Anesthesiology 2003; 99:632–7

Reversal of Neuromuscular Blockade and Simultaneous Increase in Plasma Rocuronium Concentration after the Intravenous Infusion of the Novel Reversal Agent Org 25969

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Background: **The purpose of this study was to determine the changes in the plasma concentration of rocuronium and the reversal of its neuromuscular blockade after the intravenous infusion of Org 25969, the novel neuromuscular block–reversal agent, in anesthetized guinea pigs.**

Methods: **Rocuronium was infused for 1 h at a rate of 12–19 nmol·kg^{-1}·min^{-1} to produce a steady-state 90% neuromuscular block. After 30 min, a concomitant infusion of either the reversal agent Org 25969 at a rate of 50 nmol·kg^{-1}·min^{-1} or an infusion of an equivalent volume of saline was started. The time course of plasma concentrations of rocuronium was determined by use of liquid chromatography–mass spectrometry/mass spectrometry.**

Results: **In both treatment groups, a steady-state plasma concentration of rocuronium was obtained after 30 min. In the saline-treated group, the plasma concentration of rocuronium and depth of block remained constant. In the Org 25969 group, neuromuscular block was reversed while the rocuronium infusion was ongoing. Simultaneously, an increase in the total plasma concentration of rocuronium (free and complexed) was observed, even though the infusion rate of rocuronium was not changed. Compared with the saline-treated group, a small increase in the postmortem bladder concentration of rocuronium was detected.**

Conclusions: **The authors propose that the capture of rocuronium by Org 25969 causes the rapid reversal of neuromuscular block. The reversal can be explained by the rapid transfer of free rocuronium from the effect compartment (neuromuscular junction) to the central compartment, in which it is bound to Org 25969. This explains the increase in total plasma concentration of rocuronium (free and bound to Org 25969).**

ROCURONIUM (Zemuron® or Esmeron®; Organon Inc., West Orange, New Jersey) is one of the most widely used drugs for the induction of neuromuscular block. Although rocuronium has become a drug of choice for inducing neuromuscular block, it still has the common problem of other neuromuscular blocking agents: risk of possible postoperative residual block.[1] Reversal agents are used to reverse nondepolarizing neuromuscular block to speed up recovery of neuromuscular function or to treat residual neuromuscular block.[2]

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

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Received from the Department of Pharmacology, Organon Research, Scotland, United Kingdom. Submitted for publication October 1, 2002. Accepted for publication April 14, 2003. All the work reported in this article was funded solely by Organon Research, Scotland, United Kingdom.

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Traditionally, the effect of nondepolarizing neuromuscular blocking agents is reversed by the administration of an acetylcholinesterase inhibitor, such as neostigmine. Inhibition of acetylcholinesterase in the neuromuscular junction increases the survival time of acetylcholine in the synaptic cleft, which increases the competition between acetylcholine and the neuromuscular blocking agent for the nicotinic acetylcholine receptor in favor of acetylcholine. Inhibition of acetylcholinesterase causes an increase in acetylcholine concentration in all cholinergic synapses, resulting in undesired stimulation of muscarinic and nicotinic acetylcholine receptors in other tissues, *e.g.*, the smooth muscles in the respiratory and gastrointestinal tract. Pretreatment with or concomitant administration of muscarinic receptor antagonists, such as atropine or glycopyrrolate, can be used to reduce the unwanted stimulation of muscarinic acetylcholine receptors. Acetylcholinesterase inhibitors also have disadvantages, such as long-lasting inhibition of the enzyme in certain patients and lack of effect against profound neuromuscular block[2,3]

In anesthetic practice, administration of acetylcholinesterase inhibitors concomitantly with muscarinic antagonists has been used successfully for many years for reversal of neuromuscular block, but this method of treatment is ineffective until the recurrence of the first twitch during train-of-four stimulation.[2,3] The discovery of Org 25969 (fig. 1), a modified γ-cyclodextrin, which forms tight 1:1 complexes with rocuronium, is a promising new alternative treatment. Org 25969 does not require pretreatment or concomitant administration of muscarinic antagonists and is able to reverse profound neuromuscular block.[4] The reversal of rocuronium-induced neuromuscular block by Org 25969 has been demonstrated *in vivo* in guinea pigs,[5] cats,[6] dogs,[7] monkeys,[8] and human volunteers.[9]

The aim of this study was to investigate the effects of infusion of Org 25969 on the depth of neuromuscular block and rocuronium plasma concentration (free and complexed with Org 25969) during constant rocuronium infusion. The postmortem amount of rocuronium in urine was also determined.

Materials and Methods

Chemicals

Org 25959 was synthesized as described by Bom *et al.*[10] Rocuronium and Org 24748 were synthesized in

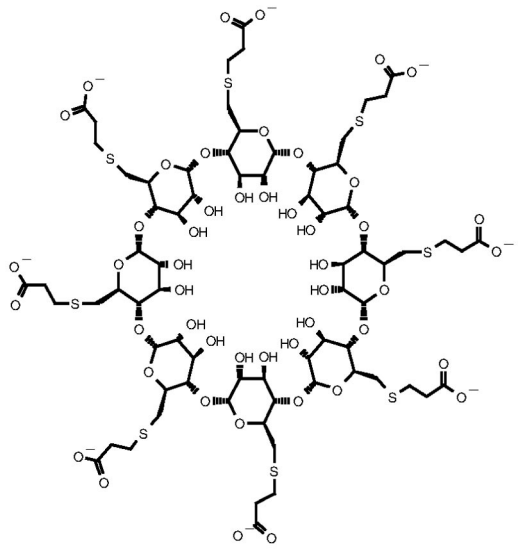

Fig. 1. Chemical structure Org 25969.

the Department of Medicinal Chemistry, Organon Research, Scotland. The purity of both compounds was approximately 99%. All other chemicals and reagents were of analytical or high-performance liquid chromatographic grade. Acetonitrile was obtained from BDH (Poole, United Kingdom); ammonium acetate, formic acid, and trifluoroacetic acid were purchased from Fisher Scientific (Loughborough, United Kingdom). Urethane was purchased from Fluka Chemical, Poole, United Kingdom, and pentobarbitone (Sagatal®) was obtained from Rhone Merieux, Harlow, United Kingdom.

Instrumentation

Rocuronium. The liquid chromatography–mass spectrometry/mass spectrometry bioanalysis was performed on a Sciex 3000 mass spectrometer equipped with Turbo-Ion spray (Warrington, United Kingdom) and Perkin Elmer series 200 Micro LC pumps, autosampler, column oven, and six-port switching valve (Beaconsfield, United Kingdom). The full bioanalysis was as described by Epemolu *et al.*[11] Briefly, the chromatographic conditions were as follows: the column used was a Jupiter C4 (50 × 4.6 mm) from Phenomenex (Cheshire, United Kingdom); the flow rate was 1.3 ml/min with a split ratio of 10:1 waste/mass spectrometer; the solvents were 20 mM ammonium acetate and acetonitrile; the gradient was 90% of 20 mM ammonium acetate for the first 1.0 min, dropping to 10% by 2.5 min. This was maintained for the next 1.0 min before being allowed to equilibrate for 0.5 min more at its initial composition. The run time was 4 min, and the injection volume was 10 μl.

The interface temperature was 300°C, and the ionization source was Turbo-Ion spray operated in positive mode. The Turbo Gas flow was set to 6 l/min. The multiple reaction-monitoring transitions used for rocuronium and Org 24748 (the internal standard) were mass-to-charge ratio (m/z) 528 to m/z 487 and m/z 310 to m/z 91, respectively. The dwell time was 200 ms, and the tuning conditions were optimized for each compound by infusion of a 1 μg/ml solution.

The assay was linear and reproducible over the range of 25–10,000 ng/ml for both plasma and urine. The lowest limit of quantification in urine and plasma was 25 ng/ml. The interday and intraday variation was lower than 20%.

Animal Preparation

All the animal work reported in this article was performed according to the United Kingdom Home Office Animals Scientific Procedures Act 1986.

Twelve male Dunkin-Hartley guinea pigs (Harlan, Bicester, United Kingdom) weighing approximately 700–850 g were anesthetized with urethane (900 mg/kg) and pentobarbitone sodium (30 mg/kg). The guinea pigs were artificially ventilated with ambient air (0.75 ml/100 g and 60 strokes/min). Arterial blood pressure was measured with a blood pressure transducer connected to a cannula placed in the left carotid artery. Heart rate was derived from the pressure signal. A cannula was placed in each jugular vein for the infusion of rocuronium and saline or Org 25969, respectively. Gastrocnemius muscle contractions were induced by single-twitch stimulation of the sciatic nerve at supramaximal voltage, at a frequency of 0.1 Hz and a pulse width of 0.25 ms.

Six animals were allocated to the Org 25969-treatment group and 6 animals were assigned to the saline group to serve as controls.

Experimental Protocols

After surgery, animals were allowed to stabilize for 30 min.

Protocols 1 and 2. The infusion of rocuronium was started at a rate of 12 nmol·kg^{-1}·min^{-1} and was subsequently increased to obtain a steady-state 90% neuromuscular block of gastrocnemius contractions.[4–6,8] The infusion lasted for 60 min.

Protocol 1. After rocuronium had been infused for 30 min, a second infusion was started with saline at a rate of 160 μl/min while the rocuronium infusion was continued (fig. 2a). After 30 min of saline infusion, the animal was killed with an overdose of anesthetic. The abdomen was opened, and the urine in the bladder was collected.

Protocol 2. After rocuronium had been infused for 30 min, a second infusion was started with Org 25969 at a rate of 50 nmol·kg^{-1}·min^{-1} (160 μl/min) while the rocuronium infusion was continued (fig. 2b). After 30 min of Org 25969 infusion, the animal was killed with an over-

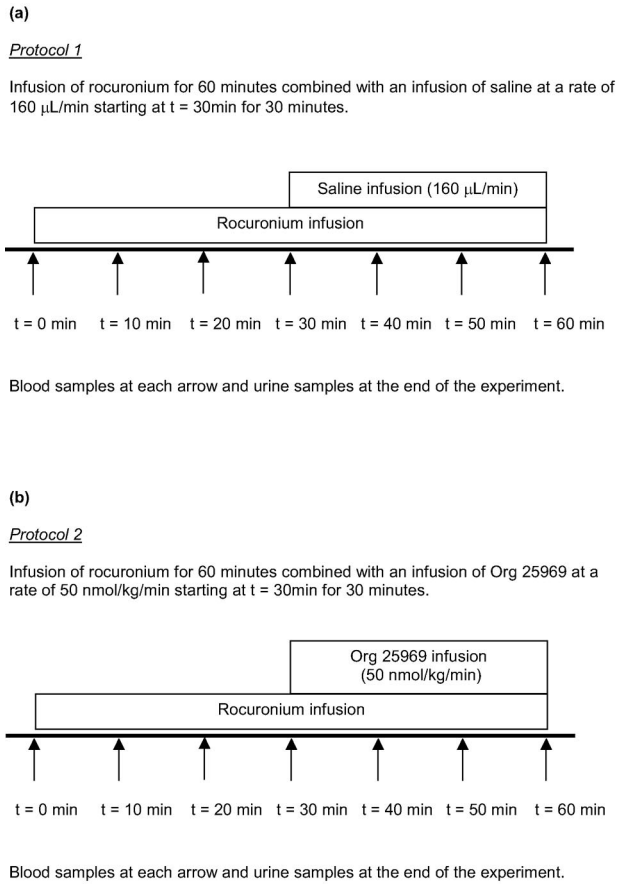

Fig. 2. Schematic diagrams of the infusion protocols.

dose of anesthetic. The abdomen was opened and the urine in the bladder was collected.

Arterial blood samples were taken at 10-min intervals over the 60-min infusion period, and the cannulae were flushed with approximately 300 μl of heparinized saline (100 U/ml). The blood samples (300 μl) were collected into tubes with EDTA and subsequently processed for plasma, which were stored frozen at −20°C until analyzed.

The urine volumes were measured, and the samples were stored frozen at −20°C until analyzed.

Vehicles Used

The vehicle used for rocuronium bromide was phosphate buffer for injection (Department of Pharmacy, Organon NV, Oss, the Netherlands). The vehicle used for Org 25969 was saline.

Statistics

The results are presented as mean value ± SD. The levels of significance of differences in parameters between two treatment groups were determined by use of the unpaired Student *t* test.

Differences between more than two measurements were analyzed by one-way ANOVA. When one-way ANOVA revealed significant differences ($P < 0.05$) between groups, the Duncan new multiple range procedure was followed.

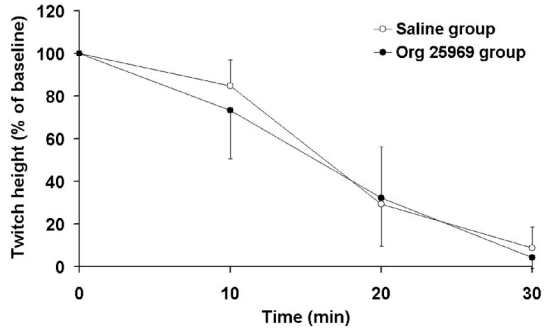

Fig. 3. Twitch height against time for the first 30 min of infusion for both the saline- and Org 25969–treated groups.

Results

Twitch Height and Plasma Concentration of Rocuronium

At the beginning of each protocol, rocuronium was infused at a rate of 12 nmol·kg^{-1}·min^{-1}. The infusion rate was adjusted to obtain steady-state 90% block within 30 min of the infusion. The twitch height and plasma concentration of rocuronium of both groups of animals during the first 30 min of rocuronium infusion are shown in figures 3 and 4, respectively. No statistically significant differences in twitch height and plasma rocuronium concentrations were observed between the two groups. There appears to be more variation in the group that received Org 25969 later during the experiment, which can be explained by the variation in the dose required to achieve 90% neuromuscular block in each guinea pig. This phenomenon is not uncommon, because this has also been described by Sparr *et al.*,[12] who showed that the infusion dose of rocuronium required to maintain 80% neuromuscular block varied fourfold in patients.

Table 1 shows the body weights and infusion rates needed to obtain steady-state 90% block of twitch height of gastrocnemius muscle contractions. No statistically

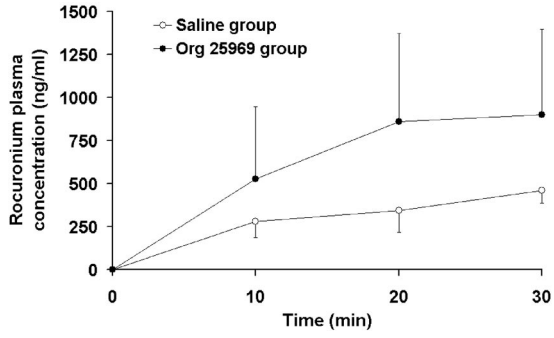

Fig. 4. Rocuronium plasma concentration against time for the first 30 min of infusion for both the saline- and Org 25969–treated groups.

Table 1. Body Weight and Infusion Rate Required to Obtain 90% Block of Twitch Height

	Saline-treated Group		Org 25969-treated Group		
	Mean ± SD	Range	Mean ± SD	Range	*P* Value
Body weight, g	778 ± 37	720–814	784 ± 14	765–795	0.70
Rocuronium infusion rate, nmol · kg^{-1} · min^{-1}	14.4 ± 2.9	12.0–18.8	14.6 ± 2.5	12.0–18.8	0.88

n = 6 animals in each treatment group. *P* value determined with the unpaired *t* test.

significant differences between the two treatment groups were observed.

After 30 min of rocuronium infusion, a parallel infusion was started in the two groups with saline or Org 25969, respectively. The twitch heights (expressed as percentage of baseline twitch height) during the infusion with saline or Org 25969 are shown in table 2.

In the saline-treated group, no significant changes in twitch height were observed. In contrast, infusion of Org 25969 (at a rate of 50 nmol·kg^{-1}·min^{-1}) caused a marked increase in twitch height over time.

The plasma concentration of rocuronium was also determined during the infusion of saline and Org 25969, respectively (table 3). In the saline-treated group, no change in plasma concentration of rocuronium was observed, confirming the steady-state situation. In the Org 25969–treated group, a marked increase in plasma concentration of rocuronium (both free and complexed to Org 25969) was observed after 50 and 60 min of infusion of rocuronium (corresponding to 20 and 30 min after the start of the Org 25969 infusion).

Figures 5 and 6 demonstrate the relationship between twitch height and plasma concentration of rocuronium in the two different treatment groups. In the saline-treated group, no significant changes in either twitch height or plasma concentration of rocuronium occurred during the saline infusion (fig. 5). In the Org 25969–treated group, a rapid reversal of twitch height occurred within 10 min after the start of the infusion. The plasma concentration of rocuronium (free and complexed to Org 25969) doubled within the 30 min of Org 25969 infusion (fig. 6).

Hemodynamic Effects

The baseline mean arterial blood pressure and heart rate values measured at the start of the rocuronium infusion are shown in table 4. No differences in baseline blood pressure and heart rate values were detected between the two treatment groups. No significant changes in hemodynamic parameters were observed in either experiment.

Amount of Rocuronium in Urine

The urine volume obtained after postmortem collection was 3.8 ± 2.0 ml in the saline-treated group *versus* 4.1 ± 2.0 ml in the Org 25969–treated group, which was not significantly different. However, in two animals of the saline-treated group and one animal in the Org

Table 2. Twitch Height Expressed as Percentage of Baseline Twitch Height

Time after Start of Rocuronium Infusion	Saline-treated Group		Org 25969-treated Group		
	Mean ± SD	Range	Mean ± SD	Range	*P* Value
After 30 min	8.7 ± 10.1	0.0–23.3	4.4 ± 5.2	0.0–11.3	0.39
After 40 min	4.1 ± 10.0	0.0–24.5	81.5 ± 22.9*	41.7–108.5	0.0001
After 50 min	2.8 ± 6.9	0.0–17.0	92.1 ± 21.0*	52.1–108.5	0.0001
After 60 min	1.6 ± 3.9	0.0–9.4	100.4 ± 7.4*	90.6–109.3	0.0001

n = 6 animals in each treatment group. *P* value determined with the unpaired *t* test.

* Significantly ($P < 0.05$) different from twitch height after 30 min (one-way ANOVA followed by Duncan's new multiple range test).

Table 3. Rocuronium Plasma Concentration during Infusion of Either Saline or ORG 25969

	Saline-treated Group		Org 25969-treated Group		
Rocuronium, ng/ml	Mean ± SD	Range	Mean ± SD	Range	*P* Value
After 30 min	460 ± 74	364–578	899 ± 497	436–1665	0.08
After 40 min	520 ± 140	329–743	1379 ± 472	857–2093	0.006
After 50 min	503 ± 163	342–799	1640 ± 563*	1022–2564	0.003
After 60 min	463 ± 98	298–580	1833 ± 501*	1155–2705	0.001

n = 6 animals in each treatment group. *P* value determined with the unpaired *t* test.

* Significantly ($P < 0.05$) different from plasma concentration after 30 min (one-way ANOVA followed by Duncan's new multiple range test).

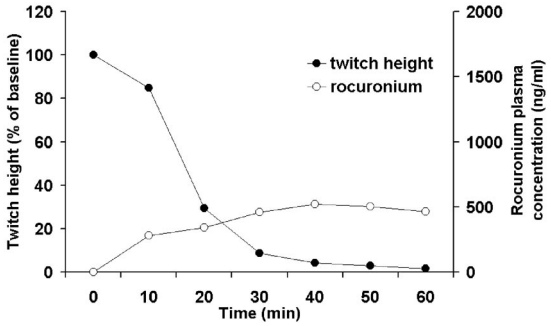

Fig. 5. Relationship between rocuronium plasma concentration and twitch height for the saline-treated group.

25969–treated group, no urine was present in the bladder, probably because of spontaneous voiding during the experiment. The total amount of rocuronium in the urine samples was 5,554 ± 8,394 ng in the saline-treated group compared with 14,255 ± 13,683 ng in the Org 25969–treated group, which was also not significantly different.

Discussion

Using a similar infusion rate of rocuronium bromide, steady-state 90% block of twitch height and plasma concentration of rocuronium was achieved within 30 min in both groups of six animals. Infusion of saline during continuous infusion of rocuronium did not cause a change in twitch height or plasma concentration of rocuronium.

Infusion of Org 25969 at a rate of 50 nmol·kg^{-1}·min^{-1} caused rapid reversal of neuromuscular block, combined with an increase in plasma concentration of rocuronium (free and bound to Org 25969). The rate of 50 nmol·kg^{-1}·min^{-1} Org 25969 was chosen on the basis of the assumption that Org 25969 not only had to form complexes with the concomitantly infused rocuronium molecules but also had to form complexes with the rocuronium molecules infused during the first 30 min. The rapid return of muscle twitch height to baseline values, together with an increase in plasma concentration of rocuronium, suggests that the increase in rocuronium concentration is not caused by an increase in free rocuronium molecules but rather by an increase in rocuronium molecules complexed to Org 25969. Unfortunately, no method is yet available to distinguish between free and bound rocuronium in plasma.

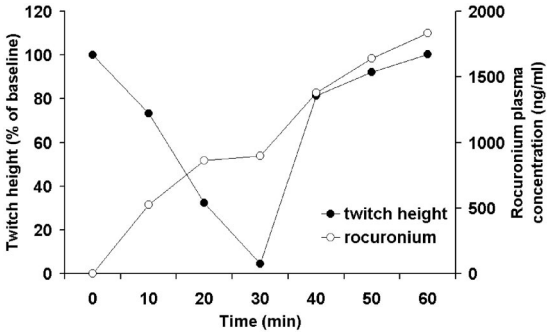

Fig. 6. Relationship between rocuronium plasma concentration and twitch height for the Org 25969–treated group.

The proposed mechanism of action of Org 25969 as a reversal agent is based on capture of free rocuronium molecules in plasma, resulting in a rapid decrease in plasma concentration of free rocuronium. This creates a concentration gradient between free rocuronium molecules in the tissue compartment and the central compartment. The tissue compartment consists of both the effect compartment (neuromuscular junction) and noneffect tissue compartment. As a result, free rocuronium molecules will rapidly return into the central compartment, *i.e.*, plasma, where these molecules are also caught by Org 25969. This leads to a rapid decrease in the occupation of the postsynaptic nicotinic acetylcholine receptors in the neuromuscular junction, resulting in reversal of neuromuscular function.

Org 25969 or saline infusion did not cause any significant hemodynamic changes, which is in good accordance with earlier observations.[4–8]

Another contributory factor is the renal clearance of the Org 25969–rocuronium complex. In the absence of Org 25969, only a limited amount of rocuronium (less than 20%) is excreted *via* the renal route.[13] It has been demonstrated that Org 25969 is excreted rapidly and dose-dependently in the urine of anesthetized guinea pigs.[11] Therefore, the excretion of captured rocuronium *via* the urine will be increased markedly. However, this could not be the main mechanism of action, because the plasma concentration of rocuronium (both free and bound to Org 25969) doubled.

Because some of the animals voided spontaneously during the experiment, it is not possible to make con-

Table 4. Baseline Mean Arterial Blood Pressure and Heart Rate at the Start of the Infusion

	Saline-treated Group		Org 25969-treated Group		
	Mean ± SD	Range	Mean ± SD	Range	*P* Value
MAP, mmHg	42 ± 8	36–56	39 ± 3	33–42	0.35
HR, beats/min	263 ± 15	240–280	287 ± 38	225–330	0.19

n = 6 animals in each treatment group. *P* value determined with the unpaired *t* test.

HR = heart rate; MAP = mean arterial blood pressure.

clusions about the amount of rocuronium excreted *via* the urine. However, the amount of rocuronium found in the urine of the Org 25969–treated group tended to be higher.

Conclusions

In summary, Org 25969 infusion caused a marked increase in the total plasma concentration of rocuronium by capture of rocuronium molecules by Org 25969 molecules. The redistribution of rocuronium from the effect compartment to the central compartment explains the rapid reversal activity of Org 25969.

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