Journal of Anesthesia (2019) 33:642–646
https://doi.org/10.1007/s00540-019-02681-x

ORIGINAL ARTICLE

Remifentanil does not inhibit sugammadex reversal after rocuronium-induced neuromuscular block in the isolated hemidiaphragm of the rat: an ex vivo study

Jae Moon Choi[1] · Ha-Jung Kim[1] · Hey Ran Choi[2] · Yong Beom Kim[3] · Hyeun Joon Bae[1] · Hong Seuk Yang[4]

Received: 9 April 2019 / Accepted: 7 September 2019 / Published online: 18 September 2019

Abstract

Purpose Sugammadex is used to reverse neuromuscular block induced by rocuronium or vecuronium by forming a stable complex. If the binding capacity of any substance to sugammadex is large enough, this molecule will displace rocuronium or vecuronium from the complex. For drugs used in anesthesia, the binding affinity of remifentanil for sugammadex was highest. The aim of the current study was to investigate the decrease in the reversal of neuromuscular blockade with sugammadex by complex formation between remifentanil and sugammadex in the model using isolated hemidiaphragm of the rat.

Methods Phrenic nerve-hemidiaphragms from 34 male Sprague–Dawley rats were allocated randomly to four groups: 0 or 100 ng/ml remifentanil with equimolar amounts of sugammadex and 0 or 100 ng/ml remifentanil with three-quarter dose of sugammadex. Muscle contraction responses were recorded during the stimulation of the phrenic nerve by train-of-four (TOF) stimulation. Rocuronium was added to the organ bath with or without 100 ng/ml remifentanil until the first height response (T1) of TOF disappeared completely. Then, equimolar amounts or three-quarter dose of sugammadex was added.

Results Remifentanil has no significant effects on the concentration–response curves of rocuronium. No significant differences were observed in the recoveries of T1 and TOF ratio with time after administration of equimolar amounts or three-quarter dose of sugammadex regardless of the presence of 100 ng/ml remifentanil.

Conclusion Clinical concentration of remifentanil does not inhibit sugammadex reversal after rocuronium-induced neuromuscular block. Sugammadex can be used safely without worrying about the interaction with remifentanil.

Keywords Sugammadex · Remifentanil · Rocuronium

Introduction

Sugammadex, a γ-cyclodextrin, is widely used to reverse neuromuscular block induced by aminosteroid neuromuscular blockers [1]. Cyclodextrins are doughnut-shaped molecules to form inclusion complexes with other substances [2]. Sugammadex binds and inactivates rocuronium or vecuronium by forming a stable 1:1 water-soluble complex by a process of encapsulation [3]. This results in a rapid decline of free rocuronium concentration in the

✉ Hong Seuk Yang
hsyang@amc.seoul.kr

Jae Moon Choi
superoxide@naver.com

Ha-Jung Kim
alexakim06@gmail.com

Hey Ran Choi
viovio04@naver.com

Yong Beom Kim
fred0314@gilhospital.com

Hyeun Joon Bae
joshpheonix1@gmail.com

[1] Department of Anesthesiology and Pain Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea

[2] Department of Anesthesiology and Pain Medicine, Inje University Seoul Paik Hospital, Seoul, Republic of Korea

[3] Department of Anesthesiology and Pain Medicine, Gil Medical Center, College of Medicine, Gachon University, Incheon, Republic of Korea

[4] Department of Anesthesiology and Pain Medicine, Sun Medical Center, Daejeon 34811, Republic of Korea

plasma, and consequently causes a shift from the neuromuscular junction to the plasma [4]. It could be possible that sugammadex may combine with pharmaceutical molecules or endogenous substances. If the binding capacity of any substance to sugammadex is large enough, this molecule will displace rocuronium or vecuronium from the complex with sugammadex and form a complex with sugammadex instead of rocuronium or vecuronium [5]. This unintended phenomenon may threaten patient safety by causing insufficient recovery of muscle relaxation or reoccurrence of neuromuscular blockade.

Binding affinity between sugammadex and various drugs was determined by Zwiers et al. using isothermal titration calorimetry [5]. They have demonstrated the binding affinity of drugs used in anesthesia, such as ketamine, pentobarbital, propofol, remifentanil, oxycodone, pethidine and naloxone. Among these drugs, the binding affinity of remifentanil for sugammadex was highest, which equates to 0.28% and 0.87% of the value for sugammadex with rocuronium and vecuronium, respectively [5]. The affinity of remifentanil for sugammadex is lower than those of rocuronium or vecuronium; however, these are the results of a pharmacokinetic–pharmacodynamic modeling approach using isothermal titration calorimetry, and therefore need to be verified through an ex vivo study.

The aim of the current study was to investigate the decrease in the reversal of neuromuscular blockade with sugammadex by complex formation between remifentanil and sugammadex in the model using isolated hemidiaphragm of the rat. In addition, we evaluated the effects of remifentanil on rocuronium-induced muscle relaxation.

Materials and methods

After obtaining the ethical approval of the Institutional Animal Care and Use Committee of Asan Medical Center, Seoul, South Korea (approval number 2016-13-110), 34 male Sprague–Dawley rats (weighing 200–300 g) were anesthetized with intraperitoneal injection of 50 mg/kg alfaxalone (Alfaxan®, Jurox Pty. Limited, Rutherford, NSW, Australia). The left diaphragm and accompanying phrenic nerve were excised en bloc, and a phrenic nerve-hemidiaphragm was suspended vertically in an organ bath containing 75-ml Krebs solution (118 mmol l^{-1} NaCl, 4.7 mmol l^{-1} KCl, 2.5 mmol l^{-1} $CaCl_2$, 1.2 mmol l^{-1} $MgSO_4$, 1.2 mmol l^{-1} KH_2PO_4, 25 mmol l^{-1} $NaHCO_3$, and 11.4 mmol l^{-1} dextrose). The bath solution was aerated continuously with 95% O_2/5% CO_2 and its pH and temperature were kept at 7.4 and 35 ℃, respectively. The proximal portion of the phrenic nerve was connected to bipolar platinum electrodes. The central tendinous portion of the hemidiaphragm was connected to a force displacement transducer (FT03, Grass, Grass Instrument Co., Quincy, Massachusetts) to measure the isometric contraction of the hemidiaphragm at a resting tension of 2 g. Using a nerve stimulator (S88, Grass) and stimulus isolation unit (SIU5, Grass), the phrenic nerve was stimulated by 2-Hz train-of-four (TOF) stimulus every 20 s (rectangular pulses of 0.2 ms duration) via the bipolar platinum electrodes with individual supramaximal square-wave pulses throughout the study. After determining the supramaximal stimulation current, the phrenic nerve was continuously stimulated by TOF until the twitch height reached a stable plateau for at least 30 min, which was defined as the baseline. The first height response of the four twitches was considered as T1 and the TOF ratio was the ratio of the fourth twitch height response to the T1. Muscle contraction responses were recorded and digitized using a PowerLab acquisition system (ADInstruments, Austin, TX, USA). T1 and TOF ratio to the TOF stimulus was calculated with LabChart 7 (ADInstruments) after data acquisition [6–11].

The isolated phrenic nerve-hemidiaphragm preparations were randomly assigned to one of four groups according to the concentration of remifentanil and sugammadex: 0 or 100 ng/ml remifentanil with equimolar amounts of sugammadex and 0 or 100 ng/ml remifentanil with three-quarter dose of sugammadex. The remifentanil group was supplemented with remifentanil in an organ bath to achieve a remifentanil concentration of 100 ng/ml. Starting with 300 μg of rocuronium, a booster dose of 150 μg rocuronium was added to the organ bath every 10 min until the T1 disappeared completely. The concentrations of rocuronium that produced depression of the first twitch to 5, 50, and 95% of baseline value in each group were defined as the effective concentration $(EC)_5$, EC_{50}, and EC_{95} of rocuronium, respectively. Equimolar amounts of sugammadex or three-quarter dose of sugammadex corresponding to the total amounts of administered rocuronium was added into the organ bath 10 min after T1 disappeared to evaluate the recovery of contractions from the rocuronium-induced neuromuscular block with or without 100 ng/ml remifentanil. We observed reversal of rocuronium-induced neuromuscular block by sugammadex for 30 min in all groups. Recovery of T1 and TOF ratio was monitored at every 5 min until 30 min.

Statistical analysis

Continuous data were tested for a normal distribution using the Shapiro–Wilk test. Data are expressed as mean ± SD. Continuous variables were compared using an analysis of variance (ANOVA). Two-way repeated-measures ANOVA was used to analyze the differences in T1 and TOF ratio between groups over time. Mauchly's sphericity test was used to validate an assumption of repeated-measures factor ANOVA. Greenhouse–Geisser corrected significance

values were used when sphericity was lacking [12]. A P value < 0.05 was used as a threshold for statistically significant differences. Statistical analyses were performed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA).

In an exploratory animal study verifying the inhibiting effect of remifentanil on sugammadex reversal after rocuronium-induced neuromuscular block, sample size calculation is difficult. We thus referred to previous studies that suggested using 8–10 animals per group to attain statistically significant data [7, 8, 10, 13–15].

Results

Table 1 lists the characteristics of tissue specimens. There were no significant differences in muscle mass, size, and the height of initial T1 among the groups. The total amount of rocuronium administered to the organ bath did not show significant differences among the groups. The results of the repeated-measures ANOVA showed insignificant effects of 100 ng/ml remifentanil on the concentration–response curves of rocuronium for T1 depression (Fig. 1). The EC_{50} of rocuronium was 13.5 ± 1.4 and 12.7 ± 2.3 in the 0 and 100 ng/ml remifentanil groups, respectively. There were no significant differences in EC_5, EC_{50}, and EC_{95} of rocuronium, and total amount of administered rocuronium (Table 2). No significant differences were observed in the recoveries of T1 and TOF ratio with time after administration of equimolar amounts of sugammadex regardless of the presence of 100 ng/ml remifentanil (Fig. 2). Additional studies were performed to determine the effect of remifentanil on the reversal of neuromuscular block below equimolar amounts of sugammadex. T1 recovery did not occur at all with half dose of sugammadex for 30 min in a preliminary experiment (data not shown), so we tested with three-quarter dose of sugammadex. There were also no significant differences in the time-course of the recoveries of T1 and TOF ratio after administration of three-quarter dose of sugammadex (Fig. 3).

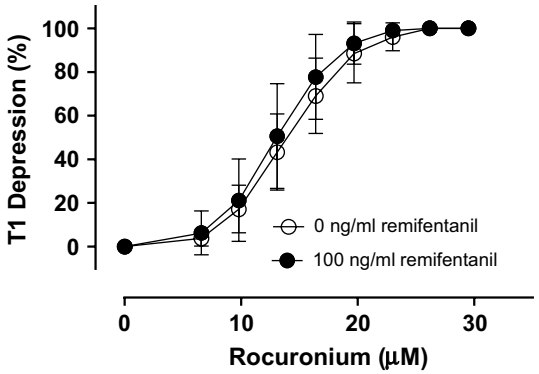

Fig. 1 Cumulative concentration–response curves of rocuronium with ($n = 16$) or without ($n = 18$) 100 ng/ml remifentanil. There were no significant differences between groups in the concentration–response curves of rocuronium for T1 depression over time. Data are presented as mean ± SD. T1, the first height response of four twitches

Discussion

In the present study, we investigated the effect of remifentanil on rocuronium-induced muscle relaxation and reversal of neuromuscular blockade with sugammadex in the isolated hemidiaphragm of the rat. The presence of 100 ng/ml remifentanil had no significant effect on muscle relaxation by rocuronium. In addition, reversal of rocuronium-induced neuromuscular block with sugammadex does not seem to be significantly influenced by clinical concentrations of remifentanil.

Rocuronium was introduced as an intermediate-acting steroidal neuromuscular blocker with a very rapid onset [16]. Various drugs interact with nondepolarizing neuromuscular blocking drugs. Magnesium sulfate, verapamil and amlodipine appear to potentiate the neuromuscular blockade induced by nondepolarizing neuromuscular blocking drugs [17, 18]. Selective estrogen receptor modulators such as tamoxifen, appear to augment the blockade induced by neuromuscular blocking drugs [19]. Calcium and steroids

Table 1 Characteristics of tissue specimens

Sugammadex	Equimolar amounts		Three-quarter amounts	
Remifentanil (ng/ml)	0 ($n = 9$)	100 ($n = 8$)	0 ($n = 9$)	100 ($n = 8$)
Muscle mass (mg)	186 ± 33	188 ± 41	200 ± 64	229 ± 87
Muscle length (mm)	9 ± 2	9 ± 1	9 ± 2	9 ± 3
Muscle width (mm)	19 ± 2	19 ± 1	19 ± 1	20 ± 1
Initial T1 (g)	16.9 ± 3.6	12.2 ± 2.7	14.0 ± 4.1	14.5 ± 4.0

Values are expressed as mean ± SD

No significant differences were observed among the groups

T1, the first height response of four twitches; n, number

Table 2 Effective concentrations of rocuronium according to remifentanil concentration

Rocuromium (μM)	Remifentanil	
	0 ng/ml ($n = 18$)	100 ng/ml ($n = 16$)
EC_5	8.9 ± 1.3	8.4 ± 2.4
EC_{50}	13.5 ± 1.4	12.7 ± 2.3
EC_{95}	18.0 ± 1.7	17.1 ± 2.1
Final concentration	22.8 ± 3.0	21.1 ± 3.2

Values are expressed as mean ± SD

No significant differences were observed between the groups

EC, effective concentration; n, number

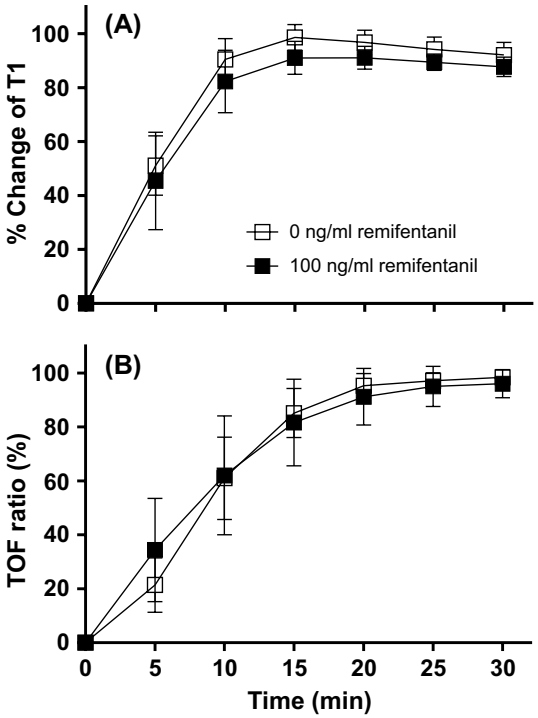

Fig. 2 Changes of T1 (**a**) and TOF ratio (**b**) for 30 min after administration of equimolar amounts of sugammadex. Insignificant effects of 100 ng/ml remifentanil on the recoveries of T1 and TOF ratio with time after administration of equimolar amounts of sugammadex were observed. Data are presented as mean ± SD. T1, the first height response of four twitches; TOF, train-of-four

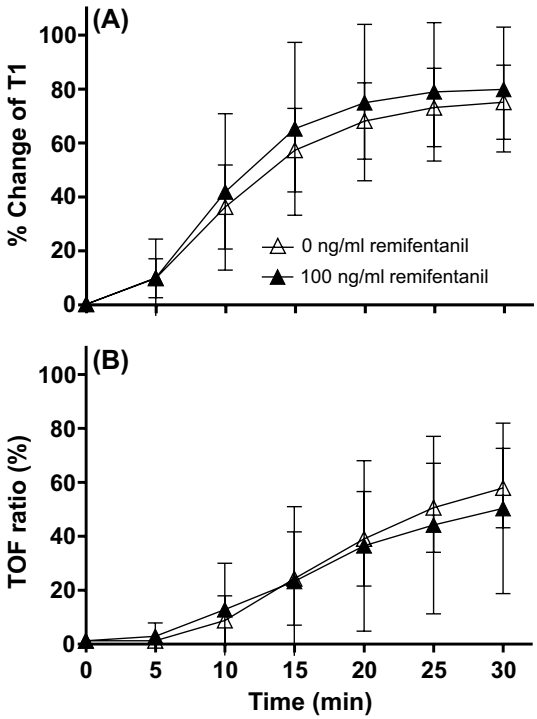

Fig. 3 Changes of T1 (**a**) and TOF ratio (**b**) for 30 min after administration of three-quarter dose of sugammadex. There were no significant differences in the recoveries of T1 and TOF ratio with time after administration of three-quarter dose of sugammadex regardless of the presence of 100 ng/ml remifentanil. Data are presented as mean ± SD. T1, the first height response of four twitches; TOF, train-of-four

antagonize the effects of nondepolarizing neuromuscular blockers [20]. In our present study, rocuronium-induced neuromuscular block was not influenced by the presence of remifentanil in an organ bath.

Sugammadex reduces the amounts of free neuromuscular blocking agents in the neuromuscular junction by forming a stable complex with rocuronium or vecuronium [1, 21]. Various compounds besides rocuronium and vecuronium demonstrated binding affinity for sugammadex, meaning that the potential for displacement of neuromuscular blocking agent from the complex with sugammadex and recurrence of neuromuscular blockade could theoretically occur. Flucloxacillin was found to have potential for displacement reactions with sugammadex [5]. However, administration of flucloxacillin did not result in recurrence of neuromuscular blockade through displacement of sugammadex from the sugammadex–neuromuscular blockade complex in healthy anaesthetized volunteers [22]. Dexamethasone, which structurally resembles the aminosteroid neuromuscular blocking agents, did not delay neuromuscular blockade reversal by sugammadex in anesthetized patients [23]. Of the drugs that may be used in anesthesia, remifentanil has the highest affinity to sugammadex [5]. Our results show that the recoveries of T1 and TOF ratio with time after administration of equimolar amounts of sugammadex were not different by the presence of 100 ng/ml remifentanil. Since the effect-site concentration of remifentanil is usually between 3 and 8 ng/ml for total intravenous anesthesia with propofol [24, 25], remifentanil at the clinical use level does not seem to influence neuromuscular blockade reversal by sugammadex.

Experiments with three-quarter dose of sugammadex were conducted to investigate the effect of remifentanil on the reversal effect of sugammadex when sugammadex was not administered in sufficient amounts. The recoveries of T1 with three-quarter dose of sugammadex were 76% of the recoveries of T1 with equimolar amounts of sugammadex corresponding to the total amounts of administered rocuronium. Even in this situation, the presence of 100 ng/ml remifentanil did not affect the reversal of muscle relaxation by sugammadex.

Our present study had several limitations. This study was an ex vivo experiment, wherein the pharmacodynamic and pharmacokinetic properties of each agent were excluded. Thus, our results may not be generalizable in an in vivo study or clinical situations in which sugammadex may interact with other drugs being used during general anesthesia. An additional limitation to our study was that we used 100 ng/ml remifentanil, which is not widely used in clinical

practice. However, experiments with a high concentration of remifentanil did not affect the reversal of rocuronium-induced neuromuscular block with sugammadex, so the use of sugammadex in the presence of remifentanil is considered safe in clinical practice.

In conclusion, clinical concentration of remifentanil does not inhibit sugammadex reversal after rocuronium-induced neuromuscular block. Sugammadex, a breakthrough drug to reverse neuromuscular blockade, can be used safely without worrying about the interaction with remifentanil in patients undergoing total intravenous anesthesia with propofol and remifentanil or in patients receiving remifentanil as an adjuvant during inhalation anesthesia.

Acknowledgements Assistance with the study: these authors are members of the Neuromuscular Physiology Research Team at the Laboratory of Animal Research, Asan Institute of Life Science, Seoul, Republic of Korea.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests in relation to this study.

References

1. Naguib M. Sugammadex: another milestone in clinical neuromuscular pharmacology. Anesth Analg. 2007;104:575–81.
2. Saokham P. Loftsson T. Gamma-cyclodextrin. Int J Pharm. 2017;516:278–92.
3. Bom A, Epemolu O, Hope F, Rutherford S, Thomson K. Selective relaxant binding agents for reversal of neuromuscular blockade. Curr Opin Pharmacol. 2007;7:298–302.
4. Gijsenbergh F, Ramael S, Houwing N, van Iersel T. First human exposure of Org 25969, a novel agent to reverse the action of rocuronium bromide. Anesthesiology. 2005;103:695–703.
5. Zwiers A, van den Heuvel M, Smeets J, Rutherford S. Assessment of the potential for displacement interactions with sugammadex: a pharmacokinetic–pharmacodynamic modelling approach. Clin Drug Investig. 2011;31:101–11.
6. Kim YB, Lee S, Lee KC, Kim HJ, Ro YJ, Yang HS. Effects of presynaptic muscarinic cholinoreceptor blockade on neuromuscular transmission as assessed by the train-of-four and the tetanic fade response to rocuronium. Clin Exp Pharmacol Physiol. 2017;44:795–802.
7. In J, Bae H, Choi H, Kim YB, Lee S, Yang HS. Dexamethasone concentration affecting rocuronium-induced neuromuscular blockade and sugammadex reversal in a rat phrenic nerve-hemidiaphragm model: an ex vivo study. Eur J Anaesthesiol. 2018;35:856–62.
8. Haerter F, Simons JC, Foerster U, Moreno Duarte I, Diaz-Gil D, Ganapati S, Eikermann-Haerter K, Ayata C, Zhang B, Blobner M, Isaacs L, Eikermann M. Comparative effectiveness of calabadion and sugammadex to reverse non-depolarizing neuromuscular-blocking agents. Anesthesiology. 2015;123:1337–499.
9. Sung TY, You HJ, Cho CK, Choi HR, Kim YB, Shin YS, Yang HS. Effects of magnesium chloride on rocuronium-induced neuromuscular blockade and sugammadex reversal in an isolated rat phrenic nerve-hemidiaphragm preparation: an in-vitro study. Eur J Anaesthesiol. 2018;35:193–9.
10. Fortier LP, Robitaille R, Donati F. Increased sensitivity to depolarization and nondepolarizing neuromuscular blocking agents in young rat hemidiaphragms. Anesthesiology. 2001;95:478–84.
11. Singh YN, Harvey AL, Marshall IG. Antibiotic-induced paralysis of the mouse phrenic nerve-hemidiaphragm preparation, and reversibility by calcium and by neostigmine. Anesthesiology. 1978;48:418–24.
12. Meyers LS, Gamst GC, Guarino AJ. Applied multivariate research: design and interpretation. 1st ed. Thousand Oaks: Sage Publications; 2005.
13. Itoh H, Shibata K, Matsumoto T, Nitta S, Nishi M, Kobayashi T, Yamamoto K. Effects of neuromuscular-blocking drugs in rats in vivo: direct measurements in the diaphragm and tibialis anterior muscle. Acta Anaesthesiol Scand. 2004;48:903–8.
14. Shiraishi S, Cho S, Akiyama D, Ichinomiya T, Shibata I, Yoshitomi O, Maekawa T, Ozawa E, Miyaaki H, Hara T. Sevoflurane has postconditioning as well as preconditioning properties against hepatic warm ischemia-reperfusion injury in rats. J Anesth. 2019;33:390–8.
15. Nishigaki A, Kawano T, Iwata H, Aoyama B, Yamanaka D, Tateiwa H, Shigematsu-Locatelli M, Eguchi S, Locatelli FM, Yokoyama M. Acute and long-term effects of haloperidol on surgery-induced neuroinflammation and cognitive deficits in aged rats. J Anesth. 2019;33:416–25.
16. Wierda JM, de Wit AP, Kuizenga K, Agoston S. Clinical observations on the neuromuscular blocking action of Org 9426, a new steroidal non-depolarizing agent. Br J Anaesth. 1990;64:521–3.
17. Ozkul Y. Influence of calcium channel blocker drugs in neuromuscular transmission. Clin Neurophysiol. 2007;118:2005–8.
18. Fuchs-Buder T, Wilder-Smith OH, Borgeat A, Tassonyi E. Interaction of magnesium sulphate with vecuronium-induced neuromuscular block. Br J Anaesth. 1995;74:405–9.
19. Naguib M, Gyasi HK. Antiestrogenic drugs and atracurium–a possible interaction? Can Anaesth Soc J. 1986;33:682–3.
20. Meyers EF. Partial recovery from pancuronium neuromuscular blockade following hydrocortisone administration. Anesthesiology. 1977;46:148–50.
21. Bom A, Bradley M, Cameron K, Clark JK, Van Egmond J, Feilden H, MacLean EJ, Muir AW, Palin R, Rees DC, Zhang MQ. A novel concept of reversing neuromuscular block: chemical encapsulation of rocuronium bromide by a cyclodextrin-based synthetic host. Angew Chem Int Ed Engl. 2002;41:266–70.
22. Kam PJ, Heuvel MW, Grobara P, Zwiers A, Jadoul JL, Clerck E, Ramael S, Peeters PA. Flucloxacillin and diclofenac do not cause recurrence of neuromuscular blockade after reversal with sugammadex. Clin Drug Investig. 2012;32:203–12.
23. Rezonja K, Mars T, Jerin A, Kozelj G, Pozar-Lukanovic N, Sostaric M. Dexamethasone does not diminish sugammadex reversal of neuromuscular block—clinical study in surgical patients undergoing general anesthesia. BMC Anesthesiol. 2016;16:101.
24. Poterman M, Scheeren TWL, van der Velde MI, Buisman PL, Allaert S, Struys M, Kalmar AF. Prophylactic atropine administration attenuates the negative haemodynamic effects of induction of anaesthesia with propofol and high-dose remifentanil: a randomised controlled trial. Eur J Anaesthesiol. 2017;34:695–701.
25. Scott HB, Choi SW, Wong GT, Irwin MG. The effect of remifentanil on propofol requirements to achieve loss of response to command vs. loss of response to pain. Anaesthesia. 2017;72:479–87.