

Effects of SZ1677, a new non-depolarizing steroidal neuromuscular blocking drug, and rocuronium on two laryngeal muscles and the anterior tibial muscle in guinea pigs

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Background: SZ1677 is a new neuromuscular blocking drug structurally related to rocuronium. We compared the effect of an ED₉₀ of SZ1677 (25 µg/kg) with that of rocuronium (100 µg/kg) in guinea pig laryngeal and peripheral muscles.

Methods: Electromyography was used to quantify neuromuscular blockade at the posterior cricoarytenoid muscle, the thyroarytenoid muscle and the anterior tibial muscle after SZ1677 ($n = 10$) and rocuronium ($n = 9$).

Results: Maximum neuromuscular blockade was similar after SZ1677 and rocuronium (83 ± 11% vs. 89 ± 11%; thyroarytenoid muscle: 91 ± 8% vs. 97 ± 3%; anterior tibial muscle: 91 ± 15% vs. 96 ± 3%, respectively). Onset time of neuromuscular blockade at the laryngeal muscles was similar for the two neuromuscular blocking drugs; it was shorter at the thyroarytenoid muscle (67 ± 32 s vs. 42 ± 40 s) than at the posterior cricoarytenoid muscle (101 ± 26 s vs. 102 ± 108 s). Onset time at the anterior tibial muscle was longer after SZ1677 (114 ± 34 s) than after rocuronium (68 ± 46 s); $P < 0.05$. Neuromuscular recovery was faster after SZ1677 (interval 25%–75%: posterior

cricoarytenoid muscle: 222 ± 66 s; thyroarytenoid muscle: 192 ± 92 s; tibial muscle 149 ± 55 s) than after rocuronium (450 ± 148 and 464 ± 183 s, 292 ± 86 s, respectively); $P < 0.05$.

Conclusions: In guinea pigs, SZ1677 offers a rapid onset of neuromuscular blockade at a laryngeal adductor muscle with a shorter duration than rocuronium. Regardless of the drug used, the course of neuromuscular blockade differs not only between peripheral muscles and the larynx but also between antagonistic laryngeal muscles. The differences seem to be species specific.

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SZ1677, a new steroidal non-depolarizing neuromuscular blocking drug, has recently been described. SZ1677 [1-(3 α -hydroxy-17 β -acetyloxy)-2 β -(1,4-dioxo-8-azaspiro-(4.5)dec-8-yl)-(5 α -androstane-16 β -yl)-1-(2-propenyl)], a pyrrolidinium bromide synthesized by Gedeon Richter Ltd (Budapest, Hungary), is structurally related to rocuronium, at present the only clinically available non-depolarizing neuromuscular blocking drug with a short onset time. SZ1677 has a rapid onset and a short duration of neuromuscular blockade in various laboratory animal species (1). The main metabolite of SZ1677 is its 17-OH derivative SZ1823, which is 27.5 times less potent than SZ1677. In the rat and the guinea pig, the duration of SZ1677 at the anterior tibial muscle did not change

with the administration of three repeated ED₉₀ doses, leading to the conclusion that SZ1677 is without cumulative effect (1). SZ1677 has less of a pre-synaptic inhibitory effect on acetylcholine release than rocuronium (2). SZ1677 has no effects on blood pressure or heart rate and, in doses up to 8 × ED₉₀, has no cardiac vagal blocking effects (1). Furthermore, SZ1677 does not increase norepinephrine release from human atrial tissue (3) and does not have antimuscarinic effects in *in vitro* experiments (4).

Ideally, pharmacodynamic characteristics of neuromuscular blocking drugs should be tested at the muscles most relevant for anaesthetic practice, e.g. the laryngeal muscles (as in this study) and the diaphragm. Complete vocal cord paralysis facilitates

intubation of the trachea and is necessary for surgical procedures at the larynx; conversely, inadequate neuromuscular recovery at the larynx, during emergence from anaesthesia, compromises airway patency and increases the risk of aspiration. In general, neuromuscular blocking drugs are investigated in easily accessible peripheral muscles, though it is well documented that different muscles differ with respect to the degree and time course of neuromuscular blockade (5).

As neuromuscular blockade at laryngeal muscles is of special interest to the anaesthesiologist, we compared the effects of SZ1677 at the anterior tibial muscle (TIB) and two antagonistic laryngeal muscles, the abducting posterior cricoarytenoid muscle (POST) and the adducting thyroarytenoid muscle (TA), with those of an equipotent dose of rocuronium in guinea pigs. This is the first study to compare the effects of neuromuscular blocking drugs on antagonistic laryngeal muscles in this animal model.

Methods

The study was approved by the University's committee on animal research (GZ 66 009/321-I/A/2/93). Twenty male Dunkin-Hartley guinea pigs (mean weight 790 ± 90 g) were used for the study. After premedication with intramuscular atropine (0.05 mg/kg), anaesthesia was induced with intramuscular ketamine hydrochloride 100 mg/kg, pentobarbitone sodium 37 mg/kg and urethane 250 mg/kg intraperitoneally. Intermittent intravenous bolus doses of pentobarbitone sodium (3–8 mg/kg) and fentanyl 5 μ g/kg were used to maintain anaesthesia.

A tracheotomy was performed in the caudal third of the trachea to allow insertion of an uncuffed tube. The lungs were ventilated with N_2O/O_2 (70%/30%) by means of a pneumatic driven ventilator for small animals. Tidal volume and ventilation frequency were adjusted individually to keep blood gases within physiological ranges (P_aCO_2 4.5–5.8 kPa, $P_aO_2 > 13.3$ kPa). Fluid loss was balanced with an infusion of Ringer's solution (3 ml/kg/h). Sodium bicarbonate was administered intravenously in cases of metabolic acidosis. A catheter was placed in the right carotid artery to record arterial pressure and for blood sampling. Rectal and laryngeal surface temperature were measured continuously and kept constant at $> 36^\circ C$ by a heated water mattress and a heating lamp.

At the end of the experiments, the animals were killed with an intravenous overdose of pentobarbitone. Technical details of the muscle and nerve preparation and the neuromuscular monitoring have been described previously (6) and are summarized below.

Preparation of the sciatic nerve and anterior tibial muscle

Briefly, the sciatic nerve was exposed and identified by electrical stimulation to facilitate correct placement of a bipolar clip stimulation electrode. The hind leg was then rigidly fixed in a half-bent position applying 50–80 g pretension to the TIB's tendon. To record the evoked electromyogram (EMG), Ag/AgCl electrodes were placed in subcutaneous pouches at the belly and origin of the TIB.

Preparation of the recurrent laryngeal nerve and laryngeal muscles

The right recurrent laryngeal nerve was exposed approximately 1 cm caudal to the cricoid cartilage and placed on a bipolar platinum electrode. The trachea was dissected and cut at the level of the 7th–8th tracheal cartilage. The posterior surface of the larynx was then carefully exposed and an electrode for the EMG recording (platinum wire 0.2 mm in diameter, insulated shaft, uninsulated blank tip) was placed in the POST under direct visual control. A similar electrode was advanced from the inferior margin of the thyroid cartilage through the cricothyroid membrane into the TA.

The indifferent electrodes for the EMG of the POST and TA were placed laterally in the muscles originating from the hyoid bone. The EMG electrode positions were considered adequate when the EMG signals were of maximum amplitude and biphasic with minimum stimulation artefacts. Before administration of neuromuscular blocking drugs, a stabilization period of 20–25 min was observed. The POST and TA electrode positions were confirmed after each experiment at a post-mortem examination.

Nerve stimulation

The sciatic and recurrent laryngeal nerve stimulation was synchronized using two separate, optically isolated nerve stimulators. Their stimulation pulses were separated by a time interval of 20–30 ms to avoid stimulation artefacts being picked up in the laryngeal EMG recordings. Supramaximal constant current pulses (1–3 mA, 0.2 ms square wave) were delivered at a rate of 0.1 Hz. Additionally, 30 s prior to relaxant administration, and also intermittently every 3 min during neuromuscular blockade, train-of-four stimulation (four pulses within 1.5 s) was applied.

Recording

The evoked EMG signals were amplified using electrically isolated pre-amplifiers (ISODAM-B, WPI,

Sarasota, FL) and, after further preprocessing in a multichannel transient recorder, charted on a Gould 3800 fast pen system (Gould, Cleveland, OH). Peak-to-peak amplitude of the evoked EMG was used to evaluate neuromuscular blockade. Arterial blood pressure and airway pressure were recorded continuously.

Administration of neuromuscular blocking drugs

Ten guinea pigs received bolus doses of SZ1677 ($ED_{90} = 25 \mu\text{g}/\text{kg}$; ED_{90} defined as a dose producing a 90% blockade at the TIB as measured by EMG); nine guinea pigs were given rocuronium (Esmeron®; $ED_{90} = 100 \mu\text{g}/\text{kg}$ N.V. Oraganon, Oss, Netherlands) (1). The neuromuscular blocking drugs were injected over a period of 5 s into the jugular vein into a running infusion of lactated Ringer's solution.

In accordance with GCRP guidelines (7), maximum drug-induced depression, onset time (defined as time from beginning of injection to maximum blockade or, in the case of $\geq 95\%$ neuromuscular block, to 95% blockade) and duration from start of injection to 25%, 50%, 75%, and 90% twitch recovery were evaluated from the charted EMG responses. Recovery indices are given as an interval of 25%–75%, i.e. duration of recovery between 25% and 75% twitch height. Calculations of recovery times were referenced to the final EMG, as the final EMG did not always recover to 100% of pre-drug EMG amplitude (8).

Statistical analysis

Data are presented as the mean (\pm standard deviation). The Kruskal–Wallis test was used for

statistical analysis. For statistical comparison of individual muscles within the respective drug group, a non-parametric test (Wilcoxon's difference test) was used. To compare the effect of the different drugs, Wilcoxon's test for unpaired samples was used. Statistical significance was accepted at $P < 0.05$. Assuming a difference of $\geq 20\%$ of the mean, we calculated the statistical power ≥ 0.8 in respect to maximal block. As a result of the large interindividual variations, statistical power was lower for onset and recovery parameters. All calculations were performed using UNISTAT 5.014 software (Unistat Ltd., London, UK).

Results

One guinea pig in the rocuronium group died before the administration of rocuronium. In another guinea pig in this group laryngeal EMG signals could not be recorded because of technical difficulties.

In all the animals investigated, haemodynamic and respiratory variables remained within the physiological range throughout the experiments, with no statistical differences between the SZ1677 and the rocuronium groups. The EMG signals recorded fulfilled the criteria of stability as outlined (7), were stable with respect to their biphasic waveform and, after recovery, corresponded with the respective control curve.

Degree of neuromuscular blockade (Table 1)

The degree of neuromuscular blockade induced by SZ1677 did not differ from that induced by

Table 1

Maximum blockade and onset time after the administration of SZ1677 25 $\mu\text{g}/\text{kg}$ and rocuronium (ROC) 100 $\mu\text{g}/\text{kg}$.

Drug		Maximal block (%)		Onset time (s)	
		SZ1677 (25 $\mu\text{g}/\text{kg}$)	ROC (100 $\mu\text{g}/\text{kg}$)	SZ1677 (25 $\mu\text{g}/\text{kg}$)	ROC (100 $\mu\text{g}/\text{kg}$)
TIB	mean	91	96	114*	68§
	SD (range)	16 (47–100)	3 (92–100)	34 (70–180)	46 (30–150)
	<i>n</i>	10	9	10	9
POST	mean	83†	89†	101‡	102
	SD (range)	11 (63–97)	11 (64–98)	26 (50–130)	108 (20–330)
	<i>n</i>	10	8	10	8
TA	mean	91	97‡	67	42
	SD (range)	8 (78–100)	3 (92–100)	32 (30–110)	40 (20–140)
	<i>n</i>	10	8	10	8

Results are presented as the mean, standard deviation (SD) and range.

n = number of experiments evaluated.

Statistical significance ($P < 0.05$): *TIB vs. TA; †POST vs. TIB; ‡POST vs. TA; §ROC vs. SZ1677.

TIB, anterior tibial muscle; POST, posterior cricoarytenoid muscle; TA, thyroarytenoid muscle.

Onset time given in seconds (s).

rocuronium in the muscles investigated. Maximal neuromuscular blockade was significantly lower at the POST than at the TIB. In addition, a significant difference between the POST and the TA with regard to maximal block was observed after rocuronium.

Onset time (Table 1)

Onset time was shorter after rocuronium than after SZ1677 at the TIB. At the laryngeal muscles, onset time was similar after the two neuromuscular blocking drugs. With SZ1677 onset time was shortest at the TA.

Recovery from neuromuscular blockade (Table 2)

Neuromuscular recovery was adequate at all muscles, as evidenced by a train-of-four (TOF) ratio of > 0.95 at the end of recovery. Neuromuscular recovery was faster after SZ1677 than after rocuronium in all muscles investigated. Complete neuromuscular recovery tended to take longer at the laryngeal muscles than at the TIB, although the duration of neuromuscular recovery to 25%, 50%, 75% and 90% did not differ significantly between the muscles.

Discussion

In this study, we compared the neuromuscular effects of an ED₉₀ dose of SZ1677 and rocuronium in antagonistic laryngeal muscles, the POST, the TA and the TIB in guinea pigs; thus, the effects of SZ1677 at different muscle groups were studied *in vivo* for the first time. We chose guinea pigs because the neuromuscular effects of steroidal neuromuscular blocking drugs in this species are considered to closely parallel those in humans, as pointed out by Nitahara et al. (9). The choice of laryngeal muscles was based on functional and technical considerations. In both animals and humans, the POST is the only vocal cord abductor. From among the laryngeal adductor muscles, the TA was selected for easy accessibility in the model used.

Comparison by the drugs investigated

After administration of SZ1677 25 µg/kg, the degree of neuromuscular blockade was similar to that after rocuronium 100 µg/kg at all three muscles. This finding confirms the ED₉₀ of SZ1677 as reported by Vizi et al. (1). It also indicates that the ED₉₀ doses of the two neuromuscular blocking drugs investigated were also equipotent not only at the peripheral muscle, the TIB, but at the laryngeal adductor and abductor muscles. At the laryngeal muscles, which are of greater interest for anesthetic practice (10), the

Table 2

Drug	Recovery 25% (s)		Interval 25%–75% (s)		Recovery 90% (s)		Final TOF ratio	
	SZ1677 (25 µg/kg)	ROC (100 µg/kg)	SZ1677 (25 µg/kg)	ROC (100 µg/kg)	SZ1677 (25 µg/kg)	ROC (100 µg/kg)	SZ1677 (25 µg/kg)	ROC (100 µg/kg)
TIB	mean 290 SD (range) 87 (180–440) n 9	580‡ 205 (300–990) 9	149 55 (90–250) 9	292*‡ 86 (130–390) 9	500 142 (310–720) 10	1060‡ 287 (480–1830) 9	1.00 0.01 (0.98–1.01) 10	0.99 0.01 (0.98–1.00) 8
POST	mean 230 SD (range) 82 (140–400) n 8	440‡ 305 (220–770) 7	222‡ 66 (110–310) 8	450‡ 148 (230–620) 7	510 158 (260–690) 10	1110‡ 447 (630–1580) 8	0.99 0.01 (0.96–1.00) 10	0.99 0.02 (0.97–1.01) 8
TA	mean 230 SD (range) 101 (120–450) n 10	680‡ 235 (330–990) 8	192 92 (80–390) 10	464‡ 183 (200–680) 8	580 175 (350–920) 10	1430‡ 551 (640–2190) 8	0.99 0.01 (0.97–1.01) 10	1.00 0.00 (1.00–1.01) 9

Results are presented as the mean, standard deviation (SD) and range. n = number of experiments evaluated. Interval 25%–75% = duration of recovery between 25% and 75% twitch height, recovery 25%, interval 25–75% and recovery 90% given in seconds (s). Final train-of-four (TOF) = TOF-ratio (T₄/T₁) at the end of recovery. Statistical significance (P < 0.05): *TIB vs. TA ; †TIB vs. TIB; ‡ROC vs. SZ1677.

onset of SZ1677 was similar to that of rocuronium. At the TIB, onset time was shorter after rocuronium than after SZ1677. In all three muscles, neuromuscular recovery was faster after SZ1677 than after rocuronium.

Currently, rocuronium is the only clinically available non-depolarizing neuromuscular blocking drug with a short onset time. Rocuronium also has an intermediate duration of effect. According to our results, SZ1677 combines a rapid onset at the clinically important laryngeal muscles, and, in contrast to rocuronium, a fast neuromuscular recovery. This is especially of interest in shorter surgical procedures. Therefore, SZ1677 could be a step towards the 'ideal' neuromuscular blocking drug as postulated by Savarese and Kitz (11).

Comparison by the muscles investigated

Another aim of this study was to compare the effects of the two neuromuscular blocking drugs at a peripheral muscle (i.e. the TIB) and at antagonistic laryngeal muscles. There are numerous clinical studies that investigated the effects of various neuromuscular blocking drugs at the larynx by relating the force of the contraction of laryngeal adductor muscles to changes in the cuff of an endotracheal tube placed between the vocal cords (5). However, it is not possible with this method to quantify the effect of neuromuscular blocking drugs on *individual* laryngeal muscles. These studies revealed, as a rule, a smaller degree and a more rapid onset of neuromuscular blockade at the larynx than at peripheral muscles. Recovery at laryngeal muscles usually occurred sooner than at the adductor pollicis (5). Though the cuff pressure method is now considered to be the gold standard in quantifying laryngeal neuromuscular blockade, this technique has certain limitations (12), and it measures the combined forces of both the adductor and abductor laryngeal muscles after simultaneous electric stimulation, which is non-physiological.

Studies that do compare the specific effects of neuromuscular blocking drugs on individual or antagonistic laryngeal muscles are few in number. These studies used different techniques, e.g. needle EMG (13–17), and more recently, phonomyography (18) and have been conducted in different species. Our investigation is the first to describe the effects of neuromuscular blocking drugs at guinea pig antagonistic laryngeal muscles.

We found that SZ1677 and rocuronium had parallel neuromuscular effects at the individual muscles: For both drugs, neuromuscular blockade was less

and onset time was longer at the POST than at the TA. This would suggest that in guinea pigs the POST is more resistant to the effects of neuromuscular blocking drugs than is the laryngeal adductor muscle TA. Neuromuscular recovery after either neuromuscular blocking drug tended to take longer at the two antagonistic laryngeal muscles than at the TIB. These results in guinea pigs are to a certain degree at variance with data from other investigations using other animal models (14–16, 19). In our guinea pig experiments, maximum neuromuscular blockade at the TA was similar to that at the TIB after both neuromuscular blocking drugs. Thus, the current assumption (5) of a lesser degree of neuromuscular blockade at laryngeal adductor muscles than at peripheral muscles does not seem to hold true for all species.

Our results in guinea pigs are in accord with most human studies as far as onset is concerned. The short onset time at the centrally located laryngeal muscles is generally attributed to a higher blood flow than in peripheral limb muscles (5, 20). Again, in cats rocuronium and vecuronium revealed a different profile, as onset of neuromuscular blockade at the adductor laryngeal muscles was slower than at the POST and TIB (14).

The pattern of neuromuscular recovery in our guinea pig study, with a somewhat slower offset of neuromuscular blockade at the antagonistic laryngeal muscles than at the peripheral muscle TIB, has not been reported in either human or other animal studies. In humans, laryngeal adductor muscles usually recover faster than the adductor pollicis (5), whereas recovery at the POST is slower than at the lateral cricoarytenoid muscle (18). In cats, laryngeal adductor muscle recovery was longer than at the TIB and POST after vecuronium and rocuronium (14).

Discussion of discrepant study results

Several explanations for these discrepant findings may be considered. First, methodology should be considered. We do not think that the results of this study were influenced by our surgical technique, because laryngeal integrity was not destroyed. Further, electrode displacement, an inherent risk of needle EMG, is unlikely to have occurred during our experiments, as the EMG signals were stable and the biphasic form of the EMG signal returned to baseline waveform after each experiment. The comparisons between laryngeal and limb muscle EMG should therefore be valid.

With the EMG technique, especially when needles are used, it is possible to measure the evoked responses of single muscles. As electromyography and mechanomyography, accelerography or phonomyography measure different physical phenomena, the estimation of neuromuscular blockade will also differ to a variable degree (21). A direct comparison of data derived by different methods is difficult and awaits further studies.

Second, the pharmacodynamics of neuromuscular blocking drugs are studied in different peripheral muscles in animals and humans. In animal studies, drug effects are tested preferably at the TIB. In clinical practice, neuromuscular blockade is usually monitored at the adductor pollicis muscle. While both muscles are peripheral or limb muscles, it is by no means clear that their course of neuromuscular blockade is equivalent as no study so far provided a direct comparison.

Third, species differences are to be considered. Although it is not yet fully understood which properties are responsible for the different sensitivity of individual muscles, interspecies differences in muscle fibre microanatomy certainly exist at laryngeal and also at peripheral muscles. Considerable interspecies variation in the proportion of type I and type II fibres in different laryngeal muscles (22), unique types of laryngeal muscle fibres not commonly found in other skeletal muscles (22), multi-motor end-plate fibres, found to a varying degree in laryngeal muscles, variable muscle fibre diameter (23) and differences in end-plate size to fibre size ratio (24) are all factors that might influence the sensitivity of muscles to neuromuscular blocking drugs. In addition, the unequal sensitivity of the POST and lateral cricoarytenoid muscle to d-tubocurarine as tested in rats has been suggested to be caused by different sensitivities at pre- and post-synaptic sites of the neuromuscular junction in this species (19). Species differences certainly are the most likely explanation for the different results obtained, with an almost identical study design in our guinea pig and cat experiments. In this context, it is interesting to note that Iwasaki et al., using a similar study design in humans (17) and dogs (16), also found differing results. In humans, the adductor pollicis muscle was more sensitive than either the cricothyroid muscle or the POST, whereas in dogs, the cricothyroid muscle was more sensitive and recovered more slowly than the peripheral TIB.

We conclude that in guinea pigs, SZ1677 shows a favourable profile of neuromuscular blockade, combining a rapid onset of blockade at the laryngeal muscles and a short duration of action. The differences in laryngeal muscle neuromuscular blockade

in the guinea pig, regardless of the neuromuscular blocking drug, seem to be species specific.

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