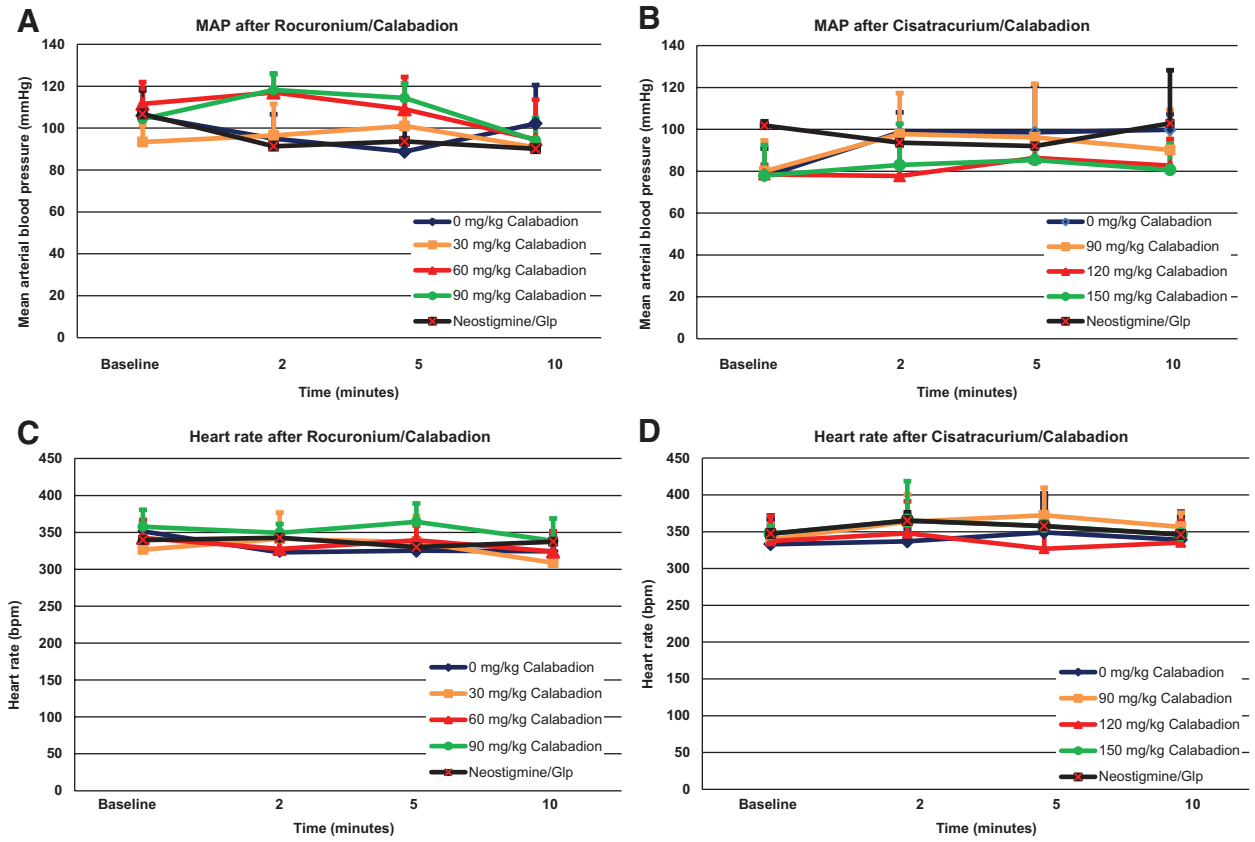

Fig. 4. Comparison of heart rate and mean arterial blood pressure (MAP) in the rocuronium (*A* and *C*) and cisatracurium (*B* and *D*) groups. No significant difference in heart rate or MAP at baseline, 2, 5, or 10 min after administration of calabadion 1 was observed. Data shown as mean ± SD.

substantial amount of calabadion 1 was eliminated by the kidney within 1 h.

Chemical encapsulation of NMBAs is a new approach for the reversal of neuromuscular blockade.[17] Chemists who pursue this line of research—supramolecular chemists[27]—have developed a variety of different containers including cyclodextrins[28,29] and used them in a variety of application areas including artificial ion-channels,[30,31] supramolecular polymers,[32] and ion and molecular sensing systems.[33] Sugammadex, the most prominent cyclodextrin, inactivates rocuronium and vecuronium by encapsulating the molecules into its lipophilic cavity and thereby reversing the neuromuscular block.[17,34] However, sugammadex does not reverse benzylisoquinolines that account for about one third of the market volume of NMBAs.

Cucurbit[n]urils (CB[n], n = 5, 6, 7, 8, or 10) are molecular containers with properties that are even superior to those of cyclodextrins. They bind to a variety of cationic and neutral species with very high affinity (K_a typically $>10^4/M$ and sometimes reaches $10^{15}/M$) and selectivity and are available in a variety of size (n = number of glycoluril units) that span and exceed those available in the cyclodextrin series.[35,36] In order to use cucurbit[n]urils to reverse NMBAs, good solubility and selectivity are important goals. Isaacs *et al.* have synthesized acyclic CB[n]-type receptors—calabadions—and studied their molecular recognition properties.[24,37–39] Based on the acyclic structure, calabadions are able to flex

Table 1. Effects of Calabadion on Arterial Blood Gas Analysis

Calabadion Dose, mg/kg	Before Injection of Calabadion 1			10 Minutes after Calabadion 1			N
	pH	pCO_2	pO_2	pH	pCO_2	pO_2	
0	7.42 ± 0.02	42 ± 6	176 ± 20	7.42 ± 0.04	41 ± 3	171 ± 27	5
30	7.42 ± 0.03	42 ± 3	185 ± 40	7.42 ± 0.03	39 ± 2	179 ± 42	5
60	7.43 ± 0.03	44 ± 6	147 ± 6	7.43 ± 0.02	40 ± 6	176 ± 76	5
90	7.42 ± 0.01	43 ± 2	176 ± 29	7.41 ± 0.01	41 ± 2	187 ± 21	5

Arterial blood gas parameters measured before and after the administration of calabadion 1. Data shown as mean ± SD.

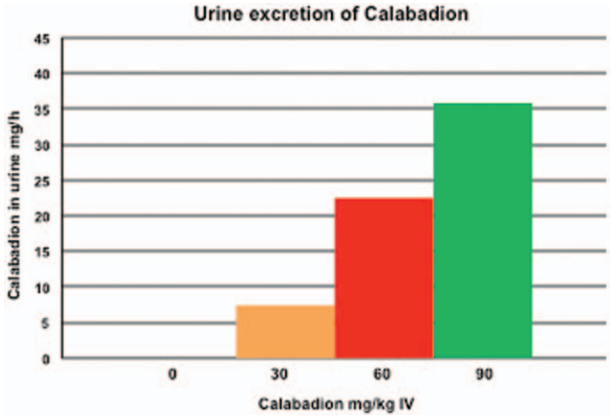

Fig. 5. Urinary excretion of calabadion 1. After administration of 30, 60, and 90 mg/kg, almost 90–100% of a single dose of calabadion 1, was recovered by the urine within 1 h of intravenous (IV) dosing.

their methylene-bridged glycoluril oligomer backbone, expand their cavity, and thereby accommodate even large guests. Accordingly, we thought that calabadions would display excellent affinity toward both steroidal and benzyl isoquinoline NMBAs by ion-dipole, π–π interactions, and the hydrophobic effect.

In contrast to its macrocyclic CB[n]s ancestors,[40] calabadion 1, which has been tested in this study, shows an excellent solubility in water (346 mM). The structure of calabadion 1 makes it complementary to dicationic drugs such as rocuronium and cisatracurium. Calabadion at 90 mg/kg produced complete recovery of the TOF-ratio after complete rocuronium- and cisatracurium-induced neuromuscular block in ~1–2 min, which is even faster than the speed of reversal observed previously after sugammadex (15 mg/kg sugammadex fully reversed complete rocuronium- [3.5 mg/kg] induced neuromuscular block in 2.5 min) in the same species under the same experimental conditions.[23] However, studies in other species are needed to confirm its efficacy, and the side effects profile of calabadions needs to be further studied.

Rocuronium, cisatracurium, neostigmine/glycopyrrolate, and calabadion 1 doses used in this study have been selected based on our previously published work[22,23] and on our pilot experiments. As rats are highly resistant to NMBAs, lower doses of NMBAs and calabadion will probably be required in other species such as humans. The speed of recovery from rocuronium-induced neuromuscular block observed in our trial seems to be even faster than values observed in our previous sugammadex trial that was conducted in the same model.[23] When interpreting the very fast speed of twitch height and TOF-ratio recovery, one has certainly to keep in mind that the encapsulation of an NMBA through calabadion is a drug–drug interaction that is limited by the circulation time. The circulation time in rats compared with humans is, with ~10 s in rats compared with 60 s in men, much faster, and therefore the reversal agent will predictably reach its target in a shorter period of time.[25,26] This might have been further promoted by the use of isoflurane as anesthetic which has been shown to increase muscle blood flow and perfusion.[41] As the speed of reversal was so fast, we decided to use 1-Hz single twitch stimulation[42] because the TOF-ratio, which is usually taken every 10–15 s, would not have provided the appropriate resolution to identity the fast recovery from deep neuromuscular block.[23] After recovery of the twitch height to 50% of baseline, we changed the stimulation frequency to TOF stimulation to increase the resolution for detecting subtle levels of neuromuscular blockade. We did not artificially ventilate the animals during surgery, and kept gas exchange normal confirmed by arterial blood gases analysis. We also measured the time to recovery of spontaneous breathing after reversal, which we believe may be a better model than TOF recovery of a clinical cannot ventilate, cannot intubate scenario. It is important to state that spontaneous breathing does not ensure sufficient breathing, but under our experimental conditions, it took only very few seconds after the first movements of the diaphragm until sufficient breathing was restored, as indicated by respiratory rate and arterial blood gas results.

The binding constant measured for the calabadion 1•rocuronium is ($K_a = 8.4 \pm 0.9 \times 10^6$/M) and ($K_a = 9.7 \pm 0.8 \times 10^5$/M) for the calabadion 1•cisatracurium complex. This lower binding affinity of calabadion 1 toward cisatracurium by a factor of 10 compared with rocuronium might explain right shift in the observed dose–response relationship. The binding constant measured for the calabadion 1•rocuronium complex is comparable with that measured for the sugammadex•rocuronium complex ($K_a = 1.1 \pm 0.2 \times 10^7$/M). Importantly, the binding affinity of calabadion 1 to rocuronium is 350-fold higher compared with acetylcholine ($K_a = 2.4 \times 10^4$/M) than to rocuronium[21] making rocuronium a much more likely target to encapsulate. In contrast to acetylcholinesterase inhibitors, calabadion 1 does not interfere with the release and metabolism of acetylcholine. As shown in our study, calabadion 1 administered in intact rats preserved stable cardiovascular and respiratory functions such as pH, arterial partial pressure of carbon dioxide and oxygen, and arterial blood pressure, and heart rate did not change in response to injection of calabadion 1.

Based on the limited, available *in vitro* and *in vivo* studies, it seems that calabadions are well tolerated. Calabadion 1 is not toxic *in vitro* to HepG2 and HEK293 cells up to 5 mM and *in vivo* in mice (maximum tolerated dose >1.23 g/kg).[24,43] Cholesterol is an essential structural component of mammalian cell membranes that is required to establish proper membrane permeability. Our ^{1}H NMR-derived spectroscopy data show that calabadion 1 does not bind to cholesterol. calabadion 1 is excreted rapidly by the kidney as shown in figure 5. This elimination kinetics is comparable with that of sugammadex.[44]

Unselective binding of containers such as cyclodextrin derivatives (*e.g.,* sugammadex) or cucurbit[n]uril type receptors (*e.g.,* calabadions) might be associated with toxic effects as well as displacement of other drugs. For example, sugammadex binds to sexual hormones such that the effects of oral anticontraceptives cannot be ensured after sugammadex reversal. The binding affinity of calabadions toward drugs commonly used in anesthesia and critical care needs to be established in future trials. At present, it has been shown that calabadion 1, like his ancestor CB[7], forms stable host–guest complexes with local anesthetics in aqueous solution *in vitro*.[37,45] If these findings are relevant *in vivo*, a second application of the calabadions may relate to treatment of local anesthetic-induced toxicity. In light of the application explored in current study, it is important to investigate in a future study whether systemic local anesthetic administration or systemic distribution may result in reparalysis after calabadion 1-induced reversal of neuromuscular block.

In summary, we have shown that calabadion 1 is a broad-spectrum reversal agent to antagonize complete neuromuscular block induced by benzylisoquinolines or steroidal NMBAs in the rat.

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