SCIENTIFIC ARTICLE

Functional respiratory imaging after neostigmine- or sugammadex-enhanced recovery from neuromuscular blockade in the anesthetised rat: a randomised controlled pilot study ☆

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Abstract

Objectives: Reductions in diaphragm activity are associated with the postoperative development of atelectasis. Neostigmine reversal is also associated with increased atelectasis. We assessed the effects of neostigmine, sugammadex, and spontaneous reversal on regional lung ventilation and airway flow.

Methods: Six Sprague−Dawley rats were paralysed with rocuronium and mechanically ventilated until recovery of the train-of-four ratio to 0.5. We administered neostigmine ($0.06\,mg.kg^{-1}$), sugammadex ($15\,mg.kg^{-1}$), or saline ($n = 2$ per group). Computed tomography scans were obtained during the breathing cycle. Three-dimensional models of lung lobes were generated using functional respiratory imaging technology, and lobar volumes were calculated during the breathing cycle. The diaphragmatic surface was segmented for the end-expiratory and end-inspiratory scans. The total change in volume was reported by the lung volume change from the end-expiratory scan to the end-inspiratory scan. Chest wall movement was defined as the lung volume change minus the volume change that resulted from diaphragm excursion.

Results: The two rats that received neostigmine exhibited a smaller relative contribution of diaphragm movement to the total change in lung volume compared with the two rats that received sugammadex or saline (chest wall contribution (%): 26.69 and 25.55 for neostigmine; −2.77 and 15.98 for sugammadex; 18.82 and 10.30 for saline).

Conclusion: This pilot study in rats demonstrated an increased relative contribution of chest wall expansion after neostigmine compared with sugammadex or saline. This smaller relative contribution of diaphragm movement may be explained by a neostigmine-induced decrease in phrenic nerve activity or by remaining occupied acetylcholine receptors after neostigmine.

Imagem funcional da respiração após aceleração da recuperação do bloqueio neuromuscular com neostigmina ou sugamadex em ratos anestesiados: estudo piloto controlado e randomizado

Resumo
Objetivos: As reduções da atividade do diafragma estão associadas ao desenvolvimento de atelectasia no período pós-operatório. A reversão com neostigmina também está associada ao aumento de atelectasia. Avaliamos os efeitos de neostigmina, sugamadex e da reversão espontânea sobre a ventilação pulmonar regional e fluxo aéreo.
Métodos: Seis ratos Sprague-Dawley foram paralisados com rocurônio e mecanicamente ventilados até a recuperação da sequência de quatro estímulos atingir relação 0,5. Administramos neostigmina (0,06 mg.kg^{-1}), sugamadex (15 mg.kg^{-1}) ou solução salina (n = 2 por grupo). As tomografias foram realizadas durante o ciclo respiratório. Modelos tridimensionais dos lobos pulmonares foram gerados usando a tecnologia de imagem funcional respiratória e os volumes lobares foram calculados durante o ciclo respiratório. A superfície diafragmática foi segmentada para as varreduras expiratória final e inspiratória final. A alteração total no volume foi relatada pela alteração do volume pulmonar da varredura expiratória final para a varredura inspiratória final. O movimento da parede torácica foi definido como a variação do volume pulmonar menos a alteração no volume resultante da excursão do diafragma.
Resultados: Os dois ratos que receberam neostigmina apresentaram uma contribuição relativa menor do movimento do diafragma para a alteração total do volume pulmonar em comparação com os dois ratos que receberam sugamadex ou solução salina (contribuição da parede torácica (%): 26,69 e 25,55 para neostigmina; -2,77 e 15.98 para sugamadex; 18,82 e 10,30 para solução salina).
Conclusão: Este estudo piloto com ratos demonstrou uma contribuição relativa aumentada de expansão da parede torácica após neostigmine em comparação com sugamadex ou solução salina. Essa contribuição relativa menor de movimento do diafragma pode ser explicada por uma redução induzida por neostigmina na atividade do nervo frênico ou por receptores de acetilcolina permanecerem ocupados após a administração de neostigmina.

Introduction

The use of Neuromuscular Blocking Agents (NMBAs) is associated with postoperative pulmonary complications at the end of surgery.[1] Respiratory complications after surgery (e.g., desaturation and atelectasis) are potentially related to the lack of diaphragm activity, which cannot be detected using peripheral Acceleromyography (AMG) monitoring.[2,3]

A previous trial by our study group associated the use of a Selective Relaxant-Binding Agent (SRBA) for the reversal of neuromuscular blockade, sugammadex, to an increase in diaphragm Electromyographic activity (EMGdi) compared with reversal with neostigmine.[4] More nicotinic acetylcholine receptors may be free from rocuronium in diaphragmatic neuromuscular junctions after SRBA

compared with neostigmine, or neostigmine may exert a direct negative impact on phrenic nerve activity.[5] These factors may independently or concomitantly exert subsequent effects on central breathing control and influence the balance between the accessory inspiratory (chest wall) muscle and diaphragm muscle activity. We performed a pilot study in rats using functional respiratory imaging (FRI, FLUIDDA, Kontich, Belgium) to assess regional lung ventilation after sugammadex, neostigmine or spontaneous reversal and further explore the balance between diaphragm and accessory inspiratory muscle activity. Micro-computed Tomography (CT) scan images provided accurate reconstructions of airway morphology in free-breathing rats.[6] High-resolution CT scans are limited to static information of the respiratory system without additional post-imaging processing. FRI enables

studies of regional flow and allows structural simulations. Different airway sections are associated with corresponding lung tissue to render a full anatomical picture. A comparison of morphological scans at different breathing levels (e.g., end-inspiratory and end-expiratory) allows the modelling of regional airway and alveolar recruitment.

Methods

This study was a randomised, controlled, parallel-group double-blinded trial in rats. We compared spontaneous (no drug) reversal with neostigmine- or sugammadex-enhanced reversal of a rocuronium-induced neuromuscular blockade. Animal protocols strictly adhered to the institutional guidelines for animal care and use for research purposes. Approval of the Committee for Medical Ethics and the Use of Experimental Animals at the University of Antwerp, Belgium was obtained (Ref. 2014-63, Chairperson Prof. Dr. P. De Deyn) on November 17, 2014. The study was registered as NCT02284412. Every effort was made to minimise the number of rats used. The experiments were conducted at the Bruker facilities (Kontich, Belgium), and qualified personnel performed all experimental animal procedures.

Experimental animal preparation

Six adult male Sprague–Dawley rats (LA number: 1100155) weighing 377–451 g were fed a standard pellet diet and water ad libitum. Room temperature and humidity were monitored daily and kept constant. Twelve-hour light and dark cycles were provided. The 6 animals were randomised to one of the following three groups in a 1:1:1 ratio according to a computer-generated randomisation list: 0.06 mg.kg^{-1} neostigmine, 15 mg.kg^{-1} sugammadex[7] or saline. All drugs were obtained from clinical supplies. Anesthesia was induced in an induction chamber using 5% isoflurane, and anesthesia was maintained with 1.5–2.5% isoflurane in air-oxygen. A tail vein and the trachea were cannulated after anesthesia induction. The rats were placed in a supine position with the head in a neutral position and connected to a breathing circuit (Harvard model 683 Small Animal Ventilator, Holliston, MA, USA). Body temperature was measured using a rectal probe and maintained at >36 °C using a thermostatically controlled heating plate. Heart rate, breathing frequency and paw skin colour were maintained within normal ranges.

The right leg was shaved, and the femoral nerve was stimulated supramaximally using subcutaneous needle electrodes (B. Braun Melsungen AG, Melsungen, Germany) for assessments of neuromuscular transmission. The evoked response of the femoral muscle was measured using accelerometry with the TOF-Watch SX (MIPM Mammendorfer Institut für Physik und Medizin GmbH, Munich, Germany), as described previously.[7] The transducer was fixed to the skin ventromedially at the proximal end of the thigh (Fig. 1). The supramaximal stimulation current was determined, and the femoral nerve was continuously stimulated at 1 Hz (8–10 mA) until the twitch height reached a stable plateau. The stimulation pattern was changed thereafter to train-of-four (TOF) stimulation (2 Hz) applied for a minimum of 3 min to calibrate the TOF-Watch SX monitor (calibration mode 1). TOF
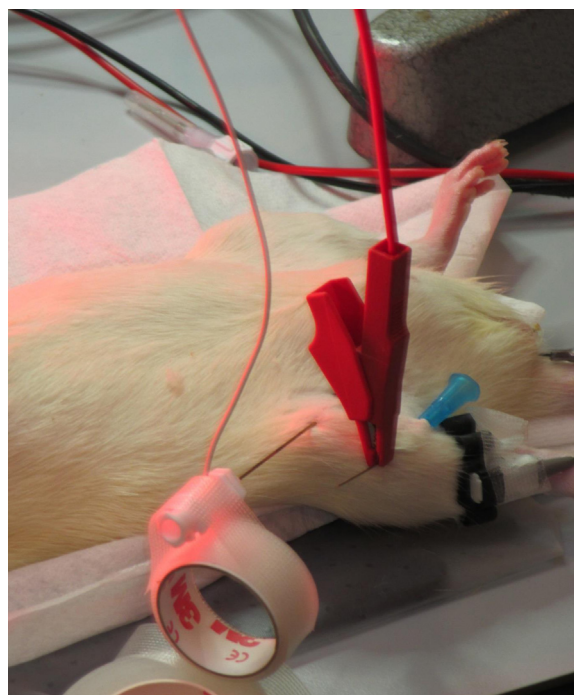

Figure 1 Assessment of neuromuscular transmission using accelerometry with the TOF Watch SX, subcutaneous needle electrodes for femoral nerve stimulation and transducer placement on the rat thigh for muscle response measurements.

stimulation was continued for at least 2 min before injection of the NMBA. Rocuronium (3.5 mg.kg^{-1}, 2 times the effective dose (ED) 90) was injected, and the lungs were ventilated using a 2.5 mL tidal volume, 90 breaths per minute ventilation scheme. TOF stimulation with a 15 s interval was continued throughout the remainder of the procedure.

We administered 0.06 mg.kg^{-1} neostigmine, 15 mg.kg^{-1} sugammadex or saline at a TOF = 0.5. Neostigmine was dosed as 0.06 mg.kg^{-1}, and glycopyrrolate was used at 0.012 mg.kg^{-1} (commercially available 5:1 co-formulation). The syringes that contained sugammadex or neostigmine/glycopyrrolate contained approximately 1 mL of fluid. Therefore, the third group received 1 mL of saline. All prepared syringes were covered with tape to mask the potential colour and fluid volume differences as an additional precaution for blinding. All drugs were administered intravenously over 5–10 s via the tail vein and flushed with 0.5 mL of saline. TOF monitoring was removed for practical reasons at a TOF ratio ≥0.9, and the animals were placed in the CT scanner in a prone position. Animals were allowed to breathe spontaneously through a custom-designed breathing system. Weaning off artificial ventilation was performed via a gradual lowering of respiratory frequency until full spontaneous breathing was observed. The animals remained under anesthesia (1.5% isoflurane), and CT scanning was initiated as soon as possible. The front paws and tail were loosely fixed aside when the rat was in the CT tube to clear these appendages from the field of view (Fig. 2). We targeted a light anesthesia depth (righting reflex lost, marked response to painful stimuli) during the remaining procedure, including the scanning period, instead of a surgical depth of anesthesia before intubation and placement of the cannulae. We
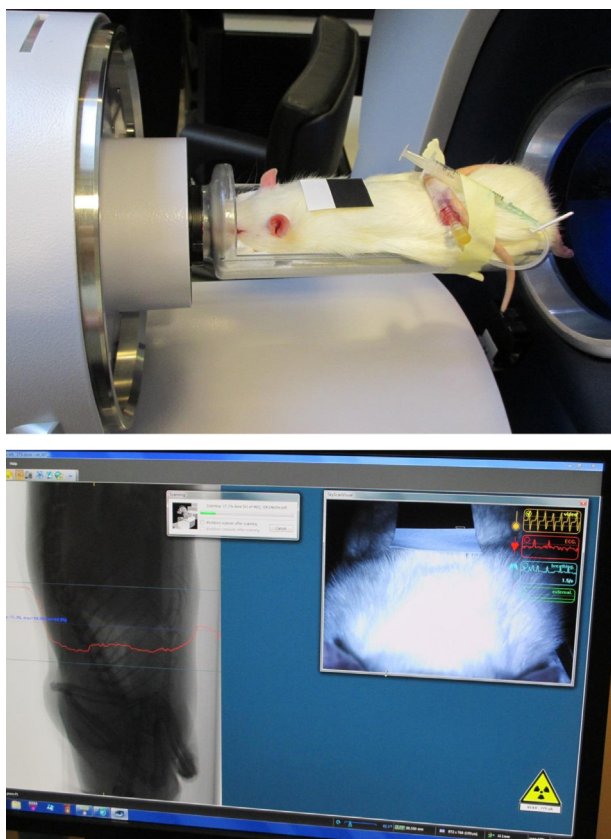
Figure 2 Top: Position of the rat in the CT tube. Bottom: The physiological monitoring system included video monitoring of the rat with real-time breathing detection. The software analyses the video stream from the point where breathing movement is visible. These movements are converted into a movement waveform to provide breathing time marks for time-resolved micro-CT reconstruction. Top and bottom: The anesthetic delivery facemask on the animal is connected to a flow sensor for direct breathing detection. Multiple projection images obtained at each angular position are sorted post-scan into breathing time bins using recorded physiological monitoring time marks.

used the respiratory frequency as parameters for anesthesia depth maintenance during the CT scanning period when the rats were inaccessible. All records were kept and stored. All animals were euthanised at the end of the procedure.

Imaging

We used the SkyScan 1278 in vivo micro-CT scanner (Bruker microCT, Kontich, Belgium). This system has integrated physiological monitoring, including breathing movement, and four-dimensional (4D) time-resolved microtomography capabilities. Dynamic scanning techniques, as used in the present 4D free-breathing CT scan protocol, exhibit the intrinsic problem of motion artefacts. We used the respiratory gating technique to reduce these artefacts. Motion of the animal's thorax was registered, and their breathing patterns were monitored to apply this gating. The gating thresholds were set on these recordings. Scans were obtained using a source voltage of 70 kV and a source current of 140 μA. The resolution was set to 100 μm. The scanner had

an 8 cm wide field of view, which spanned the entire thorax. The retrospectively synchronised ''listmode'' scan was performed with an exposure time of 40 ms, a scan rotation of 360° and step of 0.75°. Twenty-five images were acquired per step for subsequent sorting into breathing cycle phases. The time of all video-recorded breathing inhalation movement maxima and the time of acquisition of all projection images were recorded during the scan in text files with a precision of ±1 ms to facilitate listmode sorting. End-expiratory and end-inspiratory gated and binned images around tidal breathing were further processed. The respiratory wavetrain was displayed in real-time, and data were exported at the end of the scanning process for further reference. The entire scanning procedure took between 18 and 24 min. CT images were post-processed to assess segmentation and regional flow patterns and visualise and quantify the following parameters of the micro-CT images: (1) lung and lobe volumes at inspiration and expiration; (2) airway volumes at inspiration and expiration; (3) diaphragm shape and deformation with resulting lung volume change from inspiration to expiration; and (4) skeletal movement from inspiration to expiration. These parameters were used to assess differences between treatment groups.

The present study compared the effect of sugammadex, neostigmine/glycopyrrolate and spontaneous reversal on regional lung ventilation. All comparisons were performed with an exploratory intent, and no statistical analyses were performed. Data are expressed as absolute values (%).

Results

The time from rocuronium administration to recovery of the TOF ratio to 0.5 was 14 min and 14 min in the rats that received neostigmine, 15 min and 21 min in the rats that received sugammadex, and 14 min and 16 min in rats that received saline. The time from the start of neostigmine or sugammadex or saline administration to recovery of the TOF ratio to 0.9 was 120 and 90 s, 45 and 90 s, and 120 and 360 s, respectively.

Lung lobes were segmented and divided for FRI based on visible lobar fissures on the CT scan. There are five lung lobes in the rat: one Left Lobe (LL) and four lobes on the right (cranial (RCrL), middle (RML), caudal (RCL) and accessory (RAL) lobes). The accessory lobe contacts the diaphragm and apex of the heart and it is notched to accommodate the caudal caval vein.[8] Fig. 3 presents an example of the 3D reconstruction of the lung lobes for one rat. The fissure dividing the right cranial lobe and the right middle lobe was not visible on most of the scans, and the right cranial lobe often appeared collapsed. Therefore, these lobes were not separated and considered a single lobe for all subsequent analyses.

Lobar volumes were reconstructed for all rats, and total lung volumes were calculated using these models. The relative lobar growth from the end-expiratory scan level (hereafter referred to as FRC) to the end-inspiratory scan level (hereafter referred to as TLC), $V_{TLClobe} - V_{FRClobe}/V_{TLClungs} - V_{FRClungs}$, was considered a measure for the internal distribution of inhaled air. Table 1 presents lobar and total lung volume values at FRC and TLC for all rats. Internal airflow distribution was calculated for

Table 1 Lobar and total lung volumes (in mL) at the end-expiratory scan (FRC) and end-inspiratory scan (TLC).

	Neostigmine				Sugammadex				Saline			
	FRC	TLC	FRC	TLC	FRC	TLC	FRC	TLC	FRC	TLC	FRC	TLC
RCrL + RML	0.60	1.14	0.81	1.22	0.66	0.95	1.12	1.80	1.42	1.74	0.43	0.81
RCL	1.69	2.48	1.72	3.09	2.33	2.91	1.83	2.56	2.42	3.58	1.55	2.65
RAL	0.73	1.09	0.73	1.39	1.01	1.49	0.40	0.56	0.70	1.03	0.41	0.83
LL	1.81	2.58	2.26	3.73	1.70	2.24	1.84	2.60	1.82	2.51	0.52	1.07
Total	4.84	7.29	5.52	9.43	5.70	7.60	5.19	7.52	6.36	8.86	2.91	5.36

RCrL, right cranial lobe; RML, right middle lobe; RCL, right caudal lobe; RAL, right accessory lobe; LL, left lobe.

Table 2 Internal airflow distribution (%) from the end-expiratory scan (FRC) to the end-inspiratory scan (TLC).

	Neostigmine		Sugammadex		Saline	
RCrL + RML	21.77	10.31	15.29	29.14	13.07	15.49
RCL	32.24	35.27	30.72	31.31	46.42	45.06
RAL	14.69	16.74	25.55	6.84	13.28	16.82
LL	31.30	37.68	28.44	32.70	27.22	22.63

RCrL, right cranial lobe; RML, right middle lobe; RCL, right caudal lobe; RAL, right accessory lobe; LL, left lobe.

each rat using these values, and the results are presented in Table 2.

Three-dimensional models of the diaphragmatic surface were generated for FRC and TLC after the identification of the FRC and TLC scans based on the total lung volumes, as exemplified in Fig. 4 (same rat as Fig. 3). Diaphragm movement was assessed by measuring the volume contained between the two surfaces. The total change in volume is indicated by the lung volume change from FRC to TLC.
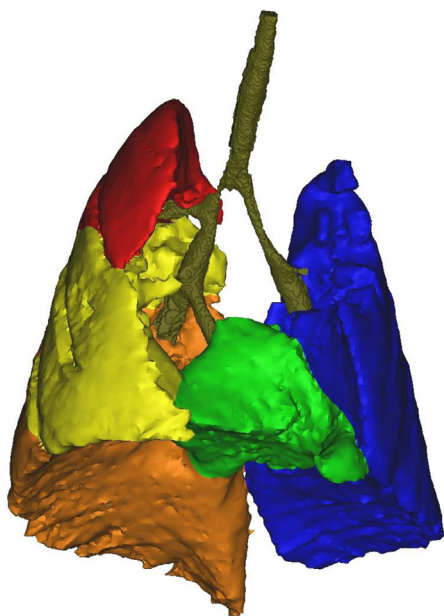
Therefore, the chest wall movement was defined as the total lung volume change minus the lung volume change resulting from diaphragmatic movement.

Table 3 summarises the calculations that were performed to obtain the relative contributions of the chest wall expansion and diaphragm movement to the total change of lung volume. The relative contribution of chest wall expansion was increased in neostigmine-treated rats compared with sugammadex- or saline-treated rats (chest wall contribution (%): 26.69 and 25.55 for neostigmine; -2.77 and 15.98 for sugammadex; 18.82 and 10.30 for saline).

Discussion

This pilot study compared the effects of sugammadex, neostigmine/glycopyrrolate and spontaneous reversal on regional lung ventilation in rats paralysed with rocuronium and reversed (or not) at a TOF ratio of 0.5. CT scans were obtained after recovery of the TOF ratio >0.9 during the spontaneous breathing cycle, and 3D models of the lung lobes were generated using FRI technology to calculate lobar volumes. The relative contributions of the chest wall
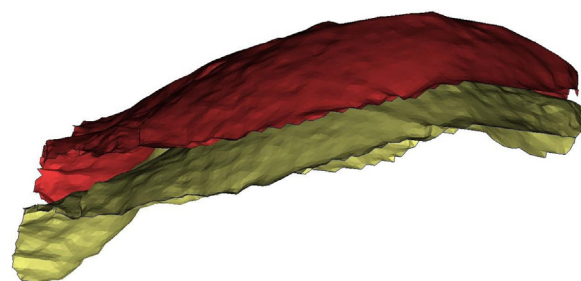

Figure 3 Three-dimensional reconstruction of the lung lobes at the end-inspiratory scan for one of the study rats. Right cranial lobe (RCrL) – red; right middle lobe (RML) – yellow; right caudal lobe (RCL) – orange; right accessory lobe (RAL) – green; left lobe (LL) – blue.

Figure 4 3D reconstruction of the diaphragm at the end-inspiratory scan (TLC) – yellow and the end-expiratory scan (FRC) – red.

Table 3 Total lung, diaphragm and chest wall volume changes from the end-expiratory scan (FRC) to the end-inspiratory scan (TLC) and the relative contribution of the chest wall expansion and diaphragm movement to the total volume change.

	Neostigmine		Sugammadex		Saline	
Total lung volume change (mL)	2.46	3.91	1.91	2.34	2.51	2.45
Diaphragm volume change (mL)	1.80	2.91	1.96	1.97	2.04	2.20
Chest wall volume change (mL)	0.66	1.00	−0.05	0.37	0.47	0.25
Diaphragm contribution (%)	73.31	74.45	102.77	84.02	81.18	89.70
Chest wall contribution (%)	26.69	25.55	−2.77	15.98	18.82	10.30

expansion and diaphragm movement to the total change of lung volume were obtained. The two rats that received neostigmine as a reversal agent displayed a smaller relative contribution of diaphragm movement to total change in lung volume compared with rats that received sugammadex or saline. Consequently, the relative contribution of chest wall expansion was increased in neostigmine rats compared with rats that received sugammadex or saline.

The exact effect of neuromuscular blockade and reversal agents on the diaphragm in a perioperative setting is not well studied. Mechanical ventilation and rocuronium play independent roles in diaphragmatic dysfunction.[9] Mechanical ventilation exerts relatively short-term effects on the rat diaphragm, including a reduction in blood flow, which impairs oxygen uptake.[10] However, good diaphragmatic function is essential in patients at the end of surgery when spontaneous breathing is resumed. The diaphragm is the most important inspiratory muscle, and reductions in diaphragm activity are associated with the development of atelectasis postoperatively.[2]

A previous study compared the effects of neostigmine/glycopyrrolate and sugammadex on EMGdi.[4] EMGdi, tidal volume and PaO_2 following tracheal extubation at a TOF ratio >0.9 decreased after neostigmine compared with sugammadex, which reflects a reduced diaphragm-driven inspiration after neostigmine. Eikermann et al.[11] demonstrated that neostigmine alone (without prior treatment with a NMBA) decreased diaphragmatic EMG activity. Neostigmine reversal was recently associated with increased atelectasis and longer postoperative hospital stays. The unwarranted use of neostigmine (neostigmine administration without appropriate guidance from neuromuscular transmission monitoring) is associated with an increased incidence of pulmonary oedema and reintubation.[12] These study results are consistent with the findings from a previous epidemiological study that revealed an absence of beneficial effects of neostigmine on postoperative oxygenation and reintubation.[1,13]

Our pilot study in rats demonstrated that unwarranted neostigmine use was not an issue because reversal was not administered earlier than a TOF ratio of 0.5, and FRI occurred after recovery of the TOF ratio >0.9. However, the two rats that received neostigmine as a reversal agent exhibited a smaller relative contribution of diaphragm movement to the total change in lung volume compared with rats that received sugammadex or saline. This result may be explained by an effect on neuromuscular transmission because the remaining occupied acetylcholine receptors after neostigmine administration may decrease the efficiency of neuromuscular coupling at the diaphragm in contrast to sugammadex. Alternatively, we hypothesise that the increased relative contribution of rat chest wall expansion after neostigmine compared with sugammadex or saline may also be explained by a neostigmine-induced decrease in phrenic nerve activity. Neostigmine reduced efferent phrenic nerve activity before neuromuscular blockade at the gastrocnemic muscle in cats.[5]

Summary

Rats in this pilot study exhibited a smaller relative contribution of diaphragm movement to the total change in lung volume after a neostigmine-enhanced recovery to a TOF ratio of at least 0.9 compared with sugammadex or saline, which consequently recruited secondary respiratory muscles as primary breathing muscle. Limitations of our pilot study include the use of an animal model, the small number of subjects, and the possible imprecision associated with the results. The relevance to human biology requires further investigation.

Conflicts of interest

TS received research grants from MSD. GC received research grants and lecture fees from MSD and previously performed funded research on sugammadex. SM, BD and KD have no competing interests related to this article. TS, GC, SM, BD and KD have no financial relationships with any organisation or company that may have an interest in the submitted work. WV is Chief Technology Officer at FLUIDDA.

Acknowledgments

References

1. Grosse-Sundrup M, Henneman JP, Sandberg WS, et al. Intermediate acting non-depolarizing neuromuscular blocking agents and risk of postoperative respiratory complications: prospective propensity score matched cohort study. BMJ. 2012;345:e6329.

2. Karayiannakis AJ, Makri GG, Mantzioka A, et al. Postoperative pulmonary function after laparoscopic and open cholecystectomy. Br J Anaesth. 1996;77:448–52.

3. Eikermann M, Gerwig M, Hasselmann C, et al. Impaired neuromuscular transmission after recovery of the train-of-four ratio. Acta Anaesthesiol Scand. 2007;51:226–34.

4. Schepens T, Cammu G, Saldien V, et al. Electromyographic activity of the diaphragm during neostigmine or sugammadex-enhanced recovery after neuromuscular blockade with rocuronium. Eur J Anaesthesiol. 2015;32:49–57.

5. Fleming NW, Henderson TR, Dretchen KL. Mechanisms of respiratory failure produced by neostigmine and diisopropyl fluorophosphates. Eur J Pharmacol. 1991;195:85–91.

6. De Backer JW, Vos WG, Burnell P, et al. Study of the variability in upper and lower airway morphology in Sprague-Dawley rats using modern micro-CT scan-based segmentation techniques. Anat Rec (Hoboken). 2009;292:720–7.

7. Eikermann M, Zaremba S, Malhotra A, et al. Neostigmine but not sugammadex impairs upper airway dilator muscle activity and breathing. Br J Anaesth. 2008;101:344–9.

8. Baker HJ, J, Lindsey JR, Weisbroth SH. The laboratory rat: biology and diseases. Waltham, MA: Elsevier Academic Press; 2013.

9. Testelmans D, Maes K, Wouters P, et al. Rocuronium exacerbates mechanical ventilation-induced diaphragm dysfunction in rats. Crit Care Med. 2006;34:3018–23.

10. Davis RT, Bruells CS, Stabley JN, et al. Mechanical ventilation reduces rat diaphragm blood flow and impairs oxygen delivery and uptake. Crit Care Med. 2012;40:2858–66.

11. Eikermann M, Fassbender P, Malhotra A, et al. Unwarranted administration of acetylcholinesterase inhibitors can impair genioglossus and diaphragm muscle function. Anesthesiology. 2007;107:621–9.

12. Sasaki N, Meyer MJ, Malviya SA, et al. Effects of neostigmine reversal of nondepolarizing neuromuscular blocking agents on postoperative respiratory outcomes: a prospective study. Anesthesiology. 2014;121:959–68.

13. Meyer MJ, Bateman BT, Kurth T, et al. Neostigmine reversal doesn't improve postoperative respiratory safety. BMJ. 2013;346:f1460.