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ORIGINAL ARTICLE

Influence of intense neuromuscular blockade on surgical conditions during laparotomy: a pig model

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Abstract
Purpose Intense neuromuscular block may improve surgical conditions in ileus laparotomies; however, it is difficult to evaluate. The aim of this study was to investigate if neuromuscular block improved surgical conditions in pigs with artificial ileus laparotomy.
Methods Six pigs were endotracheally intubated, mechanically ventilated, anesthetized with propofol and fentanyl, and randomized into two groups in a cross-over assessor-blinded design. Neuromuscular block was established with rocuronium. Artificial laparotomy for ileus was performed. We investigated the influence of intense neuromuscular block on surgical conditions with a subjective rating scale, force needed to close the fascia, incidences of abdominal contractions while suctioning the lungs, width of the wound diastase and operating time as outcome parameters.
Results In all six pigs no abdominal contractions occurred while suctioning the lungs at intense neuromuscular block. Without neuromuscular block we detected abdominal contractions seen as hiccups and bucking. In all six pigs during intense neuromuscular block we found no visible electromyographic (EMG) activity in the abdominal muscles while suctioning the lungs. Without neuromuscular block suctioning the lungs elicited brief periods of abdominal EMG activity. No difference was found in the force needed to close the fascia when comparing no neuromuscular block with intense neuromuscular block. Furthermore, no significant differences were found in the width of the diastase, operating time and subjective ratings using a four-point rating scale when comparing no neuromuscular block with intense neuromuscular block. However, these outcomes were related to the order of the suturing round.
Conclusion Intense neuromuscular block prevented abdominal muscle contractions but did not influence the force needed to close the fascia.

Keywords Neuromuscular blockade · Laparotomy · Surgical conditions · Pig model

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Introduction

The use of neuromuscular block may improve surgical conditions in laparotomy for ileus. Surgeons often experience difficulties suturing the fascia while at the same time replacing the distended intestines into the abdominal cavity, especially while closing the abdominal wall. In humans, intense neuromuscular block measured by post-tetanic count (PTC) 0–1 with acceleromyography establishes total relaxation in the diaphragm and the abdominal

muscles [1, 2] and may therefore improve the surgical conditions during abdominal wall closure.

There are various methods to evaluate surgical conditions and some studies [3, 4] have used subjective ratings where the surgeon evaluated surgical difficulties using a rating scale at certain time intervals. Additionally, operating time, number of sudden abdominal contractions, width of the diastase and force needed to close the fascia can also be useful methods to describe surgical conditions during laparotomy. A precise study design evaluating surgical conditions during laparotomy would be a cross-over setting that enabled comparison of surgical conditions in the same patient with intense neuromuscular block and without neuromuscular block. Preferably, patients should be of same weight and have a standardized laparotomy with same length of incision and same volume distending the intestines. However, such a standardized cross-over design is impossible to establish in a human setting but may be possible in animal models.

The aim of this study was to investigate if neuromuscular block improves surgical conditions in a pig model with a standardized artificial ileus laparotomy with surgical conditions reported on a subjective rating scale as the primary outcome. Other outcomes were force needed to close the fascia, incidences of sudden abdominal contractions while suctioning the lungs, width of the wound diastase and operating time.

Methods

The Animal Experiments Inspectorate approved the study (License number 2012-15-2934-00186). Before initiating the experiment, our design was evaluated in a pilot study in one pig. Following that, six female Landrace pigs weighing 28–30 kg underwent an artificial laparotomy for ileus in a non-survival design. Data regarding objective neuromuscular monitoring of intense neuromuscular block paralysing the abdominal wall and the diaphragm have previously been reported in the same six pigs [5]. They were randomized by envelope into two groups in a cross-over assessor-blinded setting. The abdominal fascia was closed during two standardized suturing rounds (by removing sutures in between). In one group the pigs received intense neuromuscular block during the first suturing round and subsequently no neuromuscular block, by reversal with sugammadex, before the second suturing round. In the other group the pigs received no neuromuscular block (placebo) during the first suturing round and intense neuromuscular block during the second suturing round. Accordingly, three pigs received sugammadex. The pigs served as their own controls with evaluation of surgical conditions and simultaneous abdominal electromyographic (EMG) recordings during intense neuromuscular block and without neuromuscular block.

Anesthetic care

Each animal was premedicated with azaperone 2 mg/kg and atropine 0.02 mg/kg intramuscularly. An intravenous catheter was placed in the ear. Anesthesia was then induced with propofol (1.8–3.6 mg/kg, average 2.1 mg/kg). Endotracheal intubation was performed and mechanical ventilation was initiated. The ventilator was adjusted to normoventilation in the pig with an end-tidal carbon dioxide (CO_2) of 7.3 kPa. A blood pressure cuff was placed on the right forelimb. We used the left forelimb for neuromuscular monitoring. Anesthesia was maintained with propofol 15 mg/kg/h and fentanyl 7 μg/kg/h. The depth of anesthesia was clinically evaluated by intermittent pain stimuli of the tail. Neuromuscular block was established with a bolus dose of rocuronium 3 mg/kg followed by infusion of rocuronium (0.7–1.8 mg/kg/h).

Neuromuscular monitoring with acceleromyography

We followed international guidelines for good clinical research practice in pharmacodynamic studies of neuromuscular blocking agents [6]. All neuromuscular measurements were performed with the TOF Watch SX (MSD, Ballerup, Denmark) connected to a computer collecting neuromuscular data (Version 2.5 INT 2007, Organon, The Netherlands). The pigs were placed in the supine position on the surgical table. Neuromuscular monitoring and verification of the intense neuromuscular block on the diaphragm and the abdominal wall muscles are described elsewhere [5].

Artificial laparotomy for ileus

All pigs underwent a standardized midline laparotomy with a 20-cm incision. To mimic ileus, a plastic tube was placed inside the intestine through an enterotomy on the duodenum. The enterotomy was closed tight around the tube with a suture. Through this tube the intestine was infused with 4,000 ml of saline (volume determined in a pilot study in one pig).

Surgical conditions

First we evaluated surgical conditions with force needed to close the fascia as the outcome parameter. Before each closure we measured the force–length relationship of the abdominal wall (Newton/cm) by stepwise pulling the fascia horizontally towards the midline of the laparotomy with a dynamometer attached to a stiff suture (made of 4 sutures

vicryl 2–0) attached to the fascia. We determined the force–length relationship of the abdominal wall six times in each pig—three times without neuromuscular block, and three times during intense neuromuscular block. To prevent displacement while pulling, the pigs were secured to the operating table and positioned on a special mattress. The stiffness of the abdominal wall was calculated as the linear slope of the force–length curve and expressed in Newton/cm.

We then evaluated surgical conditions with incidences of sudden abdominal and diaphragmatic contractions as the outcome parameter. We performed a bronchial suction test of the lungs while observing the laparotomy [1]. We stimulated the carina with a soft silicone catheter in the pig during intense neuromuscular block and without neuromuscular block. A surgeon with experience in experimental pig surgery evaluated the response on a three-point rating scale (no, mild, or severe bucking) and was blinded to the degree of neuromuscular block.

Next, we evaluated surgical conditions by using the width of the diastase as the outcome parameter. At the middle of the incision we measured the distance between the edges of the fascia using a slide gauge.

Subsequently surgical conditions were evaluated by using subjective ratings as the outcome parameter. While suturing the abdominal fascia and replacing the distended intestines the surgeon assessed the difficulties of the procedure using a four-point numerical rating scale (1, optimal; 2, good; 3, acceptable; 4, poor).

Finally, we evaluated surgical conditions by using time to close the abdominal fascia as the outcome parameter. We measured the suturing time defined as the interval from the first to the last stitch while closing the fascia.

Electromyography of the abdominal wall muscles

EMG activity of the abdominal wall muscles was recorded simultaneously during all evaluations of surgical conditions. We recorded bipolar surface EMG activity from the left and right external abdominal oblique muscles. For each measuring site, two surface Ag/AgCl electrodes (720-01-K; Medicotest, Denmark) were attached to the skin in a bipolar configuration in line with the muscle fiber direction and with an inter-electrode center distance of 2 cm. Before electrode attachment, the skin was carefully shaved, rubbed with sandpaper, and cleaned with alcohol to ensure low inter-electrode resistance. The EMG signals were recorded using an 8-channel system with a pre-amplifier (Logger Technology, Sweden), high-pass filtered at 10 Hz and low-pass filtered at 400 Hz, amplified and sampled at 1 kHz. EMG activity recorded during intense neuromuscular block and without neuromuscular block was analyzed. The 21-ms root mean square (RMS) values moved in 1-ms steps were calculated on data from the suction test and peak RMS EMG values were reported.

Sample size for evaluating surgical conditions

We estimated that without neuromuscular block, 33 % (2 out of 6) of the cases would have operating conditions rated as 1 (optimal conditions). With intense neuromuscular block, 83 % (5 out of 6) of the cases would have operating conditions rated as 1. With alfa 0.05 and power 0.80 in a one-sided test, 6 pigs must be included. The suturing time, subjective rating and difference in force needed to close the fascia during intense neuromuscular block and without neuromuscular block was compared using a Wilcoxon test. In all cases a p value of ≤ 0.05 was considered statistically significant.

Results

All six pigs completed the experiment. We found no difference between the force needed to close the fascia when comparing no neuromuscular block [stiffness 2.92 N/cm (SD 0.51)] with intense neuromuscular block [stiffness 2.77 N/cm (SD 0.53)] ($p = 0.418$, Fig. 1). The EMG recordings showed no visual activity in the external abdominal oblique muscles regardless of level of neuromuscular block. Abdominal wall stiffness was not related to the suturing round (first or second).

In all six pigs no incidences of sudden abdominal contractions occurred during the bronchial suction test at intense neuromuscular block (Table 1). Without neuromuscular block, we detected sudden abdominal contractions seen as hiccups and bucking in all pigs; these were graded as severe in five pigs and mild in one. In all six pigs during intense neuromuscular block we found no visible EMG activity in the abdominal muscles during the bronchial suction test. Without neuromuscular block the bronchial suction test elicited brief periods of EMG activity. The average RMS-EMG amplitude during the bronchial suction test without neuromuscular block was 57.7 μV (Table 1).

When comparing no neuromuscular block with intense neuromuscular block no significant differences were found in the width of the diastase, subjective ratings or suturing time (Table 2). However, we saw significantly better subjective ratings ($p = 0.034$) and shorter suturing time ($p = 0.046$) when comparing the second suturing round with the first suturing round. During the first suturing round, large parts of the intestines were displaced outside the laparotomy wound onto the surface of the skin. This was not the case during the second suturing round, where

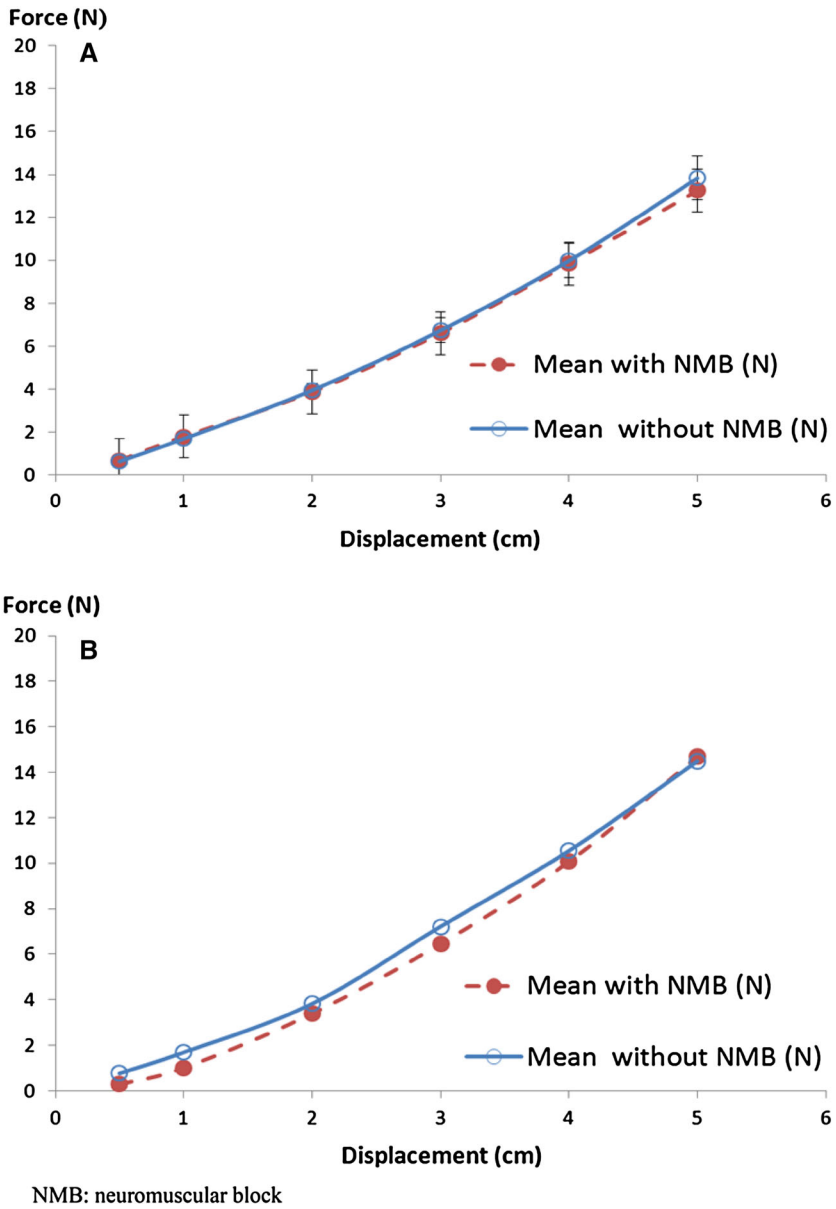

Fig. 1 **a** Mean of six pigs (*N*), **b** mean of one pig (*N*). Mean with NMB (intense NMB): $y = 3.0543x - 1.4675$, $R^2 = 0.9866$, stiffness 2.77 N/cm (SD 0.53). Mean without NMB (no NMB): $y = 3.1732x - 2.2129$, $R^2 = 0.9785$, stiffness 2.92 N/cm (SD 0.51). No difference between the fascia stiffness (N/cm) when comparing no NMB with intense NMB ($p = 0.418$). *NMB* neuromuscular block

Table 1 Evaluation of surgical conditions with incidences of sudden abdominal and diaphragmatic contractions as outcome parameters

	Electromyographic activity in abdominal muscles during bronchial suction test		Response to bronchial suction test (severe, mild, no)	
	No NMB	Intense NMB	No NMB	Intense NMB
Pig 1	Yes (123 μV)	No	Severe	No
Pig 2	Yes (17 μV)	No	Severe	No
Pig 3	Yes (27 μV)	No	Severe	No
Pig 4	Yes (95 μV)	No	Severe	No
Pig 5	Yes (28 μV)	No	Severe	No
Pig 6	Yes (56 μV)	No	Mild	No

NMB neuromuscular block

the intestines were mainly placed inside the abdominal cavity.

Discussion

In this pig study with laparotomy for artificial ileus we found that intense neuromuscular block improved surgical conditions in terms of preventing incidences of sudden abdominal muscle contractions while suctioning the lungs, but we found no relationship between the level of neuromuscular block and improvement of surgical conditions regarding the force needed to close the abdominal fascia as the stiffness of the fascia remained constant. Because of a

Table 2 Evaluation of surgical conditions with width of diastase, suturing time and subjective ratings as outcome parameters

		No NMB	Intense NMB	*p* value	1st round	2nd round	*p* value
Width of diastase (cm)	Mean (SD)	7.23 (0.76)	7.73 (0.62)	0.116	7.8 (0.5)	7.17 (0.79)	0.046
Subjective ratings (1,2,3,4)	Mean (SD)	3.17 (0.98)	2.67 (1.03)	0.396	3.67 (0.82)	2.17 (0.41)	0.034
Suturing time (sec)	Mean (SD)	806.7 (285.2)	719.2 (153.0)	0.463	918.2 (197.5)	607.7 (111.8)	0.046

Subjective ratings of surgical conditions reported on a four-point numerical rating scale while suturing the abdominal fascia and replacing the intestines (1, optimal; 2, good; 3, acceptable; 4, poor)

Suturing time: suturing of the abdominal fascia from first to last stitch

SD standard deviation, *NMB* neuromuscular block

considerable carry-over effect in the cross-over study design, we were not able to reach conclusions on relationships between level of neuromuscular block and data on subjective ratings, width of wound diastase or suturing time of the abdominal fascia.

To our knowledge this is the first study to describe the influence of intense neuromuscular block on sudden abdominal contractions and stiffness of the abdominal wall. The lack of influence of neuromuscular block on the stiffness of the abdominal wall of the pig is an important finding. First, it is of interest when trying to improve surgical conditions during closure of a laparotomy. Second, in daily clinical practice an anesthetic intervention to improve surgical conditions during closure of the abdominal wall can be the use of neuromuscular block with the aim of reducing the force needed to close the fascia.

Maintaining an appropriate anesthetic level that improves surgical conditions by preventing cough reflexes and tensions in the abdominal wall and the diaphragm can be difficult due to the cardiovascular depressant effects of the anesthetics, especially in patients with ileus. Furthermore, improvement of surgical conditions by the use of neuromuscular block in patients with severe chronic infections, such as in ileus and peritonitis, may be complicated as these patients require larger doses of neuromuscular blocking agents [7]. In our study we focused on establishing an animal model which could anatomically simulate ileus with distended intestines that are difficult to replace into the abdominal cavity and complicate suturing of the abdominal fascia. We did not aim to establish a pathophysiologic ileus condition with severe chronic infection and unstable cardiovascular and respiratory conditions.

We used a pig model as the abdominal wall stiffness is difficult to measure in human laparotomy. The absolute values of stiffness of the abdominal wall are expected to differ between pigs and humans but the effect of neuromuscular block is expected to be similar. The muscle composition of the pig differs from humans in the thickness of the muscle layers. This could have had an impact on the subjective ratings on the surgical rating scale if muscle contractions in the thin layers were too small to be felt by the surgeon. However, we recorded EMG activity while suturing the wound and detected no muscle contractions regardless of the level of neuromuscular block. Moreover, in our measurements of the force–length relationship, we calibrated the dynamometer to detect any potential differences.

We were able to measure the stiffness of the abdominal wall both with intense neuromuscular block and without neuromuscular block. The two conditions differ regarding electrophysiological response. Thus, without neuromuscular block, electrophysiological reflex response in the abdominal muscles was maintained as verified during the bronchial suction test, although no activity was found during suturing of the abdominal fascia. In contrast, with intense neuromuscular block no EMG response was found either during the bronchial suction test or during suturing of the abdominal fascia. The lack of EMG response in the abdominal muscles during suturing of the abdominal fascia, regardless of the level of neuromuscular block, may be due to an appropriately anesthetized pig. This finding may illustrate why alternatives such as increasing the depth of anesthesia, e.g., with propofol or opioids, can optimize surgical conditions in ileus laparotomy cases by inhibiting pain-induced contractions of the abdominal wall muscles. The effect of opioids on muscle contractions has been described in one study of patients scheduled for craniotomy [8]. The authors showed that increasing infusion rates of remifentanil reduced incidences of patient movement. However, even during the highest infusion rate (0.21 μg/kg/min), 21 % of the patients were still able to move. Finally, animal studies have reported a paralyzing effect on the abdominal wall due to midline laparotomy [9]. Thus, in anesthetized dogs during quiet breathing without neuromuscular block, EMG of the abdominal wall muscles recorded before and after midline laparotomy showed a considerable reduction in muscle activation after incision of the peritoneum. This could perhaps also explain the lack of EMG responses during suturing of the fascia, regardless of the level of neuromuscular block, in our pig model.

Additionally, no previous animal studies have evaluated surgical conditions in a standardized artificial ileus laparotomy model during different levels of neuromuscular block. The rapid reversal agent, sugammadex [10] makes it possible to perform surgery during intense neuromuscular block until the last suture. In this respect, our pig model is useful for testing and developing other experimental abdominal surgical procedures during intense neuromuscular block before they are transferred to patients.

Our study has certain limitations. We found a significant improvement in subjective ratings and shorter suturing time during the second suturing round compared to the first regardless of level of neuromuscular block. This was probably due to a clear difference in the visible operating field between the two situations in all pigs. During the first suturing round, regardless of level of neuromuscular block, it seemed that the intestines which were full of saline were displaced outside rather than inside the abdominal cavity. One explanation could be that after the first suturing round the saline was moved to more distal parts of the intestine or that saline was diffused from the intestinal lumen out into the abdominal cavity or absorbed and redistributed. However, in the pig model from our pilot study for estimating the saline volume for the artificial ileus laparotomy, we did not see this phenomenon. This carry-over effect of saline expanding the intestines prevented a comparison between the two situations in the same pig regarding width of the diastase, suturing time and subjective ratings. Therefore, we were only able to reach conclusions regarding the data on the stiffness of the abdominal wall and the incidences of sudden abdominal muscle contractions.

Improvement of surgical conditions by prevention of sudden abdominal contractions is highly relevant since these may cause life-threatening complications, especially if an instrument perforates a bowel or a large vessel. Our study indicates that intense neuromuscular block will potentially increase patient safety by avoiding sudden muscle contractions during surgery. This is a finding first described by Fernando et al. [1]; however, it has rarely been used in clinical practice because of the risk of prolonged emergence from anesthesia, residual block and pulmonary complications [11].

In conclusion, we found that intense neuromuscular block did not improve surgical conditions during abdominal wall closure in terms of reducing the force needed to close the fascia. However, intense neuromuscular block improved surgical conditions by preventing sudden abdominal contractions. Because of considerable carry-over effect we were not able to reach conclusions regarding the data on subjective ratings, width of diastase or suturing time for closing the abdominal fascia.

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Conflict of interest None.

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