A Novel Laparoscopic Surgery Model with a Device to Expand the Abdominal Working Space in. Rats: The Influence of Pneumoperitoneum and Skin Incision Length on Postoperative Inflammatory Cytokines

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DECLARATIONS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

ABSTRACT

Purpose: Experimental models of laparoscopic surgery generally use large animals owing to a sufficient abdominal working space. We developed a novel laparoscopic surgery model in rats. We performed intestinal anastomosis to demonstrate the feasibility and reliability of this model.

Materials and Methods: We designed a device for rats that expanded the abdominal working space and allowed us to manipulate the intraperitoneal organs by hand under direct vision with pneumoperitoneum. We performed small bowel resection and intestinal anastomosis in rats using this model. To elucidate the effects of pneumoperitoneum and skin incision length, rats were randomly divided into four groups with differing surgical techniques : small incision group, large incision group, small incision + pneumoperitoneum group, and large incision + pneumoperitoneum group. Intraoperative abdominal pressure and postoperative cytokines were measured.

Results : One experimenter completed small bowel resection and hand-sewn anastomosis under direct vision without any difficulties or assistance. Carbon dioxide pneumoperitoneum was maintained at 8-10 mmHg during surgery in both pneumoperitoneum groups.

Necropsies revealed no evidence of anastomotic leakage at 24 h after surgery The interleukin 6 and C-reactive protein concentrations were significantly greater in large incision group than in small incision group, but were not significantly different between small incision + pneumoperitoneum group and small incision group. These cytokines concentrations were the greatest in large incision + pneumoperitoneum group.

Conclusions: Our laparoscopic surgery model in rats is a simple and reliable experimental model. The length of skin, incision might be a more influential determinant of surgical invasiveness than pneumoperitoneum.

INTRODUCTION

Few studies have assessed the biological /physiological effects of minimally invasive surgical procedures that are unrelated to the purpose of the procedure. And the mechanisms of minimal invasiveness in endoscopic surgery remain unexplained. We need a reliable experimental model in order to investigate these mechanisms. Experimental models of laparoscopic surgery generally use large animals such as pigs or rabbits because a sufficient abdominal working space is necessary for the laparoscopic intraperitoneal manipulations. However, small animal models are desired because they offer benefits in

terms of cost and labor; for example [1]. We developed a novel laparoscopic surgery model with a device to expand the abdominal working space in rats. Using this device, we are able to exteriorize the rat's organs from the abdominal cavity and manipulate them directly while maintaining pneumoperitoneum. In addition, a laparoscope and surgical assistants are not necessary because the surgeon can perform all of the procedures under direct vision. In this study we used the novel model to perform small bowel resection and intestinal anastomosis with or without pneumoperitoneum in rats. The purposes of this study were to evaluate the feasibility and reliability of this novel model and to evaluate the influence of pneumoperitoneum and. skin incision length on postoperative inflammatory cytokines as markers of surgical invasiveness.

MATERIALS AND METHODS

Animals

All animal experiments were approved, by the Institutional Animal Care and Use Committee of our university (approval number 27341). We used 8-week-old male Sprague-Dawley rats (Japan SLC, Shizuoka, Japan) weighing approximately 300 g in these experiments. For 1 week before the experiment, the rats were housed 3 per cage in

a clean air-conditioned room (set temperature: 23 °C) under a 12-h light/12-h dark cycle (lights on 8:00-20:00) with free access to food and dean filtered water.

Design of the Device used to Expand the Abdominal Working Space

The device used to expand the abdominal working space consists of one column (2 cm diameter 2 cm long) and one box (30 x 30 x 25 cm) made from transparent acrylic resin (Figure 1).The column can be inserted into a 2-cm ventromedial incision on the abdomen, and the intraperitoneal organs to be manipulated are exteriorized through this column. The box has three holes. During surgery a cover rubber is attached to the column. So the column fits tightly into one hole in the base of the box, allowing the box to serve its function, which is to expand the abdominal working space. The other two holes in the left and right sides of the box are fitted with sealing devices (LAP DISC; HAKKO, Nagano, Japan): The surgeons insert their hands into the box through these holes and can manipulate the exteriorized organs under direct vision without air leaks. In addition to the column, one 3-mm port is inserted in the right flank of the rat to connect a pneumoperitoneum device for carbon dioxide insufflation (Olympus UHI-2 Insufflator; Olympus, Tokyo, Japan). Using this pneumoperitoneum device, the surgeon can

continuously monitor the abdominal pressure (— the pressure in the box) during the procedures.

Anesthesia

All procedures were performed under inhalation anesthesia with isoflurane. Anesthesia was induced with 5% isoflurane and was maintained with l%-3% isoflurane under spontaneous respiration without tracheal intubation. Spontaneous respiration was confirmed by observing movement of the chest during surgery We did not use any drug other than isoflurane in the perioperative period.

Experimental Groups and Surgical Procedures

A total of 27 rats were included in this study. The control group (M = 3) underwent general anesthesia with isoflurane for 30 min without undergoing surgery. The other 24 rats underwent surgical resection of a 20-cm section of the small bowel and hand-sewn intestinal anastomosis under general anesthesia. These rats were randomly divided into four groups (n — 6 per group) to undergo different surgical procedures, as indicated in Table 1 and Figure 2. The small incision group (SI) underwent surgery via a 2-cm skin incision without pneumoperitoneum. The large incision group (LI) underwent surgery

with a 6-cm skin incision without pneumoperitoneum. The small incision + pneumoperitoneum group (SIP) underwent surgery with a 2-cm skin incision with pneumoperitoneum. The large incision + pneumoperitoneum group (LEP) underwent surgery with a 6-cm skin incision with pneumoperitoneum. The skin incision involved transverse abdomen incision that cut the underlying muscle. For pneumoperitoneum, we used the device to expand the abdominal working space and the carbon dioxide insufflation device was set to maintain a pressure of 10 mmHg. Even for the animals in non-pneumoperitoneum groups (SI, LI), we used the device to expand the abdominal working space without establishing pneumoperitoneum and the bowel was resected after exteriorization. The duration of surgical procedures was adjusted to 30 min. All surgical procedures were performed by a single surgeon.

Measurement of Intraoperative Abdominal Pressure

In both pneumoperitoneum groups (SIF, LIP), the intraabdominal pressure was continuously monitored during surgery using a pneumoperitoneum device for carbon dioxide insufflation (Olympus UHI-2 Insufflator; Olympus, Tokyo, Japan). This pneumoperitoneum device is equipped with the high-pressure alert system.

Postoperative Quantification of Cytokines

All rats were terminally anesthetized with isoflurane at 24 h after surgery and blood samples were taken from the heart. The whole blood samples were left at room temperature until clotting, and the clots were removed by centrifuging at 1,000-2,000 x *g* for 10 min in a refrigerated centrifuge. The serum concentrations of interleukin (IL)-1, IL-6, tumor necrosis factor-a (TNFa) and C-reactive protein (CRP) were measured using enzyme-linked immunosorbent assays (ELISA). IL-1 and TNF-a were measured using Rat Quantikine IL-lb and TNFa ELISA kits (R&D systems, Minneapolis, MN, USA), IL-6 was measured using a Rat IL-6 ELISA kit (Life Technologies, Carlsbad, CA, USA), and CRP was measured using a Rat CRP ELISA kit (Immunology Consultants Laboratory; Portland, OR, USA) according to the manufacturers' instructions.

Statistical Analysis

The results are expressed as the mean ± standard deviation. Statistical analyses were performed using one-way analysis of variance followed by Tukey's honestly significant difference test. Values of $p < .05$ were considered statistically significant. JMP Pro version 11.0 (SAS institute inc, Cary, NC, USA) was used for all analyses.

RESULTS

Operative Outcomes and Intraoperative Abdominal Pressure

One surgeon was able to complete small bowel resection and hand-sewn anastomosis under direct vision without any difficulties and without requiring assistance. The duration of surgery was 30 min in all rats. The volume of bleeding was too low to be quantified in any rat. Carbon dioxide pneumoperitoneum was continuously maintained at 8-10 mmHg and there was no high-pressure warning during surgery in all rats in both pneumoperitoneum groups (SIP, LIP). All rats survived their surgery with spontaneous respiration and no complications. All rats were in a healthy condition and resumed normal feeding within 24 h of surgery. Furthermore, there were no visual differences in the postoperative conditions among the four groups. Necropsies revealed no evidence of anastomotic leakage or feces in the abdominal cavity

Postoperative Inflammatory Cytokine Concentrations

In the control group (anesthesia only), the concentrations of IL-1b, IL-6, and TNFa at 24 h after anesthesia were below the assays' limits of detection, and the CRP concentration was 360 ± 26 fig/ml. The postoperative concentrations of IL-1b and TNFa were also below the assays' limits of detection in all four surgical groups. However; the

postoperative concentrations of IL-6 and CRP were measurable in all four surgical groups and the results are listed in Table 2. The IL-6 and CRP concentrations were significantly greater in LI group than in SI group. However; the IL-6 and CRP concentrations were not significantly different between SIP group and SI group. In LIP group, the IL-6 and CRP concentrations were the greatest of the four surgical groups.

DISCUSSION

Generally, large animals are used as experimental models of laparoscopic surgery. However, small animal models are desired because the use of large animals is becoming increasingly difficult owing to restrictive legislation, public concern, and cost, among other reasons [2]. In addition, rats are widely used in biomedical research and have many advantages, including the ability to standardize experiments by strain, sex, and weight; they are genetically and physiologically similar to each other; and are relatively inexpensive and easy to house [1]. However; the small abdominal working space available for surgical manipulation presents a challenge to surgeons using rats as an animal model. Furthermore, the equipment designed for small animals, particularly the laparoscope and micro-instruments, increase the cost of the experiment. To date, most reports of laparoscopic surgery in small animals have focused, on limited procedures,

which did not involve intraperitoneal manipulation [3-6], although a standardized laparoscopic technique has been reported in rats [2]. In such circumstances, several studies have evaluated the feasibility of laparoscopic surgery with intraperitoneal manipulation for hepatectomy; splenectomy, nephrectomy and colectomy in rats [1,7-9]. However, very few studies involved intestinal anastomosis with pneumoperitoneum. This indicates that there was a limitation to the use of rats as experimental model animals. In this study we tested a simple, cost-effective laparoscopic surgery model in rats that used device to expand the abdominal working space. We performed intestinal anastomosis under pneumoperitoneum to demonstrate the feasibility and reliability of this model. In pediatric laparoscopic surgery it is often necessary to exteriorize the intestine via *a* port in the umbilicus for anastomosis [10], and our novel experimental model is based on this approach. Although the model can be improved further; we believe that this model will enable researchers to use small animals as models of laparoscopic surgery. Inflammatory cytokines are known to reflect surgical invasion. In particular, CRP was reported to be a major predictor of the clinical outcome after trauma or surgery in pediatric patients [11-13]. In this study, the *IL-lb* and TNFa concentrations were not significantly affected by the extent of surgical invasion. By contrast, both IL-6 and CRP were good markers of surgical invasiveness, because they were significantly greater in the large incision group

than those in the small incision group. Meanwhile, the concentrations of IL-6 and CRP were not significantly different between the small incision + pneumoperitoneum group and the small incision group. These results suggest that the length of skin incision is a greater determinant of surgical invasiveness than pneumoperitoneum, and this might explain the mechanism of minimal invasiveness in laparoscopic surgery.

There are some limitations in this study; The first problem is that the pressure in the box was not monitored. However, we are convinced that the pressure in the box was equal to the pressure in the abdominal cavity because this experimental device was airtight owing to the cover rubber attached to the column and the sealing devices attached to the box. In addition, the pneumoperitoneum device was equipped with the high-pressure alert system, and there was no high-pressure warning. This showed that the 3-mm port could have not been obstructed during surgery. The second problem is that a long surgery using this experimental model might be difficult. In this study; the duration of surgery was short and all rats could survive their surgery with spontaneous respiration. However ; longer experiments might need the tracheal intubation owing to the high abdominal pressure. In addition, a large quantity of Carbon, dioxide might have an enormous impact on temperature during long surgery

In conclusion, we have developed a simple and reliable animal model of laparoscopic

surgery in rats that involves a device to expand the abdominal working space. We believe that this model will contribute to further investigation into the mechanism of minimal invasiveness of laparoscopic surgery.

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Figure legend

FIGURE 1: The animal model and the equipment used to expand the abdominal working space. (a) Photograph and illustration of the animal model. The column and a 3-mm port are inserted into the abdomen for exteriorization of the organs and insufflation to maintain pneumoperitoneum, respectively. Rats were anesthetized using inhaled isoflurane with spontaneous respiration without tracheal intubation, (b) Top row, from left to right: photograph of the acrylic box, exteriorization of the intraperitoneal organs via the column, and pneumoperitoneum was achieved via a 3-mm port connected to a carbon dioxide insufflator. As shown in the right-hand photograph, the intraabdominal pressure was maintained at 10 mmHg. The column inserted in the rat's abdomen could fit tightly into the hole in the base of the acrylic box because of the cover rubber attached to the column. This allows the organs to be exteriorized and manipulated under direct vision without air leaks.

FIGURE 2: Summary of the surgical procedures in each of the four surgical groups. Small bowel incision and intestinal anastomosis was performed via a 2- or 6-cm incision with or without pneumoperitoneum

TABLE 1 Surgical procedure

Groups	Skin incision length (cm)	Pneumoperitoneum (pressure, mmHg)	Duration of surgery (min)
Small incision (n = 6)	2	–	30
Large indsion (n = 6)	6	–	30
Small incision + Pneumoperitoneum (n = 6)	2	10	30
Large incision + pneumoperitoneum (n = 6)	6	10	30

TABLE 2-1 Postoperative interleukin-6 concentrations

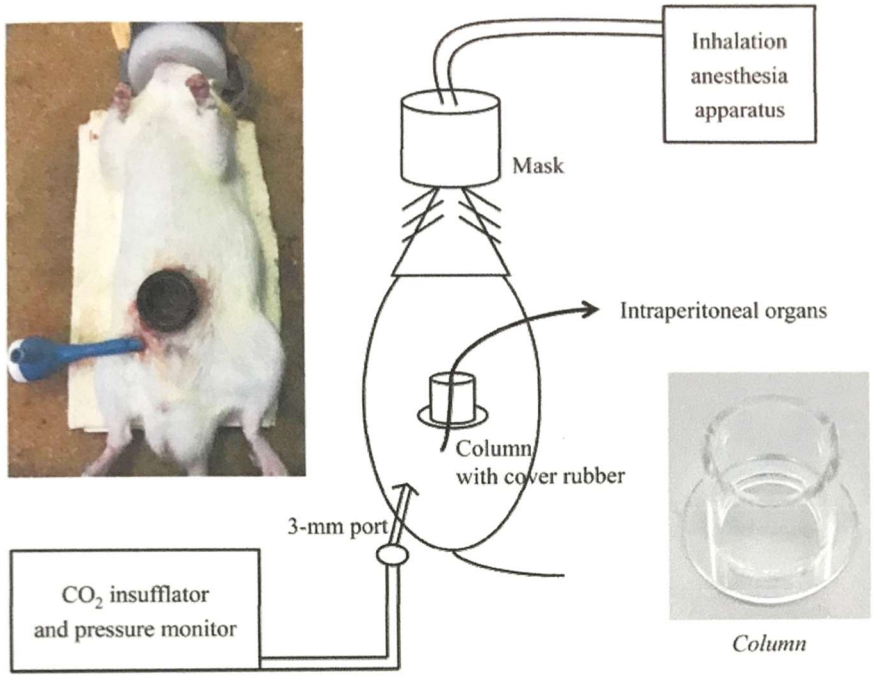
Group	IL-6 concentration (pg/ml), mean ± SD
Control (Anesthesia only)	Below LOD
Small incision	66±21
Large incision	174 ±63
Small incision + Pneumoperitoneum	141 ±64
Large incision + pncumopcriEuneum	190 ±96

TABLE 2-2 Postoperative C-reactive protein concentrations

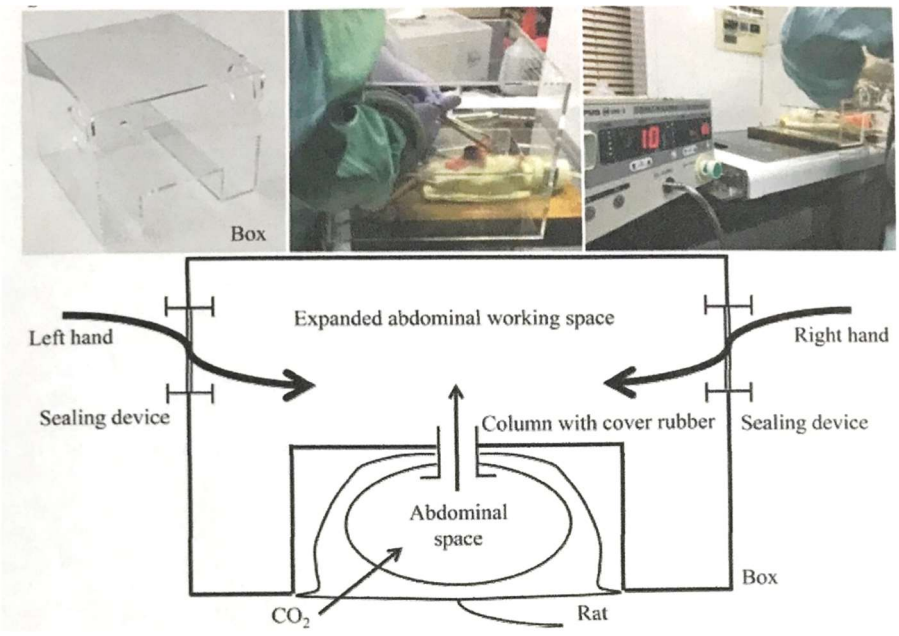
Group	(μ g/ml), mean ± SD
Control (Anesthesia only)	360 ± 26
Small incision	385 ± 2
Large incision	530 ± 81
Small incision + Pneumoperitoneum	444 ± 26
Large incision + pncumopcritoneuni	588 ± 139

FIGURE1

a


Inhalation anesthesia apparatus
Mask
Intraperitoneal organs
Column with cover rubber
3-mm port
CO_2 insufflator and pressure monitor
Column

b


Box
Expanded abdominal working space
Left hand
Right hand
Sealing device
Column with cover rubber
Sealing device
Abdominal space
Box
CO_2
Rat