

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

**New perspectives in antagonizing postoperative residual
neuromuscular block**

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The Examination takes place at Department of Anatomy, Histology and Embriology,
University of Debrecen on 7th of December 2015 at 11 a.m.

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The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal
Medicine, University of Debrecen on 7th of December 2015 at 13 p.m.

1. INTRODUCTION

D-tubocurarine, derived from curare, was first used in humans for surgical muscle relaxation by anesthetists Harold Griffith and Enid Johnson from Montreal in 1942, thereby opening a new chapter in the history of anesthesiology and surgery. Muscle relaxation, as part of the balanced anesthesia, quickly became popular all over the world. The use of muscle relaxants are now an elemental part of modern anesthesiology. Anesthesiologists are not only responsible for establishing and maintaining the proper depth of muscle relaxation for surgical interventions, but also to ensure that the patient's muscle power completely returns at the end of surgery. Postoperative residual neuromuscular block (PRNB) occurs when the patient is still affected by the muscle relaxant following the extubation at the end of the intervention.

Not only severe (TOFR <0.5), but also shallow (0.5 <TOFR <0.9) postoperative residual neuromuscular block could be a source of life-threatening complications. The reason for this is that muscles of the body are susceptible to non-depolarizing muscle relaxants in different degrees. The diaphragm is the least susceptible, which movement will reappear at a value of TOFR <0.5, and respiratory tidal volume will normalize. The striated muscle of the pharynx, tongue and upper third of esophagus are the most sensitive to muscle relaxants. The strength of these muscles normalizes only after the TOF ratio exceeds 0.9. Therefore, superficial (0.5 <TOFR <0.9) postoperative residual neuromuscular block is associated with swallowing disturbances, which can lead to aspiration. It has been shown that ventilator-associated pneumonia in intensive care units is caused by the so-called "silent" pulmonary aspiration. The same mechanism may be involved in the onset of post-operative pneumonia as well. The upper respiratory tract's "tendency to collapse" increases due to the dysfunction of the m. genioglossus when TOF ratio is under 0.9. This could lead to hypoxia, or in severe cases even to negative pressure pulmonary edema. Muscle relaxants block chemoreceptors in the glomus caroticum, which are responsible for the respiratory response in case of severe acute hypoxia by elevating the minute ventilation. The blockade of these receptors can lead to severe hypoxia if TOF ratio is below 0.9. In addition, residual muscle relaxation could cause severe subjective symptoms such as general weakness, double vision and coughing difficulties. Therefore, it is not surprising that residual muscle relaxation after operations could lead to increased post-operative morbidity and mortality ratios.

Despite all these facts postoperative residual neuromuscular block has still a very high, around 38%, prevalence. Since muscle relaxants are used in more than 400 million patients per year, the detection and elimination of postoperative residual neuromuscular block is of great importance.

The elimination of residual muscle relaxation is traditionally carried out by the inhibition of acetylcholinesterase enzyme, thereby increasing the amount of acetylcholine in the neuromuscular junction. In this way acetylcholine will be able to displace muscle relaxant molecules from the receptor and the block will be terminated. However, the use of neostigmine is restricted by its numerous serious side effects and the so-called "ceiling effect", when the drug develops its maximal potential by inhibiting all available acetylcholinesterase enzymes, thus further drug administration won't improve the efficiency. Therefore, the use of neostigmine is not recommended in too deep, deeper than TOF count of

2, muscle blocks. However, if neostigmine is administered in too superficial block, or after it has been subsided, the drug could induce neuromuscular block by itself.

Sugammadex is a new type of reversal agent, a modified γ -cyclodextrin compound, which can encapsulate certain molecules. Sugammadex was developed to bind with rocuronium molecules and form complexes, which has a very low dissociation rate. Bounding up with free rocuronium molecules in the plasma the concentration of muscle relaxant decreases. Relaxant molecules diffuse from the neuromuscular junction into the plasma according to the concentration gradient, thus their number diminishes in the synapse, the acetylcholine molecules become prevailing and the block terminates. This process occurs rapidly, within a few minutes. Sugammadex is capable of binding to other amino-steroidal muscle relaxant molecules as well with different association constants than to rocuronium. However, it is important to note that sugammadex does not encapsulate benzylisoquinoline and depolarizing muscle relaxants, therefore it is not suitable to reverse blocks that were created by such molecules. Sugammadex, unlike neostigmine, has the ability to suspend neuromuscular blocks of any depths, if adequate doses are applied. It also has a very favorable side effect profile.

In our study we investigated the possibility of reversing postoperative residual neuromuscular block induced by two different muscle relaxants with the antagonistic effect of sugammadex.

2. AIM OF THE STUDY

2.1. Reversal of residual neuromuscular blockade with sugammadex

Doses of sugammadex required to reverse intense (TOFC 0, PTC 0), deep (TOFC 0, PTC 1-2), moderate (TOFC 2) and shallow (TOFR >0,5) rocuronium-induced neuromuscular blockade have been established. However, no adequate doses for the reversal on reappearance of four twitches of train-of-four stimulation (threshold TOF-count-four) have been established.

The primary aim of the study was to determine the time required for 0.5 mg/kg, 1.0 mg/kg and 2.0 mg/kg sugammadex to reverse a residual level block to a non-normalized TOF ratio of 1.0. Rapid reversal (≤ 2.0 min average upper limit of 5.0 min) was the primary endpoint and slower reversal (≤ 5.0 min average upper limit of 10 min) was the secondary endpoint of the study.

The secondary aim was to compare these times to the times required for the control treatment of neostigmine to similarly reverse a threshold TOFC 4 degree blockade.

2.2. Reversal of pipecuronium-induced moderate neuromuscular block with sugammadex during sevoflurane maintenance anesthesia

Sugammadex has a high affinity for pipecuronium in vitro. Whether sugammadex is useful for the reversal of pipecuronium-induced neuromuscular blockade in vivo is still unknown.

We hypothesized that the sugammadex would also reverse rapidly and effectively moderate pipecuronium block.

In this study we aimed to establish an appropriate dose of sugammadex in order to reverse a moderate (TOFC-2) pipecuronium-induced neuromuscular block. We hypothesized that the dose of sugammadex recommended for the reversal of rocuronium-induced block (1.0 to 4.0 mg/kg) would also reverse the effect of pipecuronium.

The secondary aim of the study was to investigate whether reversing of pipecuronium block with sugammadex has any influence on the occurrence of PRNB or recurrent neuromuscular blockade.

3. MATERIALS AND METHODS

3.1. Reversal of residual neuromuscular blockade with sugammadex

This single center, randomized, controlled, double-blind, four groups parallel-arm study was approved by the ethics committee of the medical faculty of the University of Debrecen, Hungary (DEOEC RKEB/IKEB: 3315-1/2011), and the National Institute of Pharmaceutics (OGYI/17316-1/2011). The study is classified as EudraCT Number: 2011-001683-22. The investigation was conducted at the University Hospital of Debrecen, Hungary between April 2011 and April 2012. The service of anesthesiology recruited eighty patients.

Participants recruiting: Inclusion criteria were: age 18-65 years, Body Mass Index 18.5-25.0 kg/m², American Society of Anesthesiologists physical status I to III, and scheduled for elective surgery with an expected duration greater than 50 min under general anesthesia with intubation of the trachea. Patients with suspected difficult airway, bronchial asthma, chronic obstructive pulmonary disease, known neuromuscular disease, suspected malignant hyperthermia, hepatic or renal dysfunction, glaucoma, allergy to the medication used in this trial, taking of medicaments that might influence the effect of NMB agents, pregnant, or breastfeeding state were not included.

Randomization: Subjects were allocated in a 1:1 ratio to the four study groups. Patients randomly received 0.5, 1.0 and 2.0 mg/kg of sugammadex or 0.05 mg/kg of neostigmine in a mixture with 0.015 mg/kg of atropine. In order to ensure equal numbers of patients per group, permuted-block randomization was used.

Preparing patients: Noninvasive blood pressure, electrocardiogram, and oxygen saturation monitoring were performed. Anesthesia was induced with intravenous propofol (1.5 to 2.5 mg/kg) and fentanyl (2 µg/kg) and maintained with inhaled sevoflurane (1.1 to 1.8 vol%) in air/oxygen mixture and intravenous fentanyl according to clinical need. Patients' lungs were artificially ventilated by face mask until intubation of the trachea to maintain oxygen saturation >96%, to obtain stable end-expiratory sevoflurane concentration and to ensure normocapnia. Body temperature was maintained at 36.0 °C or higher.

Neuromuscular monitoring was carried out according to international consensus guidelines, monitoring the adductor pollicis muscle in response to ulnar nerve stimulation using the TOF-

WATCH- SX® device (Organon Teknika B.V., Boxtel, Holland). After stabilization of the acceleromyographic recording 0.6 mg/kg of rocuronium was injected. The trachea was intubated when the TOFC was 0. During surgery 0.1 to 0.15 mg/kg of rocuronium was injected as needed when the TOFC exceeded 1.

Reversal: At the end of surgery, spontaneous recovery from the NMB was allowed. The study medication was injected when the 4th twitch of TOF returned at three consecutive TOF measurements. Reversal of the displayed TOF ratio to 1.0 was considered as efficacy endpoint of the study. The time intervals between the start of injection of the study drug and the reversal of TOF ratios to 1.0 were recorded. To ensure satisfactory reversal of the neuromuscular blockade displayed TOF ratios at recovery were divided by control TOF ratios measured before the administration of rocuronium (“normalization”). After the recovery of TOFR 1,0 the administration of sevoflurane was stopped and the patient’s trachea was extubated once the patient was awake. If the TOF ratio did not reach 1.0 within 15 min, 2.0 mg/kg of sugammadex (rescue treatment) was injected to prevent PRNB. If the TOF ratio returned below 0.9, recurrent block was recorded.

Postoperative monitoring: After the extubation of the trachea, patients were kept in the recovery room for at least 60 min under close surveillance for recurrence of any sign of muscle weakness or critical respiratory or circulatory events. Oxygen saturation, respiratory rate, heart rate and non-invasive blood pressure were monitored. After discharge from the recovery room, the patients were followed for 24 hours in order to detect late adverse events.

Outcome measures: The efficacy endpoint of the study was to reach TOFR 1,0. The time intervals between the start of injection of the study drug and the reversal of displayed TOF ratios to 1.0 were investigated. Rapid reversal (≤ 2.0 min average upper limit of 5.0 min) was the primary endpoint and slower reversal (≤ 5.0 min average upper limit of 10 min) was the secondary endpoint of the study.

Data analysis: Recovery from rocuronium-induced threshold TOFC-4 NMB was studied in the per-protocol population. We calculated normalized TOF values at antagonism and at recovery. We analyzed data by using parametric statistical tests only when the assumptions of these tests were met by the data. Otherwise, we used non-parametric statistics or log-transformed the data to comply with the parametric assumptions. We tested the normality of response variables using the Kolmogorov-Smirnov test and the equality of variances using Levene’s test. The time of the reversal of TOF ratios was measured in seconds, converted to minutes and was log-transformed (\log_{10}) to achieve the equality of variances for use in parametric statistical tests. We first analyzed whether patients’ characteristics and treatment factors that could have influenced the results differed among experimental groups (untransformed data and Kruskal-Wallis tests) and then we analyzed the times of recovery (log-transformed data and ANOVA). We used Tukey’s HSD (honestly significant difference) procedure for the post-hoc comparison of means among experimental groups. For the analysis of primary and secondary outcome variables (rapid reversal and slower reversal) we selected the patients in categories according to predetermined criteria. Rapid reversal versus not rapid reversal was compared between the sugammadex groups (pooled data) and the control group (neostigmine), using relative risk calculations (RR). In addition, we compared the incidence

of rapid and slower reversal in patients, who received sugammadex (pooled data), using the odds ratio calculation (OR). In all statistical analyses, we used SPSS version 17.0 for Windows (IBM Corporation, Armonk, New York, United States). Two-tailed probabilities ($\alpha=0.05$) are reported in the text.

3.2. Reversal of pipecuronium-induced moderate neuromuscular block with sugammadex during sevoflurane maintenance anesthesia

This single-center, randomized, double-blind, five-groups, parallel-arm study was approved by the local ethics committee at the University of Debrecen, Hungary (DEOEC RKEB/IKEB 3585-2012), and by the National Institute of Pharmaceutics (OGYI/25260-1/2012). The study is classified under EUDRACT number 2012-00029-14. The investigations and data collection were carried out at the University Hospital of Debrecen, Hungary, between 2012 August and 2014 January. The service of anesthesiology recruited fifty patients.

Participants recruiting: Inclusion and exclusion criteria were the same in two our study, except one. In this study patients scheduled for elective surgery with an expected duration of 90 min or more were included.

Randomization: Subjects were allocated in a 1:1 ratio to the five study groups. Patients randomly received 1.0; 2.0; 3.0; 4.0 mg/kg of sugammadex or placebo. In order to ensure equal numbers of patients per group, permuted-block randomization was used.

Preparing patients and neuromuscular monitoring was the same in two our study, with the difference that in this study 0.06 mg/kg pipecuronium was injected after the stabilization of the acceleromyographic signal. During surgery 0.01 mg/kg of pipecuronium was given at TOF count 1 to maintain muscle relaxation as needed. Acceleromyographic data were recorded and stored on a computer using TOF-Watch SX® software version 2.2 INT (Organon Ireland Ltd. Dublin, Ireland).

Reversal: At the end of surgery we allowed spontaneous recovery of the block and, once 2 twitches appeared (TOFC 2) the anesthesiologist, who had prepared the study drug, injected it upon the request of the anesthesiologist who was responsible for the patient and blinded to the drug. The TOF ratio and T1 amplitude (the first of four twitches to TOF stimulation) were recorded and analyzed later. Once the displayed TOF ratio was 1.0 we discontinued the inhalation of sevoflurane. The trachea was extubated when the patient had awakened. Rescue treatment (0.015 mg/kg atropine and 0.05 mg/kg neostigmine) was administered in the case of incomplete recovery (stagnation of TOF ratio at < 0.9 for at least 5 minutes), but at latest after 45 min.

Postoperative assessment: After extubation of the trachea, patients were transferred to the recovery room. In the recovery room a different anesthesiologist who was blinded to the study drug monitored the patients, and performed acceleromyographic and clinical assessment of the neuromuscular function. Postoperative acceleromyographic recordings were commenced (time zero) without recalibration of the device and were repeated every 20 min until 60 min. At each point in time three consecutive TOF stimuli were delivered at 15 s intervals and the

averages of three evoked TOF ratios were considered. Data were recorded and stored on a computer, and analyzed off-line. TOF ratios less than 0.9 were defined as PRNB. Oxygen saturation, non-invasive blood pressure, electrocardiogram and respiratory rate were monitored. After discharge from the recovery room, the patients were observed for 24 h in order to detect late adverse events.

Outcome measures: Primary outcome measure was the time from the start of injection of the study drug to normalized TOF ratio of 0.9. Secondary outcome measure was the time from the start of injection of the study drug to final T1 (the first of maximal T1 values at stable signal). Alternative outcome measure was the time necessary to achieve non-normalized TOF ratio of ≥ 1.0 . Postoperative outcome measure was RPONB defined as non-normalized TOF ratio less than 0.9, with or without muscle weakness or critical respiratory events.

Data analysis: We used chi-square tests to compare proportions across categories and Fisher's exact test for 2x2 contingency tables. For continuous variables, parametric statistical tests were used if their assumptions were met by the data. We used Bartlett's test to check the homogeneity of variances and the Shapiro-Wilk test to check normality. Because most background variables (patient data, perioperative variables) showed either deviation from normality or non-homogeneous variances, we used Kruskal-Wallis tests to compare background variables across experimental groups. For response variables, we log-transformed data to ensure the homogeneity of variances when it was necessary and used one-way ANOVAs to compare groups. Finally, we used repeated-measures ANOVAs to test for temporal changes in variables in the postoperative period. All statistical analyses were conducted in the R statistical environment (version 2.15.2).

4. RESULTS

4.1. Reversal of residual neuromuscular blockade with sugammadex

In total, the study drugs were injected in 80 patients. Five patients were excluded. In four patients the TOF ratio did not reach 1.0 within 15 min after the injection of neostigmine, and therefore 2 mg/kg sugammadex was given as a rescue medication to prevent PRNB. These patients were in the neostigmine group. In one patient (0.5 mg/kg sugammadex group) the study drug was injected at a TOF ratio of 0.6 (minor protocol violation). With the five patients excluded from the final efficacy analysis, 75 patients were finally analyzed for TOF recovery.

The four experimental groups did not differ in any of the factors that could have influenced the results (sex, age, Body Mass Index, ASA physical status score), or in treatment: total rocuronium dose, time from last rocuronium dose to antagonism, concentrations of sevoflurane at induction and at antagonism, TOF ratios at antagonism and at recovery ($p > 0.085$, Tables 1 to 3).

The times of reversal to non-normalized TOF ratios of 1.0 (normalized TOF ratios 0.98 to 1.0) after injection of 0.5, 1.0 and 2.0 mg/kg of sugammadex were 4.1 ± 1.9 min (mean \pm SD), 2.1 ± 0.8 min and 1.8 ± 0.9 min, respectively. The time of reversal to non-normalized TOF ratios of 1.0 (normalized TOF ratio 1.0) after 0.05 mg/kg of neostigmine was 8.5 ± 3.5 min. The times of reversal differed significantly among the four experimental groups ($p <$

0.001). Patients receiving sugammadex 1.0 mg/kg or 2.0 mg/kg recovered significantly faster from the TOFC 4 block than patients with sugammadex 0.5 mg/kg or patients with neostigmine 0.05 mg/kg ($p < 0.001$). The difference in times of recovery between sugammadex of 0.5 mg/kg and neostigmine of 0.05 mg/kg was also significant ($p < 0.001$). There was no significant difference in times of recovery between 1.0 mg/kg and 2.0 mg/kg sugammadex ($p = 0.581$).

The incidence of rapid reversal (primary endpoint) after sugammadex treatment was higher than after neostigmine treatment ($p = 0.022$). The incidence of rapid reversal after sugammadex treatment was higher than the incidence of slower reversal (secondary endpoint) ($p < 0.001$).

No recurrent NMB or post-operative critical respiratory or circulatory events occurred in our patients.

4.2. Reversal of pipecuronium-induced moderate neuromuscular block with sugammadex during sevoflurane maintenance anesthesia

In total, the study drugs were injected in 50 patients. Three patients were excluded. One minor protocol violation occurred in the 1.0 mg/kg sugammadex group and in the placebo group and one technical failure occurred in the 4.0 mg/kg sugammadex group. These patients were excluded from the final analysis.

The experimental groups also did not differ in any of the factors that could have influenced the results (sex, age, body mass index, height, weight, control TOF ratio, control T1 (%), ASA physical state score) ($p > 0.1$) or in treatment (total pipecuronium dose, sevoflurane concentration at induction or at antagonism) ($p > 0.3$). The time interval from the injection of pipecuronium to the appearance of two twitches of the TOF (the time-point of administration of study drugs) did not differ significantly among the experimental groups ($p = 0.57$).

Among the placebo patients no one accomplished spontaneously satisfactory recovery, and thus each patient ($n=9$) received rescue medication after 32 ± 9.8 min (mean \pm S.D.) at ceiling of the TOF ratio around 0.2 ± 0.08 . Subsequently, normalized TOF ratio of 0.9 was achieved in all patients within 10.7 ± 4.8 min. These data, however, were inappropriate for comparison with the sugammadex groups and therefore were excluded from the analysis of reversal times. Finally, the data of 38 sugammadex patients were analyzed for reversal, and the cohort of 47 patients was evaluated for PRNB. In addition, we also monitored those three patients who were excluded due to protocol violation or technical failure.

Time intervals to TOF ratio 0.9 and 1.0 (Primary outcome): All patients who had received sugammadex recovered to normalized TOF ratio of 0.9 within 5 min. There was a weak but statistically significant difference in time to TOF ratio 0.9 among the groups ($p = 0.044$) because times were shorter in the group receiving 3.0 mg/kg sugammadex than in the 1.0 mg/kg sugammadex group. The duration of recovery to TOF ratio 1.0 and to maximal T1 was slightly shorter in the 3.0 mg/kg and 4.0 mg/kg groups than in the 1.0 mg/kg and 2.0 mg/kg groups, however, the differences were not significant.

Secondary outcome: Reversal to final T1 height took significantly longer in all sugammadex groups to take place than the reversal of the corresponding TOF ratio ($0,0003 < p < 0,018$), but there was no significant difference in reversal time among the four sugammadex groups ($p=0,327$).

Alternative outcome: The reversal times were not significantly different in the sugammadex groups irrespective of whether the endpoint was normalized 0.9 or non-normalized TOF ratio 1.0 ($p = 0.24$).

Postoperative outcome: TOF ratio of less than 0.9 did not occur in any treatment group at any measurement time during the first postoperative 60 min; the average TOF ratio was 1.0 or more overall. No early or late adverse events were observed.

5. DISCUSSION

Studies dealing with residual postoperative muscle relaxation have become more and more widely discussed among publications in the field of anesthesiology. It has been shown that even superficial muscle relaxation, with a TOF ratio between 0.5 and 0.9 could also be a source of serious complications. Some of the complications may arise immediately after the postoperative period has begun (desaturation, respiratory failure, weakness, visual disturbances) which can be easily solved if the patient is carefully observed. However, it happens frequently that consequences of PRNB emerge first only a few days after the intervention (pneumonia, atelectasis). In this case, the source of complication is not entirely clear, the prior existence of residual muscle relaxation is often not considered as a causative factor. Since the consequences of PRNB are often very severe and could be even fatal, it is important to change the anesthetists' entrenched bad habits, previously learned and outdated theories.

Nowadays we are part of an era when paradigm shift take place among professionals. Two out of the three fundamental elements of this paradigm shift are emphasizing the reasonableness of intra- and postoperative neuromuscular monitoring and the necessity of reversal in order to prevent postoperative residual neuromuscular block. The second element of the paradigm shift refers to the importance of reversal. The recent spread of sugammadex, a new reversing agent, is converting the professionals' attitude in some degree. The drug is widely available nowadays, but due to the high purchase price its use is limited all around the world. However, results of dose-defining studies show that in some cases (in superficial neuromuscular blocks) lower doses also ensure safe conditions for the reversal at lower costs. The third element is only conceived by a few professionals yet: the prevailing principle that intermediate-acting muscle relaxants should be preferred in daily practice, even at a cost of less favorable side effect profile, has been called into question lately. The new reversing agent sugammadex's effectiveness against long-acting pipecuronium justifies the extensive usage of this low side-effect profile muscle relaxant. We tried to contribute to this paradigm shift and thereby the safety of anesthesia with the results of our clinical trials.

The first element of paradigm shift is emphasizing the need for neuromuscular monitoring during surgery as well as during the postoperative period. It has been proven by several

studies that the use of quantitative monitors reduces the incidence of PRNB. Nowadays acceleromyography is a convenient and widely used form of quantitative monitoring. Since acceleromyography is a non-invasive, relatively inexpensive and easily operated technique, its application would be reasonable during and after each and every general anesthesia, where muscle relaxation occurs. However, several surveys show that muscle strength monitoring is carried out only in some of the operations. The reluctance of professionals is incomprehensible, but it is likely that as long as neuromuscular monitoring won't be compulsory only a fraction of anesthetists are going to use it. This is why the proper reversal of muscle relaxation at the end of surgeries is of great importance.

The traditional "method" of antagonizing muscle relaxants is to elevate the amount of acetylcholine, which forces relaxant molecules to dissociate from acetylcholine receptors, thus the patient's muscle strength returns. However, the muscle relaxant molecules are not going to disappear from the neuromuscular junction, and therefore the decrease of acetylcholine concentration results in the return of muscle relaxation. Neostigmine is a reversal agent with the same operating principle, which increases acetylcholine concentration by blocking acetylcholinesterase enzymes. Nevertheless, during its application a number of side effects may occur due to its agonist effect on muscarinic acetylcholine receptors. These side effects could be very serious, therefore many anesthesiologists try to avoid the administration of neostigmine, or apply only a fraction of the recommended dose. Neostigmine cannot reverse too deep neuromuscular blocks and can even lead to neuromuscular block by itself, if the muscle relaxation is too superficial, or when the muscular block has already subsided. Therefore it is possible that the administration of this reversal agent, without proper monitoring, won't decrease the incidence of postoperative residual block. This explains also that the usage of neostigmine, without monitoring the degree of muscle relaxation, has increased the risk of postoperative respiratory failure, as demonstrated by a retrospective study with large sample size.

Longstanding researches for a more ideal antagonist agent can be traced back to the above listed reasons. Sugammadex, released in the early 2000s, could meet most of criteria that an ideal reversal agent should possess. Its mechanism of action is completely different from neostigmine. It encapsulates the relaxant drugs within its molecule and forms a complex with them. The intermolecular bonding is immensely strong, an almost new molecule is formed in the process, which is excreted unaltered through the urine from the body. Sugammadex was developed to bind with rocuronium molecules, but it is capable of antagonizing other aminosteroidal muscle relaxants as well. Sugammadex possesses a very favorable side effect profile, it is free of the serious adverse symptoms that emerge after the administration of neostigmine. It can be given at any degree of neuromuscular block, it is capable of antagonizing even a complete muscular relaxation applied immediately after the administration of the relaxant. Typically it takes effect rapidly within a few minutes at any degree of muscular block, while this often takes more than 15 minutes for neostigmine. Sugammadex has a disadvantage, namely it is only capable of reversing aminosteroid non-depolarizing muscle relaxants. In addition, its very high purchase price limits the widespread use. Due to the latter mentioned reason, the possibility of using a lower dose of sugammadex, to reverse superficial neuromuscular blocks, has arisen. This idea is based on the fact that bonding between

sugammadex and rocuronium has a one to one molecular interaction ratio. In theory, therefore, a lower dose of sugammadex is sufficient to antagonize a superficial block caused by rocuronium, since less muscle relaxant molecules need to be encapsulated in order to terminate the block.

Earlier dose-defining studies have determined the effective doses of sugammadex to reverse complete (TOFC 0, PTC 0), deep (TOFC 0, PTC 1-2), moderate (TOFC 2) and superficial (TOFR > 0.5) rocuronium blocks. These doses are 16 mg/kg, 4 mg/kg, 2 mg/kg and 0.25 mg/kg respectively. Nevertheless, at the end of surgery a different depth of relaxation, a so-called residual block is often registered, when all four muscle responses (TOFC 4) appear after TOF stimulation, and the acceleromyography either does not show measurable TOF ratio, or detects only a very low one. The ideal dose of sugammadex to antagonize residual rocuronium block has not been determined yet, such investigation has never been carried out before. The manufacturer, due to the lack of reliable studies, recommends the administration of 2 mg/kg dose of sugammadex to reverse a TOF count of 2 and more superficial rocuronium blocks.

In our first study we investigated the effectiveness of 0.5 - 1 - 2 mg/kg doses of sugammadex to antagonize residual (TOFC 4) rocuronium block. Placebo controls were not examined, given the fact that the efficacy of sugammadex in reversing rocuronium block has been proven earlier. We have determined the effectiveness of neostigmine instead of a placebo control. Neostigmine was administered in full-dose (0.05 mg/kg). The anesthesia was maintained with the use of sevoflurane gas.

After the administration of reversal agent, the required time period was examined to reach a TOF ratio of 1.0 displayed by the acceleromyograph. The dose of sugammadex for quick recovery was set as a primary endpoint (≤ 2 minutes on average, but up to 5 minutes), and the dose for slower recovery as a secondary endpoint (≤ 5 minutes on average, but up to 10 minutes). These time intervals were determined according to the time periods set by earlier dose-defining studies, in order to make our result easier to compare with the values of previous studies.

Previous dose-defining studies have measured the time required to return to the non-normalized TOF ratio 0.9. During the application of acceleromyography however, it is often observable that the initial value exceeds TOF ratio 1.0. This phenomenon may arise from the indirect calculation of the force of muscle contraction. Previous studies have confirmed the hypothesis that during the use of acceleromyography, returning to the TOF ration 0.9 is not sufficient. However, there are two methods which can increase the accuracy of acceleromyography: normalization, when the value is compared with the baseline, and the return to TOFR 1.0 instead of 0.9 after preliminary calibration. In our study, therefore, we determined the return to TOFR 1.0 as an endpoint. However, the results were also normalized during the analysis.

In our study, the time required to reach TOFR 1.0 following the administration of 1 and 2 mg/kg of sugammadex as antagonizing agent did not differ significantly. The non-normalized TOFR has returned to 1.0 after the use of 1 mg/kg sugammadex in 2.1 ± 0.8 minutes (mean \pm

SD). The shortest recovery time was 1.2 minute, while the longest was 4.5 minutes. Nineteen out of twenty patients in this group have met the criteria of rapid recovery. After the administration of 0.5 mg/kg dose of sugammadex the reversal time was 4.1 ± 1.9 (mean \pm SD) minutes (min-max: 1.7 -8.0 min). In this group 84% of the patients have met the criteria of slow, while 16% the criteria of rapid recovery. It has to be noted that slow recovery, when muscle relaxation subsides within 10 minutes, is a clinically acceptable time interval.

Our study showed that reversal time took much longer following the use of neostigmine when compared to sugammadex. Residual rocuronium block was reversed approximately twice as fast even with the lowest dose of sugammadex (0.5 mg/kg) than with neostigmine. The criteria of rapid recovery was not fulfilled even a single time among patients of the neostigmine group, and slow, within 10 minutes, reversal was observed only in 37.5% of the cases. Even more prolonged time intervals were registered in case of other group members. In addition, in four cases TOFR has not returned to 1.0 15 minutes after the reversal agent was injected, so they have received a so called "rescue medication", which was 2 mg/kg sugammadex. If we had waited for complete recovery in case of those four patients, overall time results would have been even longer in this group. In our study the use of sevoflurane anesthesia has further delayed the effect of neostigmine. Several studies demonstrated the fact that sevoflurane does not counteract with sugammadex, while it significantly prolongs recovery time after the administration of neostigmine.

Residual neuromuscular block, with a degree of TOFC 4, often occurs at the end of surgery. Neuromuscular blocks with such degree are easily recognizable even with qualitative monitoring (the four muscle responses are detectable visually or by palpation), so it can be reversed easily within minutes with the help of previously defined doses of sugammadex. Based on the results of our study, if rocuronium is used as muscle relaxant, patients can be extubated only 5 minutes after four twitches appear with TOF stimulation and 1 mg/kg of sugammadex has been administered. Ten minutes should be waited after the injection of 0.5 mg/kg sugammadex as a safety period. A significantly prolonged recovery time should be expected if residual rocuronium block is antagonized with neostigmine, especially when volatile anesthetics are used. This has been proven by our study as well. Therefore, the use of neostigmine as a reversal agent should be avoided in case of neuromuscular blocks with such depth, if quantitative monitoring is not available.

It is important to note that our results pertain only to neuromuscular blocks which were antagonized by rocuronium. Time intervals needed to reverse neuromuscular blocks, caused by other types of steroid-based muscle relaxants, could differ from our results.

The third element of the previously mentioned paradigm shift is the review of long-acting muscle relaxants. At the end of the 1990s a prospective, multi-center study proved that the incidence of severe residual neuromuscular block (TOFR <0.7) is significantly higher after the application of long-acting muscle relaxant (pancuronium), which also significantly elevated the occurrence of postoperative pneumonia. Due to this publication the use of long-acting muscle relaxants has strongly decreased all over the world. As a result of the almost exclusive use of medium-acting muscle relaxants the frequency of residual paralysis was expected to reduce, nonetheless, several studies have confirmed the high incidence of PRNB.

The explanation of this phenomenon could be the muscle relaxants' unpredictable duration of action, which is influenced by several factors, and it can last longer even after the administration of a single dose, than it is expected by anesthetists. The probability of PRNB occurrence increases with the repeated administration of the muscle relaxant, or when applied through infusion, thus after longer surgical interventions. Therefore, the use of long-acting muscle relaxants should be considered in case of prolonged surgeries, which provides an adequate depth of neuromuscular block as long as the operation lasts. In this context the question arises, why to choose a medium-acting muscle relaxant at all for long surgeries. Due to the repeated dosage the advantages, such as shorter duration of action and decreased chance of PRNB risk, disappear, and other equally important aspects of drug selection may be highlighted, such as side effects, vagolytic effect and the incidence of anaphylactic reactions. The drug choice would be evident if there were any long-acting muscle relaxant with a favorable side effect profile and which could be antagonized reliably within a few minutes. Rocuronium is becoming increasingly popular among medium-acting muscle relaxants all over the world. This popularity is due to its quick onset, but first of all to its ability to be able to reverse with sugammadex within a few minutes. Unfortunately, because of its extensive use the frequency of anaphylactic reactions caused by the drug is continuously increasing.

Results of in vitro studies, carried out during the development of sugammadex, show that its affinity towards pipecuronium is about ten times stronger than towards rocuronium. However, sugammadex's potency to antagonize neuromuscular block caused by pipecuronium has not been tested under in vivo circumstances yet.

Our second study was conducted with the aim to determine the sugammadex dose suitable for reversing moderate (TOFC 2) pipecuronium block. We decided to investigate the antagonism of moderate level (TOFC 2) pipecuronium block, because the majority of previous studies, determining the necessary dose of sugammadex to reverse rocuronium block, have examined this level of neuromuscular block as well. The efficacy of 2 mg/kg dose of sugammadex was chosen to be studied because this is the recommended dose to reverse similar depth of neuromuscular block created by vecuronium and rocuronium. Pipecuronium is an approximately six to seven times more potent muscle relaxant than rocuronium, therefore, less pipecuronium molecules are required to reach the same level of neuromuscular block than rocuronium. Since the sugammadex - muscle relaxant interaction has a one to one molecular ratio, theoretically less sugammadex molecule is required to antagonize a similar level of neuromuscular block caused by pipecuronium than by rocuronium. Based on this fact we decided to investigate 1 mg/kg sugammadex as the lowest dose. Regarding the fact, that the efficacy of sugammadex to eliminate pipecuronium block has not been tested in vivo yet, we decided to test higher doses of sugammadex (3 mg/kg and 4 mg/kg) and a placebo group as well. The placebo group was necessary to compare the results with the time required for spontaneous reversal of the block. We hypothesized that after the administration of placebo or a lower dose of sugammadex the reversal time of the block can be prolonged, therefore the use of neostigmine as "rescue medication" was allowed in this cases.

In our study we managed to demonstrate that sugammadex effectively antagonizes moderate (TOFC 2) level of neuromuscular block caused by pipecuronium. The primary endpoint for

efficacy, namely the time required for returning to the normalized value of 0.9 TOFR, following the administration of the lowest dose (1 mg/kg) of sugammadex, took 2.3 ± 0.95 (mean \pm SD) minutes. The ratio of TOF has returned to the normalized 0.9 value within 2 minutes in 79% of patients receiving sugammadex and less than 5 minutes in the remaining 21%. It can be declared that 1 or 2 mg/kg doses of sugammadex can adequately reverse a pipecuronium block with a TOFC 2 level. Despite the long duration of pipecuronium, muscle relaxation has not reappeared in any case after antagonization.

All patients in the placebo group needed "rescue medication" approximately half an hour after saline administration. At this time the level of neuromuscular block corresponded with a residual block and TOFR was 0.2 on average. The normalized TOF ratio reached 0.9 value after 11.6 ± 5.5 minutes (mean \pm SD) (min-max: 2.8 to 20.3 min) following the administration of neostigmine. This study confirms the results of our previous study, namely that the antagonism of residual neuromuscular block with neostigmine often takes longer than 15 minutes, especially if anesthesia is maintained with the help of volatile anesthetics.

The primary endpoint of the study was to determine the time period required to reach a 0.9 normalized TOFR, following the administration of reversal agent. As previously mentioned, nowadays it is widely accepted, that TOF ratio should return to a normalized 0.9 or non-normalized 1.0 value, if acceleromyography is applied, in order to rule out the residual effect of muscle relaxants. In our study, no significant difference was detected between the return time to normalized 0.9 or non-normalized 1.0 value. The process of normalization is often complicated under surgical circumstances, therefore waiting until TOF returns to 1.0 could be more suitable to exclude a possible neuromuscular block. This has been confirmed by our investigation as well.

New and original observations were made by our study concerning the monitoring. The power of the first muscle contraction after TOF stimulation is characterized by the T1 value. Generally T1 returns prior to the recovery of TOF ratio, after the spontaneous termination of neuromuscular block or administration of neostigmine. It has been observed recently, that this order is reversed following the application of sugammadex. The secondary endpoint of our study was to determine the time period required to reach the highest, final T1 signal after the administration of a reversal agent. This time interval proved to be 5-8 minutes longer than the recovery time of the normalized TOFR 0.9. Thus, this phenomenon is specific not to pipecuronium but to sugammadex. The background of this phenomenon is not fully understood yet. The possible explanation is the following: while the phenomenon of fatigue is related to the inhibition of presynaptic receptors, the height of T1 is characterized by the degree of antagonized postsynaptic receptors. In vitro studies have demonstrated that non-depolarizing muscle relaxants bind to postsynaptic ($\alpha 1$ $\beta 1 \delta \gamma$) acetylcholine receptors with a much greater affinity than to presynaptic ($\alpha 3 \beta 2$) receptors. When sugammadex is used in high concentrations only very few molecules remain in the synaptic gap and rather bind to postsynaptic receptors. Therefore, T1 value remains even lower, although fatigue is no longer detectable. The reversal of neuromuscular block is complete when both T1 and TOFR have returned to their baselines. The clinical implications of this is unclear yet, because the reappearance of TOFR 1.0 is followed by the return of T1 to its baseline within a few

minutes. No residual postoperative curarization was detected by our study. However it is conceivable, that under special circumstances the "slipping" in the return of T1 could have clinical implications. Theoretically the return of T1 could be significantly delayed if magnesium or aminoglycoside antibiotics were given after the operation, or in case of neuromuscular diseases such as myasthenia gravis, which can lead to the consequences of PRNB. Therefore, although T1 monitoring is still in its early stage, the return of T1 could become a criteria to rule out the presence of PRNB, as its importance will be clarified and monitoring systems will evolve.

We have tried to contribute to a safer anesthesia and support the elements of the above mentioned paradigm shift with both of our studies. The efficacy of a new, reduced dose of sugammadex was investigated during the reversal of residual rocuronium block. Furthermore, the efficacy of the reversal agent has been investigated with a brand new indication, namely to antagonize neuromuscular block caused by the long-acting pipecuronium.

6. SUMMARY

Postoperative residual neuromuscular block (PRNB) may lead to severe complications, therefore it is important to recognize and antagonize it at the end of the surgery. In our study, we analyzed the efficacy of sugammadex in different states of neuromuscular blockade performed by two different muscle relaxants (rocuronium and pipecuronium). Our findings were as follows:

1. We were the first to antagonize TOFC 4 rocuronium block with different doses of sugammadex, collect and analyze data regarding it. Our team was the first to prove, that TOFC 4 residual rocuronium block can be reversed with low dose sugammadex.
2. We found that there was no significant difference in the times needed to reach non-normalized TOFR 1.0 after antagonizing TOFC 4 rocuronium blockade with 1.0 or 2.0 mg/kg sugammadex.
3. We proved that TOFC 4 residual rocuronium block can be safely antagonized with 1 mg/kg sugammadex in 5 minutes.
4. The safe time for reversal in TOFC 4 rocuronium block is 10 minutes, after using 0.5 mg/kg sugammadex. Antagonizing with neostigmin after sevoflurane anaesthesia results in very slow recovery.
5. We were the first to study the possibility of reversing moderate pipecuronium blockade with sugammadex, following sevoflurane anaesthesia.
6. Our study group was the first to prove that pipecuronium blockade can be adequately reversed with 1 or 2 mg/kg sugammadex.
7. We proved that reversing pipecuronium blockade with sugammadex does not lead to postoperative neuromuscular blockade, although pipecuronium has a long duration of action.

8. We also found that TOFR 1.0 is identical with normalized TOFR 0.9 regarding the safety of reversal.

9. Finally, we proved that the reversal with sugammadex is different from the classical reversal, as T1 returns later than TOFR.

As for the practical significance of our research, now it seems possible to use lower doses of sugammadex, resulting in lower hospital costs. The chance of reversing pipecuronium blockade with sugammadex makes the wider usage of this muscle relaxant possible. Our results also include new ways of monitoring.

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List of publications related to the dissertation

1. Tassonyi, E., **Pongrácz, A.**, Nemes, R., Asztalos, L., Lengyel, S., Fülesdi, B.: Reversal of Pipecuronium-Induced Moderate Neuromuscular Block with Sugammadex in the Presence of a Sevoflurane Anesthetic.
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2. **Pongrácz, A.**, Szatmári, S., Nemes, R., Fülesdi, B., Tassonyi, E.: Reversal of neuromuscular blockade with sugammadex at the reappearance of four twitches to train-of-four stimulation.
Anesthesiology. 119 (1), 36-42, 2013.
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List of other publications

3. Sárkány, P., Tassonyi, E., Nemes, R., Timkó, A., **Pongrácz, A.**, Fülesdi, B.: Testing rocuronium-induced neuromuscular blockade at the stapedius muscle using stapedius reflex measurements.

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