Studies for reduction of surgical invasion

(especially on laparoscopic surgery and reduction of bleeding volume)

Thanikran Suwannachote

手術侵襲軽減のための研究

（特に腹腔鏡下手術および出血量減少に関して）

タニカン・スワンナチョウ

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Thanikran Suwannachote

審査委員

伊藤直之

胡　東良

夏堀雅宏

佐野忠士

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Introduction

Surgical invasion, such as bleeding or tissue trauma, around surgical sites is a major cause of postoperative complications. In particular, intraoperative bleeding is an inevitable type of surgical invasion. Therefore, reducing bleeding and tissue trauma during surgery are important for preventing surgical complications, especially in major surgery, such as spleen or liver surgery, since these organs act as reservoirs of blood components in animals, and hence, massive perioperative or postoperative hemorrhaging can occur during such surgery [2]. Several techniques, such as pharmacological tools [12], electrocautery, and electrosurgery, have been used to reduce intraoperative blood loss.

Epinephrine is one of the pharmacological tools used to achieve local hemostasis, e.g., in dental surgery. It produces vasoconstrictive effects by stimulating both α_1- and α_2- adrenoceptors in arteriole walls via the sympathetic nervous system. α_1-adrenocepters are also found on the splenic surface [1]. Therefore, this study investigated the effects of directly administering epinephrine onto the spleen on bleeding and hemodynamics during splenectomy. Furthermore, comparisons of the operation time, splenic weight and volume, incision length, inflammatory reactions, and surgical stress were performed between open splenectomy and laparoscopic splenectomy involving the direct administration of epinephrine onto the spleen.

Currently, minimally invasive surgery (MIS) involving laparoscopy is employed in both human and veterinary medicine. Laparoscopic surgery has various advantages over conventional surgery, e.g., it results in reductions in surgical morbidity, tissue invasion, and intraoperative bleeding; shorter hospital stays; and a lower risk of postoperative complications, such as surgical wound infection. Laparoscopic surgery, including laparoscopic-assisted surgery, is used to examine the internal organs [9]; perform abdominal or thoracic surgery [6, 8], e.g., to remove foreign bodies or conduct biopsies of the visceral organs; as well as for neutering,

cryptorchidectomy, cholecystectomy, gastropexy, and splenectomy [10]. Several studies have compared conventional and laparoscopic surgical techniques, and they found that laparoscopic surgery resulted in significant reductions in intraoperative bleeding, surgical invasion, and postoperative pain and a lower risk of postoperative complications [3, 4, 7, 11, 13].

In this study, we aimed to investigate surgical techniques that can be used to reduce intraoperative blood loss and improve postoperative outcomes in dogs. Ovariohysterectomy (OVH) and ovariectomy (OVE) are types of neutering surgery, which are used to sterilize dogs and cats in the veterinary field. In dogs that are at high risk of gastric-dilatation volvulus (GDV), it is recommended that such surgical procedures should be carried out in combination with gastropexy (to prevent GDV). The mortality rate and recurrence rate of GDV are high; therefore, preventing the condition is important [15]. In this study, several neutering and gastropexy techniques were performed, and their postoperative outcomes were compared.

Laparoscopic surgery can be combined with other surgical techniques or the use of hemostatic devices, for instance, disposable clips, linear stapling devices, or vessel-sealing devices [14]. Furthermore, the use of a bipolar blood vessel-sealing device, which facilitates blood vessel-sealing during surgery, has been shown to be feasible and to reduce operation times in laparoscopic surgery. In addition, the use of this device is associated with a low incidence of complications. Achieving hemostatic control during surgery is key to ensuring the success of surgical interventions and for reducing complications and risks associated with the potential adverse side effects of blood transfusions. In order to reduce intraoperative bleeding during hepatectomy, many surgeons temporarily block the blood supply to the liver [5]. The Pringle maneuver is one of the methods used to block blood flow into the liver. It results in hepatic ischemia and can cause hepatic necrosis. Moreover, blocking blood flow into the liver can result in death in dogs. Therefore, in this study the effects of performing the Pringle maneuver during

laparoscopic surgery on hemodynamics and postoperative liver function were investigated in dogs using ischemic periods of different durations.

This study was carried out to examine whether laparoscopic surgery reduced intraoperative bleeding, tissue invasion, and/or inflammatory reactions in dogs and to examine the surgical techniques that can be used to reduce intraoperative blood loss in dogs without causing adverse hemodynamic effects.

Chapter 1 Comparison of the postoperative outcomes in different neutering surgery combined with prophylactic gastropexy in dogs

Introduction

Gastric dilatation-volvulus (GDV) syndrome is a life-threatening condition caused by sudden stomach dilation/torsion. It is considered that giant and large breed dogs (e.g., German Shepherd, Rottweiler, Great Dane, Saint Bernard, and Golden Retriever) have many genetic predispositions to the condition, which can also arise after splenectomy [49], but GDV can also occur in small dog breeds. Therefore, surgical procedures that help to prevent GDV should be developed. Performing prophylactic gastropexy in combination with other surgical procedures can help to prevent GDV in dogs who are susceptible to GDV [63]. Several gastropexy techniques have been reported to be effective at preventing GDV [24]. Ovariohysterectomy (OVH) and ovariectomy (OVE) are the most common surgical procedures used to neuter companion animals, and they can be combined with gastropexy to reduce the morbidity rate [63, 73]. Therefore, performing concomitant prophylactic gastropexy during elective OVH or OVE might have benefits in dog breeds that are highly predisposed to GDV.

Minimally invasive surgery, which includes laparoscopic surgery, is widely used in humans, as it has various advantages over conventional surgery, e.g., it causes fewer postoperative complications, results in earlier recovery, reduces the frequency of surgical wound infections, and shortens the hospitalization period. On the other hand, such procedures are less common in the veterinary field. However, the successful use of laparoscopic surgery in the veterinary field, such as for cholecystectomy, abdominal organ biopsy, cystotomy, splenectomy, and gastropexy as well as for OVH and OVE, has been reported previously [22, 27, 41, 74, 75]. Hence, in dogs performing gastropexy combined with OVH or OVE using laparoscopic techniques might help to reduce postoperative complications and reduce the occurrence of GDV.

Although it has been reported that laparoscopic surgery is also less invasive than laparotomy-based surgery in the veterinary field, its usefulness has not yet been established. Therefore, in this study we evaluated and compared the postoperative outcomes of laparotomy in combination with OVH and gastropexy (OOVHG), laparoscopic-assisted OVH and gastropexy (LAOVHG), and laparoscopic OVE and gastropexy (LOVEG). We assessed the operation time, surgical wound length, and the University of Melbourne Pain Scale (UMPS) as indices of postoperative pain. In addition, the total leukocyte count, lymphocyte count, serum interleukin-6 (IL-6) level, plasma C-reactive protein (CRP) level, serum cortisol level, and glucose level were evaluated as indices of inflammation. We hypothesized that the operation time, trauma at the invasion site, inflammation, and stress response would be reduced in the LOVEG group dogs compared with those seen in the other groups.

Materials and methods

1. Animal selection

Fifteen intact clinically healthy female beagles (8.7 kg-11 kg) were selected for this study. Their health was determined via routine clinical examinations. The animals were divided into 3 groups; i.e., group 1: 5 dogs were subjected to OOVHG, group 2: 5 dogs were subjected to LAOVHG, and group 3: 5 dogs were subjected to LOVEG. All of the animals were fed twice a day and had free access to water. However, all of the animals had their food restricted for ≥12 hours (hr) prior to the operation. All of the experimental animals used in this study were handled according to the experimental animal guidelines of Kitasato University.

2. Anesthetic and analgesic protocols

The anesthesia protocol was the same in all three groups. In this experiment, we used atropine sulfate (0.025 mg/kg, intravenous [IV], atropine; Mitsubishi Tanabe Pharma, Japan), midazolam (0.1 mg/kg, IV, Dormicum®; Astellas Pharma, Tokyo, Japan), and butorphanol tartrate (0.1 mg/kg, IV, Vetorphale®; Meiji Seika, Tokyo, Japan) as premedications. General anesthesia was induced with propofol (6 mg/kg, IV, animal propofol; Mylan Seiyaku, Tokyo, Japan). An endotracheal tube was inserted, and general anesthesia was maintained via the inhalation of 2.5% isoflurane (Isoflu®; Dainippon Sumitomo Pharma, Osaka, Japan). The animals were ventilated with 100% oxygen, and lactated Ringer's solution was infused (10 ml/kg/h, Lactec®; Otsuka Pharmaceutical Co., Tokyo, Japan) throughout the surgical procedure until the animals were awake. As for analgesia management, after intubating the patient we administered meloxicam (0.2 mg/kg, subcutaneous [SC], Metacam®; Vetmedica Japan Co., Ltd.) for prophylactic analgesia.

3. Surgical procedures

OOVHG group: The animals were placed in the dorsal recumbent position, and the surgical site (in the ventral abdomen, from the xiphoid process to the pubis) was prepared with a sterile technique. At first, OVH was performed. A ventral midline incision was made from 2 cm on the caudal side of the xiphoid process to the lower abdomen to approach the right ovary, before the suspensory ligament was separated, and the ovarian artery and vein were ligated and transected using an ultrasonic coagulation and incision device. Then, the ovary was removed, and the same procedure was repeated on the left side. On the uterine body, the uterine artery and uterine cervix were ligated using an absorbable suture material (2/0-3/0). Then, the uterus and its components were excised. Subsequently, circumcostral gastropexy was performed, the seromuscular layer of the pyloric antrum was dissected, and a seromuscular flap was created along the greater curvature of the stomach. A parallel tunnel was made through the right last rib at the level of the costochondral junction. The end of the seromuscular flap was brought from the cranial to the caudal side of the tunnel, passed around the rib, and sutured back to the pyloric antrum using 2/0 monofilament absorbable sutures. After that, the abdominal cavity was closed by suturing it according to the conventional method.

In the groups in which laparoscopic techniques were employed, a skin incision was made 0.5 cm on the right side of the umbilicus to allow a Veress needle to be inserted. In order to confirm the correct placement of the Veress needle, sterile saline was injected into the abdominal cavity via the Veress needle, and it was confirmed that the saline flowed freely. The insufflation of CO_2 was carried out via the Veress needle to achieve an intra-abdominal pressure of 10 mmHg using a pneumoperitoneum device (UHI-2, OLYMPUS). Thereafter, a trocar (diameter: 5 mm; CORE) was inserted at a point located halfway between the umbilicus and the os pubis along the midline of the lower abdomen (hereafter referred to as the middle port). A 5-mm rigid endoscope (Karl Storz Endoscope, OLYMPUS®) was inserted through the middle port to visualize the intra-

abdominal organs and to guide the insertion of the next trocar. Then, a trocar (diameter: 5 mm; CORE) was placed 5 cm on the caudal side of the middle port (hereafter referred to as the caudal port), and another trocar (diameter: 10 mm; Endopath Xcel Ethicon Endo-Surgery, Inc.) was placed 1 cm on the caudal side of the umbilicus (hereafter referred to as the cranial port). From this point, the surgical procedures differed among the groups.

LAOVHG group: A 5-mm rigid endoscope was inserted through the middle port so that the uterus could be checked on a monitor (OVE 142, OLYMPUS), and then an ultrasonic coagulation and incision device (SonoSurg-IU, OLYMPUS) was inserted through the cranial port. Five-mm laparoscopic forceps (Karl Storz Endoscope) were inserted into the caudal port to expose and grasp the left ovarian pedicle. The transection and sealing of the ovariouterine complex were performed intraperitoneally. The same procedure was also carried out on the right side. The uterine horn and associated tissues were taken out of the body through the caudal port. The uterine body was ligated and divided via a conventional procedure. Next, gastropexy was performed. One 5-mm caliber trocar (CORE) was placed on the caudal side of the right last rib (hereafter referred to as the right port). Then, a 5-mm rigid endoscope (OLYMPUS) was inserted into the cranial port, and the location of the pyloric antrum was confirmed on the monitor (OVE 142, OLYMPUS). Five-mm laparoscopic forceps (Karl Storz Endoscope) were inserted through the right port to grasp the pyloric antrum. An incision was made at the side of the right port to allow the pyloric antrum to be grasped with Allis tissue forceps, and then the laparoscopic forceps and trocar were removed. The right port was expanded to expose the pyloric antrum from the outside of the body, and the seromuscular layer and abdominal transverse muscle were sutured together in a simple continuous pattern with 3-0 monofilament absorbable sutures (Mono-Dox®). After the procedure had been completed, all of the trocars and residual CO_2 were removed. The peritoneum and skin were sutured according to the conventional method.

LOVEG group: The left ovary was explored, and the ovarian bursa was grasped with 5-mm laparoscopic forceps (Karl Storz Endoscope) through the caudal port. The proper ovarian ligament, ovarian pedicle, and suspensory ligament were divided using an ultrasonic coagulation and incision device (SonoSurg-IU, OLYMPUS) through the cranial port. Then, the sealing device was removed, and the 5-mm laparoscopic forceps were reintroduced to allow the left ovary to be gently removed through the cranial port. OVE was performed on the right side using the same technique as on the left side. During the gastropexy procedure, the surgeon stood on the left side of the animal in order to easily expose the correct gastropexy site on the right ventral body wall. The rigid endoscope remained in the middle port, and a 5-mm needle holder (laparoscopy needle holder, straight; Ethicon®) was inserted into the cranial port, and the 5-mm laparoscopic forceps were inserted into the caudal port and used to grasp the pyloric antrum. Then, 3-0 non-absorbable knotless barbed sutures (Covidien) were used to suture the pyloric antrum and the abdominal transverse muscle together in a simple continuous pattern. The pneumoperitoneum and trocar were removed after the procedure, and the wound was sutured according to the conventional method.

4. Postoperative management

All of the animals were intravenously administered butorphanol tartrate (0.1 mg/kg, IV, Vetorphale®; Meiji Seika, Tokyo, Japan) immediately after the completion of surgery. Meloxicam (0.1 mg/kg, SC, Metacam®; Vetmedica Japan Co., Ltd.) was administered on the first day after the operation and orally twice a day for 7 consecutive days after the operation. Ampicillin (20 mg/kg, Viccillin Dry Syrup, Meiji Seika, Tokyo, Japan) was also administered. The animals were fed normally from the first day after surgery. The surgical wound was cleaned at least once a day, and the stitches were removed on the 7th day after surgery.

5. Measurement parameters and evaluation methods

In each case, a blood sample was collected from the cephalic vein or the external saphenous vein before the operation and used to obtain pre-operative values, and further blood samples were obtained at 1, 3, and 6 hr after surgery, as well as at 1, 3, 5, and 7 days after surgery. The blood used to measure the level of hematocrit and to obtain complete blood cell counts was treated with ethylenediaminetetraacetic acid (EDTA)-2Na, whereas that used to obtain the blood biochemical measurements was collected in blood tubes. To measure the level of hematocrit, a capillary tube (Terumo®) was used to collect blood from an EDTA-2Na-treated blood sample, before being subjected to centrifugation at 12,000 rpm for 5 minutes with a hematocrit centrifuge (KH-120 M, Kubota). Then, the hematocrit level was measured using a hematocrit measurement plate. Blood cell counts were obtained using an automated cytometer (Celltack-α, Nihon Kohden, Japan). To obtain the IL-6, CRP, cortisol, and glucose level measurements, serum was separated from blood that had been allowed to clot by leaving it at room temperature for approximately 20 minutes. Then, the blood was centrifuged at 3,000 rpm at 4 °C for 5 minutes, and the supernatant was immediately transferred to a clean Eppendorf tube and cryopreserved at -35 °C until the measurements were obtained.

a. Operation time and incision length

The operation time was recorded from the first incision to the end of the abdominal suturing. The length of the skin incision(s) was measured in each case and used as the size of the surgical wound. In the laparotomy group, the total length of the wound was taken as the surgical wound size, and in the laparoscopic groups the lengths of the skin incisions for each port were measured.

b. Total leukocyte count and lymphocyte count

The total leukocyte count was determined preoperatively and at 1, 3, and 6 postoperative hours and 1, 3, 5, and 7 postoperative days using a Celltack α automatic cell counter (MEK-6358). At each time point, the number of lymphocytes was calculated as a percentage of the total number of leukocytes by examining blood smear samples.

c. IL-6 level

The IL-6 level was measured using a canine-specific IL-6 enzyme-linked immunosorbent assay (ELISA) kit (Quantikine canine IL-6; R&D System, Minneapolis, MN, U.S.A.). The IL-6 measurements were carried out according to the method described in the instructions included with the ELISA kit, and were determined with a microplate reader based on the absorbance seen at a wavelength of 450 nm (Opsys MR microplate reader, Dynex). The IL-6 level was measured preoperatively and at 1, 3, and 6 postoperative hours and 1, 3, and 5 postoperative days.

d. CRP level

The CRP level was assessed using specific measurement apparatus (Laser CRP-2, Arrows), an immunoturbidimetric method, and a reagent kit for measuring canine CRP (Arrows). The measurements were carried out according to the method described in the accompanying instruction manual. The CRP level was measured preoperatively and at 1, 3, and 6 postoperative hours and 1, 3, and 5 postoperative days.

e. Serum cortisol concentration

The serum cortisol concentration measurements were carried out using a solid-phase radioimmunoassay method. They were obtained preoperatively and at 1, 3, and 6 postoperative hours and 1, 3, and 5 postoperative days.

f. Glucose level

The glucose level measurements were carried out using an automatic biochemical analyzer (AU400, OLYMPUS). They were obtained preoperatively and at 1, 3, and 6 postoperative hours and 1, 3, and 5 postoperative days.

g. Pain score

Pain score assessments were performed preoperatively and at 1, 3, and 6 postoperative hours and 1, 3, and 5 postoperative days. The pain scores were obtained using the UMPS score (Table 1-1)

6. Statistical analysis

All results are expressed as mean±standard deviation (SD) values. Statistical comparisons among the groups were performed via one-way analysis of variance, and multiple comparisons tests were conducted using the Bonferroni method. In comparisons with the preoperative level, the paired t-test was used. P-values of <0.05 were regarded as significant.

Results

1. Operation time and incision length

The operation time was 75.2±6.7 minutes, 55.2±8.2 minutes, and 43.2±10.1 minutes in the OOVHG group, LAOVHG group, and LOVEG group, respectively. Significant differences ($P<0.05$) were detected between the OOVHG and LAOVHG groups, and between the OOVHG and LOVEG groups, but not between the LAOVHG and LOVEG groups (Table 1-2).

The mean length of the surgical wound was 19.7±1.8 cm, 5.8±1.7 cm, and 2.9±0.4 cm in the OOVHG group, LAOVHG group, and LOVEG group, respectively. There were significant differences ($P<0.05$) between the OOVHG and LAOVHG groups, and between the OOVHG and LOVEG groups, but not between the LAOVHG and LOVEG groups (Table 1-3).

2. Total leukocyte and lymphocyte counts

The total leukocyte count tended to increase until 1 day after surgery in all three groups, and then decreased to the preoperative level over time. On day 7 after surgery, a significant difference ($P<0.05$) in the total leukocyte count was detected between the OOVHG and LAOVHG groups. Conversely, no significant differences in the total leukocyte count were detected between the LOVEG group and the other groups (Figure 1-1).

On the other hand, the lymphocyte counts of all three groups tended to decrease until 6 hr after surgery, and after that they gradually increased to the preoperative level on day 1 after surgery. The LOVEG group exhibited higher lymphocyte counts than the other groups at 3, 5, and 7 days after surgery. Moreover, significant differences ($P<0.05$) in the lymphocyte count were detected between the OOVHG and LAOVHG groups, and between the OOVHG and LOVEG

groups at 6 hr after surgery. No such difference was found between the LAOVHG and LOVEG groups (Figure 1-2).

3. Interleukin-6 (IL-6) level

The IL-6 levels of all three groups tended to increase immediately after surgery and then tended to decrease. The highest IL-6 level (178.4±103.1 pg/ml) was seen at 3 hr after surgery in the OOVHG group. Significant differences ($P<0.05$) in the IL-6 level among the three groups and between the OOVHG and LOVEG groups were observed at 1 and 3 hr after surgery, as well as between the OOVHG and LAOVHG groups at 3 hr after surgery. Furthermore, the LOVEG group exhibited the lowest IL-6 levels of all groups at all time points after surgery (Figure 1-3).

4. C-reactive protein (CRP) level

The CRP levels of all groups tended to increase until 1 day after surgery, and after that they gradually normalized. Significant differences ($P<0.05$) in the CRP level between the OOVHG and LOVEG groups were observed during the preoperative period and at 1 day after surgery, as well as between the LOVEG and LAOVHG groups at 1 day after surgery. Moreover, the highest CRP level was seen in the OOVHG group (10.1±4.7 mg/dl) on day 1 after surgery. On the other hand, the CRP level of the LAOVHG group was similar to that of the LOVEG group (Figure 1-4).

5. Serum cortisol level

The serum cortisol level peaked at 1 hr after surgery and then gradually decreased to the preoperative level in all groups on day 1 after surgery, and significant differences ($P<0.05$) in the

serum cortisol level were detected between the OOVHG and LOVEG group at 1, 3, and 6 hr after surgery. Moreover, significant differences ($P<0.05$) in the serum cortisol level were observed between the LAOVHG and LOVEG group at 3 and 6 hr after surgery. The highest serum cortisol level (19.6±3.4 μg/dl) was found in the OOVHG group at 1 hr after surgery, whereas the LOVEG group exhibited the lowest serum cortisol levels of all groups at all time points until the end of the experiment (Figure 1-5).

6. Glucose level

The glucose level tended to increase after surgery in all groups, and after that it decreased to the preoperative level on day 1 after surgery. The highest glucose level (171.8±55.7 mg/dl) was seen in the LAOVHG group at 1 hr after surgery. However, no significant differences among the groups were observed. Moreover, the LOVEG group displayed the lowest glucose levels throughout the experiment (Figure 1-6).

7. Pain scores

In all groups, high pain scores were seen at 1 hr after surgery, but the pain scores had decreased to the preoperative level by 1 day after surgery. However, there were significant differences ($P<0.05$) in the pain score among the groups at 1 and 3 hr after surgery; between the OOVHG and LOVEG groups at 1 hr after surgery; and between the OOVHG and LAOVHG groups, and between the OOVHG and LOVEG groups at 3 hr after surgery. The highest mean pain score was observed in the OOVHG group (11±5.7) at 1 hr after surgery, and the OOVHG group exhibited higher pain scores than the other groups at all time points. The LOVEG group had the lowest pain scores at all time points throughout the experiment (Figure 1-7).

Discussion

Gastropexy is used to treat gastric dilatation (GD) or GDV in predisposed dog breeds (mainly large dog breeds). Several gastropexy techniques have been described, and the outcomes of these methods have been reported [2, 24, 80]. In addition, prophylactic gastropexy is effective at preventing the occurrence and recurrence of GDV [79]. Usually, it is recommended that prophylactic gastropexy should be performed in patients that undergo OVH or OVE. Furthermore, minimally invasive surgical techniques have been used to perform the latter procedures in the veterinary field. Therefore, in the present study we compared the outcomes of three different methods for performing concomitant neutering and gastropexy in healthy dogs. We aimed to show the effectiveness of laparoscopic surgery in the veterinary field.

The operation time and surgical wound size were significantly shorter in the LOVEG group than in the other groups (Tables 1-2 and 1-3). Several studies have reported that laparoscopic surgery took longer than conventional surgery [19, 20, 22]. However, another study found that the operation time of OVH did not differ significantly between the laparoscopic and laparotomy groups [22]. The operation time can be used as a parameter of surgical stress; i.e., a longer operation time is correlated with greater surgical stress. Moreover, in the current study the surgical wounds were largest in the OOVHG group; therefore, it could be considered that the operation took longer in this group than in the other groups. Using barbed sutures in gastropexy procedures has been reported to reduce the length of the procedure compared with using intracorporeal tied knots [69]. We used barbed sutures, which might have reduced the length of the LOVEG procedure. Although there was no significant difference in the operation time between the LAOVHG and LOVEG groups, in cats it was reported that laparoscopic-assisted OVH (LAOVH) took significantly longer than laparoscopic OVE (LOVE) [13]. Therefore, LOVEG is considered to be a procedure that can be performed with minimal surgical wounds and

that can drastically shorten the suturing time compared with laparotomy or laparoscopic-assisted surgery. However, the time required for laparoscopic surgery depends on the surgeon's experience.

Leukocytosis after surgery represents a clinical response to inflammation, which mainly involves an increase in the neutrophil count. In this study, increased leukocyte counts were seen after surgery all three groups, and there were significant differences in the leukocyte count between the OOVHG and LAOVHG groups on day 7 after surgery. However, the leukocyte counts of all three groups remained within the normal range, and the animals in this study did not exhibit abnormal clinical symptoms. Therefore, such changes could represent a normal response to inflammation. Furthermore, in the current study the lymphocyte count differed significantly among the three groups at 6 hr after surgery, and the lowest lymphocyte count was seen in the OOVHG group (Figure 1-2). Our results are in accordance with those of previous studies, which reported that the lymphocyte count exhibited an inverse relationship with the serum cortisol level after surgery [32]. Surgical stress can induce the release of stress hormones, which suppress immune functions. Moreover, anesthetic agents have also been reported to suppress immune functions; however, their effects might not be significant in patients with normal immune function [18]. Since lymphocytes play important roles in the immune system, we conclude that the transient lymphopenia observed in the present study might have been influenced by cortisol causing lymphocytes to be redistributed from the circulation to tissues. The lymphocyte counts of each group gradually returned to the preoperative level after surgery.

IL-6 is a proinflammatory cytokine, which is released in the early stages of systemic inflammation, such as after trauma or surgery, and so it is a useful indicator of inflammation [3, 8]. In the current study, the IL-6 level peaked at 3 hr after surgery in the OOVHG and LOVEG groups, and the mean IL-6 level of the OOVHG group (178.4±92.3 pg/ml) was about 6 times higher than that of the LOVEG group (30.5±24.2 pg/ml), and the LOVEG group had the lowest

IL-6 levels of all groups at all postoperative time points. On the other hand, the IL-6 levels of the LAOVHG group peaked (89.5±103.9 pg/ml) at 6 hr after surgery (Fig. 1-3). A previous study found that serum IL-6 levels peaked at 2-4 hr after surgical invasion and that they were related to the length of the surgical wound and the type of surgical procedure [17, 40]. In the present study, the IL-6 level tended to increase early after surgery, peaked at 3-6 hr after surgery, and then gradually decreased to the preoperative level. Furthermore, the volume of blood loss in the perioperative period was reported to be correlated with the IL-6 level [42].

CRP is an acute-phase protein, which is released by hepatocytes in response to stimulation by IL-6 [17]. It has been used as a real-time marker of inflammatory activity, and it exhibits greater sensitivity for detecting inflammatory activity than the total leukocyte count. Elevated CRP levels have been detected in neoplastic disease, immune-mediated disease, and after surgery [82] and can fall after appropriate treatment. In the current study, the CRP levels of each group peaked shortly after the IL-6 level. A comparison study between open and laparoscopic surgery also reported that postoperative IL-6 and CRP levels were lower after laparoscopic surgery than after conventional surgery [35]. Our results support those of the latter study.

The levels of cortisol, which is released by the adrenal gland, and glucose were used as systemic stress response indices in the present study. It was reported that the serum cortisol level rises rapidly following surgery and peaks at 4-6 hr after surgery [21]. In the current study, the highest cortisol level was seen in the OOVHG group (19.6±3.4 μg/dl) at 1 hr after surgery (Fig 1-5). Several studies have reported that increases in the cortisol level after surgery are indicative of surgical stress, which suppresses immunity [22, 28, 37, 83], and they also showed that laparoscopic surgery resulted in lower cortisol levels than conventional surgery. Moreover, in a study comparing different nephrectomy procedures in a canine model it was shown that longer operation times can cause higher cortisol levels [83]. Our results are in accordance with these

studies. On the other hand, another study reported that the cortisol concentration was not correlated with the duration of surgical operations [42]. Although in the present study higher glucose concentrations were seen after surgery in all groups, no significant differences among the groups were detected at any time point. Moreover, the highest glucose level (171.8±55.7 mg/dl) was seen in the LAOVHG group at 1 hr after surgery, whereas the LOVEG group exhibited the lowest glucose levels at all time points in the postoperative period. The hyperglycemia seen after surgery is caused by increased cortisol levels due to the inhibition of insulin secretion, or the induction of glucose production in the liver [21]. Prolonged hyperglycemia after surgery might increase the risk of surgical wound infections or delayed wound healing. However, the glucose concentration data obtained in the current study might not be useful as an index of surgical stress or pain.

The UMPS was used to evaluate postoperative pain in animals in a study in which both behavior and physiology were assessed [33]. In the present study, significantly higher UMPS scores were observed in the OOVHG group at 3 hr after surgery and throughout the study, while the LOVEG group displayed the lowest UMPS scores at all time points. Previous studies have reported that postoperative pain was greater after laparotomy than after laparoscopic surgery [13, 22]. In animals, using the UMPS alone might be insufficient for evaluating postoperative pain; however, in the current study marked postoperative pain was seen in the OOVHG group at 1-3 hr after surgery.

Iatrogenic damage is a complication of laparoscopic procedures, and it is usually caused by the visceral organs being lacerated when a trocar is inserted into the abdominal or thorax. The other complications of laparoscopic procedures include gas emboli from pneumoperitoneum and intra-abdominal hemorrhaging, which can necessitate conversion to conventional surgery. However, in the present study, we did not encounter any complications after laparoscopic surgery.

Our results suggest that in cases involving concomitant neutering and gastropexy performing laparoscopic OVE combined with concomitant prophylactic gastropexy using barbed sutures is a feasible way of reducing the operation time, the inflammatory response, postoperative pain, and surgical stress, as it is minimally invasive and involves smaller surgical wounds compared with other surgical procedures. This approach might also help to prevent GDV in dogs that are at high risk of developing the condition. Based on our results, we consider that in future the LOVEG technique, which is safe and easy to perform, could feasibly be applied to clinical cases.

Chapter 2 The response of the spleen and hemodynamic changes after directly administering epinephrine

Introduction

Immune-mediated hemolytic anemia (IMHA) and immune-mediated thrombocytopenia (IMT) are common autoimmune disorders in small animals [46]. At present, the treatments for IMHA and IMT involve the administration of immunosuppressants. However, previous studies have described the successful use of splenectomy to treat hematological disorders in dogs, which was based on the dogs' transfusion requirements reducing after the procedure [25, 36]. Therefore, splenectomy might be an alternative treatment for IMHA/IMT. Moreover, the spleen plays an important role as a reservoir of red blood cells (RBCs) [12], which are redistributed into the circulation when the body goes into a fight or flight state, for example, when a patient goes into hypovolemic shock, exercises, or experiences stress [76]. As we known, the spleen is contracted in those situation and expelled its content into the systemic circulation, resulting in the splenic volume to decrease, increase in circulation hematocrit and RBCs mass [31, 38, 76]. Furthermore, effects of these situations which stimulated the splenic nerve resulting in increase in CO and SV [14]. Therefore, reduction the splenic volume before performing splenectomy might be decrease perioperative and postoperative complications following splenectomy.

In previous studies involving humans [71] and seals [11, 39], the size and volume of the spleen decreased after exercise due to the effects of catecholamines. Epinephrine is an endogenous catecholamine, which stimulates both α- and β-adrenoceptors and activates the sympathetic nervous system, causing smooth muscle and blood vessels to contract. In this study, we investigated its effects on splenic muscle [60]. Also, an abundance of α_1 adrenoceptors are present on the splenic surface, which is composed of smooth muscle. Thus, epinephrine can be used to induce splenic contraction to reduce the splenic volume and cause blood component to be

released from the spleen into the systemic circulation. Moreover, the use of epinephrine during splenectomy might reduce the amount of intraoperative blood loss and the degree of trauma at the surgical site, including prevention anemia condition following splenectomy. However, the hemodynamic effects of administering epinephrine drops onto the spleen have not been adequately studied.

In the present study, computed tomography (CT) and a method for extracting the spleen area from CT scans and calculating splenic volume were used to measure splenic volume accurately [9]. First, the effect of dripping epinephrine directly onto the spleen on splenic contraction was investigated. Next, the hemodynamic effects of directly dripping different doses of epinephrine onto the spleen were evaluated using blood circulation monitoring to investigate whether it carries any risks.

Experiment 1. Measurement of splenic volume using CT after the dropwise administration of epinephrine

Materials and Methods

1. Animal selection

Six clinically healthy beagles (12.6-14.6 kg) were selected for this experiment. Their health was determined via routine clinical examinations. The animals were divided into 2 groups; i.e., 3 dogs were administered 10 μg/kg epinephrine (the EP10 group), and the other 3 dogs were administered 100 μg/kg epinephrine (the EP100 group). The animals were fed twice a day and had free access to water. However, all of the animals had their food restricted ≥12 hours prior to the operation.

2. Anesthetic and analgesic protocol

The anesthesia protocol was the same in both groups. In this experiment, we used atropine sulfate (0.05 mg/kg, intravenous [IV], atropine; Mitsubishi Tanabe Pharma, Japan), midazolam (0.1 mg/kg, IV, Dormicum®; Astellas Pharma, Tokyo, Japan), and butorphanol tartrate (0.1 mg/kg, IV, Vetorphale®; Meiji Seika, Tokyo, Japan) as premedications. Ampicillin sodium (20 mg/kg, IV, Viccillin®; Meiji Seika, Tokyo, Japan) was administered at the time of induction. General anesthesia was induced with propofol (6 mg/kg, IV, animal propofol; Mylan Seiyaku, Tokyo, Japan). An endotracheal tube was inserted, and general anesthesia was maintained via the inhalation of 2.0% isoflurane (Isoflu®; Dainippon Sumitomo Pharma, Osaka, Japan). The animals were ventilated with 100% oxygen, and lactated Ringer's solution was infused (7 ml/kg/h, Lactec G®; Otsuka Pharmaceutical Co., Tokyo, Japan) throughout the surgical procedure until the

animals were awake. As for analgesia management, we administered meloxicam (0.2 mg/kg, subcutaneous [SC], Metacam®; Vetmedica Japan Co., Ltd.) for prophylactic analgesia.

3. Surgical procedure

Laparotomy was performed in this experiment. A previously reported surgical procedure [26] was employed. A large ventral midline laparotomy was started from the xiphoid process and extended to a point caudal to the umbilicus to enable complete abdominal exploration. After approaching and examining the spleen, the abdomen was packed with sterile wet gauze, and splenic volume was measured on a CT scan. After the CT scan had been performed, the spleen was taken out from the abdominal cavity and placed on wet gauze. Then, epinephrine (dose of epinephrine depend on the group and then diluted and scaled up to 2 ml with normal saline) was dropwise onto the splenic surface and left for 5 minutes, before the spleen was placed back into the abdominal cavity, and splenic volume was measured on CT again. When the procedure had been completed, the abdomen was sutured.

We performed the procedure in the same manner in both groups.

4. Postoperative management

From 6 hours after surgery, ampicillin sodium (20 mg/kg, SC, Viccillin®; Meiji Seika, Tokyo, Japan) was administered twice a day for 3 consecutive days. The animals were fed normally from the first day after surgery. The surgical wound was cleaned at least once a day, and the stitches were removed on the 14th day after surgery.

5. Data parameters and measurement methods

To calculate the splenic volume, 16 row multi slice CT (Aqulion 16 Toshiba Medical Systems, Tochigi, Japan) was used. In Dynamic CT mode, the tube voltage was set at 120 kV, the current tube was set at 150 mA, the imaging rotation speed was set at 0.5 second/one rotation, and the slice thickness was set at 0.5 mm.

The dog was placed in a supine position under general anesthesia, and photography was carried out. The ROI of the spleen region in the cross sectional image was set and using DICOM viewer software OsiriX (Pixmeo SARL, Geneva area, Swiss). After that, a 3D stereo model was created, ROI analysis was performed, and calculated the splenic volume.

Results

The mean splenic volume decreased to 53.9% of the baseline volume in the EP10 group and significantly decreased to 15.7% of the baseline volume in the EP100 group. The percentage shrinkage varied between individuals (Figure 2-1).

Experiment 2. Evaluation of hemodynamic changes after the dropwise administration of epinephrine onto the spleen

Materials and methods

1. Animal selection

Ten clinically healthy adult beagles (9.8-12.0 kg) were used in this experiment. Their health was determined via routine clinical examinations. The animals were divided into 2 groups; i.e., 5 dogs were administered 10 μg/kg epinephrine (the EP10 group), and the other 5 dogs were administered 100 μg/kg epinephrine (the EP100 group). The animals were fed twice a day and allowed free access to water. However, they were fasted for 12 hours before the operation.

2. Anesthesia and analgesic protocol

We used the same anesthesia protocol in both groups. In this experiment, atropine sulfate (0.05 mg/kg, IV, atropine; Mitsubishi Tanabe Pharma, Japan), midazolam (0.1 mg/kg, IV, Dormicum®; Astellas Pharma, Tokyo, Japan), and butorphanol tartrate (0.1 mg/kg, IV, Vetorphale®; Meiji Seika, Tokyo, Japan) were administered as premedications. Ampicillin sodium (20 mg/kg, IV, Viccillin®; Meiji Seika, Tokyo, Japan) was administered at the time of induction. General anesthesia was induced with propofol (6 mg/kg, IV, animal propofol; Mylan Seiyaku, Tokyo, Japan). An endotracheal tube was inserted, and general anesthesia was maintained via the inhalation of 2.0% isoflurane (Isoflu®; Dainippon Sumitomo Pharmaceutical). Lactated ringer's solution (7 ml/kg/h, Lactec G®; Otsuka Pharmaceutical Co; Tokyo, Japan) was infused throughout the surgical procedure until the animals were awake. For analgesia

management, we administered meloxicam (0.2 mg/kg, SC, Metacam®; Vetmedica Japan Co., Ltd.) at the induction stage.

3. Measurement of hemodynamics

To perform the hemodynamic assessments, we placed the dog in the left lateral recumbent position. An incision was made in the right neck, and the jugular vein and carotid artery were approached. A pulmonary arterial catheter (SG catheter 131H-7F; Nihon Kohden, Japan) was used to measure cardiac output (CO). It was inserted into the right jugular vein, and its tip was placed in the pulmonary artery. The catheter's position was confirmed by observing the characteristic pulmonary arterial pressure waves, and then the catheter was connected to a thermodilution CO meter (MTC-6100; Nihon Kohden, Japan). To measure aortic pressure, we used a blood pressure monitor (OMP-7201K; Nihon Kohden, Japan), and a 7F catheter was inserted into the carotid artery, with its tip placed in the aorta.

After inserting the thermodilution catheter and placing the dog in the dorsal recumbent position, a laparotomy was performed by making an incision along the ventral midline from the xiphoid process to a point caudal to the umbilicus to enable complete abdominal exploration. Then, we approached the left kidney to visualize the left renal artery, and the probe of an electromagnetic blood flow meter (MFV-3200; Nihon Kohden, Japan) was placed in the left renal artery. In the sterile saline experiment, Heart rate (HR), CO, pulmonary artery pressure (PAP), aortic pressure (AP), and renal blood flow (RBF) were recorded, and then the surgeon exposed the spleen and removed it from the abdominal cavity. After waiting approximately 5 minutes for a steady state to be achieved, 2 ml of sterile saline were dripped onto the splenic surface, and then the spleen was returned to the abdominal cavity. The same parameters were recorded at 5, 10, and 15 minutes after sterile saline had been dripped onto the spleen. When all of the parameters had been recorded, the animal was kept in an anesthetized state for 15 minutes. Next, their

hemodynamic parameters were measured again, before epinephrine (dose of epinephrine depend on the group and then diluted and scaled up to 2 ml with normal saline) was dripped onto the splenic surface. The same parameters were recorded at 5, 10, and 15 minutes after epinephrine had been dripped onto the splenic surface. After the procedure was finished, the thermodilution catheter and the electromagnetic blood flow probe were removed, and the abdomen and neck were sutured.

We performed the surgical procedure in the same manner in both groups.

4. Postoperative management

From 6 hours after the completion of the surgery, ampicillin sodium (20 mg/kg, SC, Viccillin®; Meiji Seika, Tokyo, Japan) was administered twice a day for 3 consecutive days. Food consumption was restarted from the first day after surgery, and the surgical wound was kept clean. On the 14th day after surgery, the stitches were removed.

5. Hemodynamic parameters and evaluation methods

HR, PAP, AP, CO, and RBF were assessed as hemodynamic parameters in this experiment. We used an electrocardiograph (FUKUDA ME BIO-SCOPE AM120) to measure HR. PAP and AP were evaluated with a thermodilution catheter connected to a blood pressure monitoring kit (TW7000 NKC; Nihon Kohden, Japan). To assess CO, 3 ml of saline with a temperature of 0°C were rapidly injected into the pulmonary artery via the thermodilution catheter, which was connected to a thermodilution CO monitor (MTC-6100; Nihon Kohden, Japan), and CO was calculated as the time until the temperature of the blood returned to normal. To measure RBF, an electromagnetic blood flow meter was used to assess blood flow through the left renal artery.

In addition, we also evaluated the cardiac index (CI), stroke volume (SV), systemic vascular resistance (SVR), and pulmonary vascular resistance (PVR) as hemodynamic parameters, which were calculated using the formulas described below:

1. Cardiac index = $\frac{\text{Cardiac output}}{\text{Body surface area}}$ (l/min/m^2)
2. Stroke volume = $\frac{\text{Cardiac in dex}}{\text{Heart rate}}$ (ml/beat/m^2)
3. Systemic vascular resistance = $\frac{\text{Mean aortic pressure}}{\text{Cardiac index}}$ ×80 (dynes-sec-cm^{-5}/m^2)
4. Pulmonary vascular resistance = $\frac{\text{Mean pulmonary artery pressure}}{\text{Cardiac index}}$ ×80 (dynes-sec-cm^{-5}/m^2)

6. Statistical analysis

All results are expressed as percentage changes and as the mean±standard error. For comparisons with the baseline values, the paired t test was used, and p-values of <0.05 were considered to indicate a significant difference.

Results

1. Heart rate

The mean heart rate did not differ significantly between the groups or among the time points in any group (Table 2-1).

2. Mean aortic pressure (MAP) and mean pulmonary artery pressure (MPAP)

The mean aortic pressure (MAP) level was significantly increased at 5, 10, and 15 minutes after the administration of 10 μg/kg epinephrine ($P<0.05$). The highest MAP level was seen at 5 minutes (28.7±4.4%), and then the MAP tended to decrease to the baseline level. On the other hand, in the EP100 group the MAP gradually increased and remained elevated until the end of the experiment (Table 2-1).

In the EP10 group, the mean pulmonary artery pressure (MPAP) peaked ($P<0.05$) at 5 minutes after the administration of epinephrine, when it was 39.7±4.6% higher than the baseline level, and then gradually decreased back to the baseline level. In the EP100 group, the MPAP was significantly increased (by 60.2±23.8%, 57.9±11.7%, and 62.8±12.5%, respectively) at 5, 10, and 15 minutes after the administration of epinephrine ($P<0.05$) and remained elevated until the end of the experiment (Table 2-1).

3. Renal blood flow (RBF)

In the sterile saline experiment involving the EP10 group, RBF was significantly higher than the baseline level at 10 and 15 minutes (by 6.1±1.8% and 7.7±2.4%, respectively). Conversely, no significant changes in RBF from the baseline level were seen at these time points in the sterile saline experiment involving the EP100 group or after epinephrine treatment in the

EP10 or EP100 group. However, obviously decreasing RBF was detected in the EP100 group (Table 2-1).

4. Cardiac index (CI) and stroke volume (SV)

In the sterile saline experiment involving the EP100 group, significant increases in CI and SV from their baseline levels (by 12.3±5.1% and 11.5±2.3%, respectively) were seen. In the EP100 group, significant increases ($P<0.05$) in CI and SV from their baseline levels (by 57.8±10.5% and 53.6±2.4%, respectively) were observed at 15 minutes after the administration of epinephrine (Table 2-1).

5. Systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR)

In the sterile saline experiment involving the EP10 group, a significant reduction in SVR (by 15.47±5.67%) compared with the baseline level was observed. There was no significant change in SVR or PVR from their baseline levels in the sterile saline experiment involving the EP100 group or after epinephrine treatment in the EP10 or EP100 group. However, in the EP100 group a marked reduction in SVR (by 30.16±5.7%) compared with the baseline level was noted after the administration of epinephrine (Table 2-1).

Discussion

The purpose of this study was to investigate the effects of epinephrine on the spleen and hemodynamics by comparing the effects of the dropwise administration of 10 μg/kg and 100 μg/kg epinephrine onto the splenic surface. First, the direct effect of epinephrine on splenic contraction was examined. Both 10 μg/kg and 100 μg/kg epinephrine induced splenic contraction. Six healthy adult beagles underwent laparotomy under inhalation anesthesia, and the volume of the spleen was measured on CT before and after the administration of 10 μg/kg or 100 μg/kg epinephrine.

In this study, mean reductions in splenic volume of 46.1% and 84.4% were seen after the administration of epinephrine in the EP10 and EP100 groups, respectively. Our results are consistent with those obtained in seals [11], in which it was found that the administration of a high-dose (1.0 μg/kg) epinephrine infusion resulted in mean reductions in splenic volume of 26.3% in hooded seals and 15% in harp seals. In humans, a low-dose epinephrine (0.06 μg/kg/min) infusion induced a 47% reduction in splenic volume within 9 minutes [5]. Similarly, the splenic contraction that occurs during exercise, breath holding, fighting, or stressful conditions has been described in humans [71], dogs [60, 76], and seals [11, 39]. Cabanac et al. [11] reported that the seal spleen contracted strongly when stimulated with epinephrine. In addition, they suggested that this process involved α-adrenoceptors, while β-adrenoceptors did not play an important role. The mechanism responsible for splenic contraction was described as follows: a catecholamine selectively binds to α-adrenoceptors within the spleen and capsule, which stimulates the sympathetic nervous system and induces splenic contraction [11, 39, 60, 71]. Furthermore, Hurford et al. [39] reported that in seals the size of the spleen was reduced by epinephrine infusions and diving, and the changes in splenic size and volume were inversely correlated with the plasma epinephrine concentration.

In humans, the volumes of visceral organs are normally measured with ultrasonography. However, CT is also useful for measuring the volumes of visceral organs; i.e., it has been reported that CT provides volumetric measurements that are accurate within ±5% [34]. In the veterinary field, we also use CT to measure splenic volume because it is highly sensitive and specific and makes measuring splenic volume simple [7]. It was reported that splenic volume measurements obtained with CT in dogs exhibited accuracy values of ±5% [54]. Since, the effects of epinephrine dropwise onto the splenic surface induce the splenic volume to decrease similarly to infusion of epinephrine. Increased in circulation hematocrit and red cell mass following infusion epinephrine has been reported [31]. Therefore, reduction the splenic volume using with dropwise epinephrine might be reduce intraoperative blood loss and prevented anemia condition after splenectomy.

The purpose of our second experiment was to investigate the hemodynamic effects of the direct dropwise administration of epinephrine onto the splenic surface. Ten healthy adult beagles underwent laparotomy under inhalation anesthesia, and hemodynamic measurements were obtained. This experiment indicated that administering epinephrine at a dose of 10 μg/kg had less effect on hemodynamics than administering epinephrine at a dose of 100 μg/kg.

Epinephrine is a neurotransmitter and can deactivate and activate sympathetic receptors (α- and β-adrenoceptors), which results in hemodynamic changes [30]. The epinephrine-induced activation of the α-adrenoceptors located in the walls of blood vessels and smooth muscle results in the constriction of blood vessels throughout the body, and hence, elevated blood pressure. In contrast, the activation of β-adrenoceptors in the heart causes increases in heart rate and SV, and the release of renin to maintain blood pressure [30].

In the present study, no significant change in mean heart rate was detected after the administration of epinephrine. However, the EP100 group exhibited a higher mean heart rate than the EP10 group, which agrees with several studies involving epinephrine infusions or

combinations of local anesthetics and epinephrine [1, 16, 72]. Thus, epinephrine increases heart rate in a dose-dependent manner.

One of the effects of epinephrine is to induce vasoconstriction, which is mediated by α-adrenoceptors. In addition, it causes a dose-dependent reduction in RBF when it is administered as an intrarenal infusion. On the other hand, β-adrenoceptor stimulation results in renal vasodilation. A study of sheep showed that an intrarenal epinephrine infusion had no effect on hemodynamics. Moreover, the stimulation of α-adrenoceptors caused a 50% reduction in RBF due to renal vasoconstriction [56]. In another study, increasing the epinephrine infusion rate was found to cause a reduction in the glomerular filtration rate (GFR) [55]. Renal vasoconstriction due to α-adrenoceptor stimulation and an increase in MAP can cause reductions in blood flow to the kidneys. In the present study, RBF was significantly increased by 6.1±1.8% and 7.7±2.4% at 10 and 15 minutes, respectively, in the sterile saline experiment involving the EP10 group. This might have been caused by the activation of β-adrenoceptors, but further investigation is required to confirm this. On the contrary, a marked reduction in RBF (-24.8±24.6%) was seen in the EP100 group at 5 minutes after the administration of epinephrine, and no change in MAP was observed in the EP100 group. Thus, it is presumed that the reduction in RBF detected in our study was caused by direct stimulation of the α-adrenoceptors in the renal artery. It should be noted that sudden reductions in RBF and GFR carry a risk of acute renal injury.

Although the infusion of epinephrine was previously shown to increase SVR in a dose-dependent manner [53], a marked reduction in SVR (-30.2±5.7%) was detected in the EP100 group in the current study. We considered that the effect observed in the present study was similar to those induced by low-dose epinephrine infusions in previous studies [5, 6, 72]. The latter studies suggested that the observed reductions in SVR had been caused by vasodilation or baroreceptor activation associated with the highly sensitive effects of low-dose epinephrine on β_2-adrenoceptors. As for our findings, we suggest that epinephrine stimulated α_1-adrenoceptors

on the spleen, presumably because it was dripped directly onto the spleen, and then the spleen contracted and expelled its contents into the systemic circulation, resulting in concomitant increases in venous return and SVR [60]. Furthermore, the rise in SVR would have led to an increase in CO [30]. In turn, the increase in CO and changes in blood pressure would have activated baroreceptors, leading to a reduction in SVR. A significant reduction in SVR (by 15.5±5.7%) was seen in the sterile saline experiment involving the EP10 group, but we assumed that this phenomenon was caused by the activation of β_2-adrenoceptors.

Several studies have detected reductions in MAP following the infusion of epinephrine [5, 6, 72]. These results contrast with ours, as the MAP of the EP10 group peaked at 5 minutes after the administration of epinephrine, but a tendency to decline to the baseline level was seen after that. On the other hand, in the EP100 group the MAP gradually increased and remained elevated until the end of the experiment. We assumed that the differences in the epinephrine administration method were responsible for this discrepancy. The infusion of epinephrine can activate α- or β-adrenoceptors in all organs. However, the dropwise administration of epinephrine represents a form of topical use; therefore, the concentration of the agent is higher in the area where it is applied than in the circulation. In the present study, the dropwise administration of epinephrine onto the spleen induced increases in MAP and MPAP followed by rises in SV and CO.

In conclusion, the dropwise administration of 100 μg/kg epinephrine onto the spleen caused a marked reduction in splenic volume; i.e., the spleen decreased to 15.7% of its normal volume, while 10 μg/kg epinephrine caused the spleen to decrease to 53.9% of its normal volume. In addition, due to time of the splenic volume recovery to baseline volume within 15-20 min after dropwise epinephrine onto the splenic surface. Therefore, we can be performed the splenectomy during that time with small size of the spleen. Although, in the statistic did not shown significant different on HR, MAP, RBF, SVR, or PVR level from baseline in the EP100 group, but dripping

epinephrine 100 μg/kg onto the spleen caused a more marked reduction in RBF and SVR than dripping 10 μg/kg epinephrine onto the spleen. In addition, 100 μg/kg epinephrine induced marked increases in MPAP, CI, and SV, whereas 10 μg/kg epinephrine only had weak hemodynamic effects. For the reason of sudden reductions in RBF and GFR, and they can carry a risk of acute renal injury. Therefore, our results suggest that directly dripping 10 μg/kg epinephrine onto the spleen before performing splenectomy is feasible and safe in patients with hematological disorders to reduce perioperative and postoperative complications.

Chapter 3 Comparison of postoperative outcomes between laparotomy and laparoscopic splenectomy in dogs.

Introduction

Splenectomy is a surgical procedure that is used to treat hematologic disorders and various splenic conditions in dogs and cats, including splenomegaly, splenic masses, splenic trauma. In the veterinary field, splenectomy is usually performed in animal with splenic tumors, e.g., hemagiosarcoma, hematoma or hemangioma. In contrast to the situation in humans, splenectomy is also commonly used to treat animals with hematologic disorders [47, 61], as it results in a higher survival rate and less recurrence than medical treatment [59]. The successful treatment of hematologic disorder with splenectomy has been reported in dogs [25, 36]. However, various postoperative complication can occur after splenectomy, including ventricular arrhythmia, pancreatic fistula formation, and thromboemboli [50]. As for surgical complications, intra-abdominal hemorrhaging in the intraoperative or postoperative period is the primary concern [62].

Moreover, the spleen plays important roles in several functions, e.g., hematopoiesis, red blood cell destruction, immunologic functions, and red blood cells storage [12]. In particular, it functions as a blood reservoir; i.e., it contains approximately 10 to 20% of the blood in the body, including one-third of all red blood cells [44] and about 30% of all platelets. Therefore, performing splenectomy not only causes intraoperative blood loss, but also results in the blood within the spleen being lost, which can lead to severe anemia. Furthermore, in a study involving long-term observation following splenectomy, in which the immune function of the spleen was considered, overwhelming postoperative splenectomy infections, which can be life-threatening, occurred frequently [10, 64]. On the other hand, a study of the effects of splenic autotransplantation suggested that regeneration of the spleen might prevent such complications

[65]. However, attempts should be made to reduce the invasiveness of splenectomy and the associated intraoperative blood loss in order to avoid severe postoperative complications.

Currently, minimally invasive surgery, including laparoscopic surgery, is being widely used in humans, as it has various advantages over conventional surgery, e.g., it causes less pain and involves smaller surgical wounds, faster recovery from surgery, and reduced surgical trauma [66, 70]. For this reason, laparoscopic surgery has started to be applied to various surgical procedures for companion animals, for instance, abdominal organ biopsies, ovariohysterectomy, ovariectomy, gastropexy, cryptorchidectomy, and splenectomy [51]. Several studies have reported successful outcomes for laparoscopic splenectomy in dogs and cats [4, 58, 81]. Laparoscopic splenectomy, which involves less intraoperative bleeding than open splenectomy and can be achieved with small surgical incisions, is considered to be a very useful treatment alternative to conventional open splenectomy, which requires laparotomy to be performed.

The purpose of this study was to evaluate and compare the outcomes of open and laparoscopic splenectomy in the dogs. We hypothesize that laparoscopic splenectomy can be used to reduce bleeding in the perioperative period and reduce the risk of postoperative complications.

Materials and methods

1. Animals selection

Clinically healthy beagles were selected for this study. Their health was determined via routine clinical examinations. The animals were divided into 2 groups; i.e., 5 dogs (mean weight: 11.5±0.6 kg, range: 11-12.3 kg) were included in the open surgery (OS) group, and the other 5 dogs (mean weight: 12.0±0.8 kg, range: 11-13 kg) were included in the laparoscopic surgery (LS) group. The animals were fed twice a day and had free access to water. However, all of the animals had their food restricted at ≥12 hours prior to the operation. All of the experimental animals used in this study were handled according to the experimental animal guidelines of Kitasato University.

2. Anesthesia and analgesic protocol

In this study, we used the same anesthesia protocol in both groups, which involved the administration of atropine sulfate (0.05 mg/kg, intravenous [IV], Atropine®; Mitsubishi Tanabe Pharma, Japan) followed by midazolam (0.1 mg/kg, IV, Dolmicam®; Astellas Pharma, Tokyo, Japan). Enrofloxacin (5 mg/kg, subcutaneous [SC] Bytril®; Bayer AG) was administered during induction as a prophylaxis. General anesthesia was induced using propofol (6 mg/kg, IV, animal propofol; Mylan Seiyaku, Tokyo, Japan). An endotracheal tube was inserted, and general anesthesia was maintained via the inhalation of 2.0% isoflurane (Isofur®; Dainippon Sumitomo Pharma, Osaka, Japan). The animals were ventilated with 100% oxygen. Lactated Ringer's solution was infused (10 ml/kg/hr, Lactec®; Otsuka Pharmaceutical Co., Tokyo, Japan) throughout the surgical procedure, and then the infusion rate was reduced to 5 ml/kg/hr for 6 hours in the postoperative period until the animals woke up. As for the analgesic agents, we administered meloxicam (0.2 mg/kg, SC, Metacam®; Vetomedica Japan Co., Ltd.) during

induction for prophylactic analgesia, and the administration of fentanyl (10 μg/kg/hr, IV, fentanyl injection; Daiichi Sankyo Propharma Co., Tokyo, Japan) was started during the preoperative period. The fentanyl infusion rate was reduced to 5 μg/kg/hr for 6 hours in the postoperative period.

3. Surgical procedure

The OS group: Laparotomy was performed according the previously reported surgical procedure [7]. The animal was placed in the dorsal recumbent position, and the ventral area was prepared for the operation with an aseptic technique. A large ventral midline laparotomy was started from the xiphoid process and extended to a point caudal to the umbilicus to enable complete abdominal exploration. After approaching and examining the spleen, the splenic vessels were separated and ligated. After that, the spleen was excised from the abdominal cavity, and the abdominal wall was sutured.

The LS group: The animal was placed in the same position as in the OS procedure. Pneumoperitoneum (intraperitoneal pressure: 10 mmHg) was induced with carbon dioxide (CO_2) gas using a pneumoperitoneum device (STORZ) via a Veress needle, which was inserted into the right side of the periumbilical area. Thereafter, a trocar (diameter: 12 mm; XCEL) was inserted into the abdominal cavity on the ventral midline (hereafter referred to as the caudal port). A 5-mm rigid endoscope (OLYMPUS) was inserted through the caudal port to explore and visualize the abdominal cavity. Then, a trocar (diameter: 5 mm; CORE) was inserted into the abdominal cavity on the right side of the umbilicus (hereafter referred to as the umbilicus port), and another trocar (diameter: 5 mm; CORE) was inserted into the abdominal cavity halfway between the umbilicus port and the caudal port (hereafter referred to as the middle port). The procedure was performed under direct visualization via the endoscope. Two 5-mm grasping forceps were

introduced through the umbilicus and middle ports and advanced toward the spleen. After that, the location of the spleen was confirmed on a monitor, and a 27-G needle was inserted through the abdomen to allow the dropwise administration of 10 μg/kg of epinephrine onto the splenic surface. The rigid endoscope was removed from the caudal port and inserted through the umbilical port, and an ultrasonic coagulation device was inserted through the caudal port. The splenic vessels were isolated and dissected using the ultrasonic coagulation device, and a retrieval bag was inserted through the caudal port to accommodate the spleen, and the trocar was pulled out of the caudal port. An incision was made at the caudal port to allow the spleen to be removed from the abdominal cavity. After that, all of the trocars and residual CO_2 were removed. The peritoneum and skin were sutured according to the conventional method.

4. Postoperative management

Enrofloxacin (5 mg/kg, SC, Bytril®; Bayer AG) was administered once a day for 3 consecutive days. The animals were fed normally from the first day after surgery. The surgical wound was cleaned at least once a day, and the stitches were removed on the 7th day after surgery.

5. Measurement of splenic weight and volume

Splenic weight was measured using a general-purpose electronic balance (HF-2000; A&D). On the other hand, the volume of the spleen was measured by placing the spleen into a plastic container filled with water. The volume of the spleen was determined by measuring the volume of water that was displaced when the spleen was placed in the container.

6. Operation time and incision length

The operation time was recorded from the beginning of the incision to the end of the abdominal suturing. The length of the skin incision was taken as the surgical wound size.

7. Blood cell count, hematocrit level, and serum cortisol concentration measurement

A blood sample was collected from the cephalic vein or the external saphenous vein before the operation to obtain preoperative measurements, and then further samples were obtained at 1, 3, and 6 hours, and on the 1^{st}, 3^{rd}, 5^{th}, and 7^{th} days after surgery. The collected blood was treated with ethylenediaminetetraacetic acid (EDTA)-2Na and used to measure the level of hematocrit, obtain complete blood cell counts, and assess blood biochemistry. A capillary tube (Terumo®) was used to collect blood from an EDTA-2Na-treated sample, before being subjected to centrifugation at 12,000 rpm for 5 minutes with a hematocrit centrifuge (KH-120 M, Kubota). Thereafter, the hematocrit level was measured using a hematocrit measurement plate. Blood cell counts were obtained using an automated cytometer (Celltack α, Nihon Kohden, Japan). To measure the serum cortisol concentration, serum was separated from the collected blood by allowing the blood to clot by leaving it at room temperature for approximately 20 minutes. The serum was then centrifuged at 3,000 rpm for 10 minutes to separate the supernatant, which was immediately collected in a clean Eppendorf tube and frozen until the measurements were obtained. The serum cortisol concentration was measured using a solid-phase radioimmunoassay.

8. Statistical analysis

All results are expressed as mean±standard deviation values. For comparisons with the preoperative values, the paired t test was used, and p-values of <0.05 were considered to indicate

a significant difference. The Student's t test was used to analyze variance and determine the significance of differences between the groups at all time points, and p-values of <0.05 were considered to indicate a significant difference.

Results

1. Splenic weight and volume

The mean splenic weight was 199.6±66.7g (101-263 g) and 162.0±17.5 g (132.7-177.2 g) in the OS and LS groups, respectively. There was no significant difference between the groups. The ratio of splenic weight to body weight was 1.8±0.6% (0.8-2.4%) and 1.4±0.2% (1.1-1.5%) in the OS and LS groups, respectively.

The mean splenic volume was significantly smaller ($P<0.05$) in the LS group (138.2±26.5 cm^3 (99-164 cm^3)) than in the OS (202.2±44.4 cm^3 (160-250 cm^3)) (Table 3-1).

2. Operation time and incision length

The mean operation time was 42.0±3.8 mins (38-46 mins) and 53.8±18.3 mins (32-74 mins) in the OS group and LS group, respectively. There was no significant difference between the groups. The incision length was significantly smaller ($P<0.01$) in the LS group (5.7±0.8 cm) than in the OS group (9.6±0.4 cm) (Table 3-1).

3. Hematocrit level (Hct)

Compared with the preoperative Hct level, significantly lower ($P<0.05$) Hct values were seen at all time points in the OS group. In contrast, no significant differences were observed in the LS group. There were no significant differences between the groups at any time point, but a tendency towards a lower value in the OS group was observed (Table 3-2).

4. Platelet count (PLT)

Compared with the preoperative value, the PLT was significantly increased ($P<0.05$) at 3 h and 7 d after surgery in the OS group and at 3, 5, and 7 d after surgery in the LS group (Table 3-2). No significant difference between the groups was seen.

5. White blood cell count (WBC)

Significant differences in the WBC compared with the preoperative value ($P<0.05$) were seen at 3 and 6 h and at 1 d after surgery in the OS group. In the LS group, significant differences compared with the preoperative value were seen at 3 and 6 h, and at 1, 3, and 5 d after surgery ($P<0.05$). A significant difference between ($P<0.05$) the groups was seen at 6 h after surgery (Table 3-2).

6. Red blood cell count (RBC)

Compared with the preoperative value, the RBC was significantly lower ($P<0.05$) at 6 h and 1 d after surgery in the LS group. In addition, the RBC was significantly higher ($P<0.05$) in the LS group than in the OS group at 3 d after surgery (Figure 3-1).

7. Serum cortisol concentration

At 3 h after surgery, significant increases ($P<0.05$) in the serum cortisol level compared with the preoperative value were seen in the OS and LS groups. Moreover, a significant difference ($P<0.05$) between the groups was also seen at 3 h after surgery. The serum cortisol level of each group peaked at 3 h after surgery and then tended to decrease to its original level (Figure 3-2).

Discussion

Splenectomy is a surgical procedure, in which the spleen is removed from the abdominal cavity. It is used to treat several diseases. The most common peri-/postoperative complication of this procedure is hemorrhaging. Furthermore, in dogs the spleen acts as a store of red blood cells. Hence, performing splenectomy not only causes intraoperative blood loss, but also results in the blood within the spleen being lost, which can lead to severe anemia and a high risk of postoperative complications. Therefore, ways of reducing of intraoperative hemorrhaging during splenectomy should be considered. The current study compared an open splenectomy procedure with a laparoscopic splenectomy procedure by evaluating the operative time, surgical wound size, splenic volume, and splenic weight, and by performing preoperative/postoperative comparisons of the WBC, RBC, PLT, Hct level, and serum cortisol concentration.

The operative time did not differ significantly between the groups. However, the duration of the laparoscopic surgical procedure was related to the experience of the surgeon. In this study, the surgical wound size was significantly smaller in the LS group than in the OS group (Table 3-1). A higher frequency of surgical site infections has been reported after open surgery than after laparoscopic surgery [52]. However, the risk of surgical site infections is relate to several factors, including the duration of surgery.

Leukocytosis, in which the proliferation and differentiation of myeloid cells are promoted by inflammatory cytokines, and the number of neutrophils in the peripheral blood increases, can occur after surgery. In addition, endogenous corticosteroids, such as cortisol, which is secreted from the adrenal cortex in response to invasion, might be associated with postoperative increases in the number of leukocytes. Therefore, the occurrence of leukocytosis after splenectomy in animals could be a normal response to inflammation [15]. In the present study, epinephrine was applied to the splenic surface during laparoscopic splenectomy in order to cause

the spleen to contract and release blood components into the systemic circulation, which might have caused leukocytosis. However, the levels of these components were within the normal range and tended to decrease to preoperative levels over time (Table 3-2).

In dogs, the spleen plays an important role as a blood reservoir [12]. A previous study revealed that one-third of red blood cells are stored in the spleen during resting [44]. In the current study, the LS group exhibited higher postoperative RBC than the OS group at all time points, and a significant difference in the RBC was seen between the groups at 3 d after surgery. The Hct levels of the OS group were significantly lower that the preoperative value ($P<0.05$) at all time points in the postoperative period, whereas in the LS group the Hct level was not significantly altered at any time point in the postoperative period. Although, there were no significant intergroup differences in the Hct level, one dog in the OS group exhibited a very low Hct value (25%) on the 1st day after surgery, and its Hct level did not return to the preoperative level until the end of the experiment (7 days after surgery). Anemia is a common postoperative condition, especially after splenectomy, which not only involves intraoperative bleeding, but also loss of the spleen [57]. Therefore, the above results suggest that LS causes less intraoperative bleeding than OS.

In dogs, the spleen has not served as a dynamic reservoir of platelets [60], unlike the human spleen, which stores approximately 30% of all platelets, and this can increase to 50 to 90% in cases of splenomegaly [78]. However, thrombocytosis, which often occurs after splenectomy, can induce platelet hyperaggregation [43]. Elevated PLT after surgery might be associated with subacute postoperative complications, while an elevated WBC is an acute condition [29]. In this study, high PLT were seen without elevated WBC. This situation often persisted for a few weeks, but then the animals' PLT tended to return to normal levels.

Cortisol is a stress hormone and is secreted from the adrenal cortex. The level of cortisol increases after surgical invasion due to stimulation by adrenocorticotropic hormone. Since

cortisol has anti-inflammatory effects, this could interfere with inflammatory processes, e.g., it could reduce the immunological activity of white blood cells [21]. A study of cats reported that cats that underwent longer surgical procedures exhibited higher cortisol concentrations than cats that underwent shorter surgical procedures [68]. In our study, the cortisol concentration peaked at 3 h after surgery in both groups, and a highly significant difference was seen between the cortisol concentrations of the OS and LS groups. Moreover, the OS group displayed higher cortisol concentrations than the LS group at all time points. However, after 3 h the cortisol level tended to decrease to the preoperative level in both groups. The serum cortisol concentration can peak at 4-6 h after surgery, depending on the degree of trauma.

In conclusion, the outcomes of this study showed that laparoscopic splenectomy combined with directly administration 10 μg/kg epinephrine onto the spleen has advantages over open splenectomy, e.g., it results in reduced intraoperative hemorrhaging; less surgical invasion-related stress; lower serum cortisol concentrations; and a lower risk of postoperative complications, such as anemia. Our results suggest that laparoscopic splenectomy combined with directly administration 10 μg/kg epinephrine onto the spleen can reduce the amount of blood loss during surgery and lower the risk of postoperative complications after splenectomy. In the future, LS might be considered useful in the veterinary field, although it will be necessary to improve the technique before its application in clinical cases.

Chapter 4 Influence of the duration of blood flow blockade by the laparoscopic Pringle maneuver on hemodynamics and postoperative blood test results in dogs

Introduction

Hemorrhaging is a general complication of hepatectomy. In humans, intraoperative blood loss can be reduced by inhibiting hemorrhaging from the venous system by reducing central venous pressure [20, 29] and using devices such as ultrasonically activated scalpels and vessel-sealing systems [19, 21, 25]. In addition to these methods, the hepatic ischemia method, in which blood flow into the liver is blocked, is widely used for such purposes [1, 5].

In particular, the Pringle maneuver, in which the portal vein and hepatic artery are both blocked, is useful for controlling intraoperative hemorrhaging because it is a simple procedure [24]. When the Pringle maneuver is conducted in humans, the portal vein and hepatic artery are generally blocked for 15 minutes, before being released for 5 minutes, and then the procedure is repeated. The influence of the intermittent blockade of blood flow into the liver for about 15 minutes is mild, and the effects of this procedure on the body are considered to be small, although liver enzyme levels transiently increase. In humans, continuous blood flow blockade using the Pringle maneuver is limited to a maximum of 60 minutes for normal livers and 30 minutes for patients with liver disease, such as hepatic cirrhosis [17]. In dogs, it has been reported that it is possible to continuously block blood flow into the liver using the laparotomic Pringle maneuver for 20 minutes [13], and during hepatectomy repeated 10-15-minute periods of hepatic blood flow blockade followed by 5-10-minute periods of release are considered acceptable. However, blocking the blood flow into the liver for ≥10 minutes has a marked influence on hemodynamics, and the optimal duration of such periods is unclear.

Laparoscopic surgery has various advantages over open surgery, e.g., it involves smaller surgical wounds, less pain, and faster recovery after surgery [26, 27]. In addition, in human

laparoscopic hepatectomy retrograde hemorrhaging from the hepatic vein can be prevented by increasing the intra-abdominal pressure using pneumoperitoneum, and combining this approach with the Pringle maneuver reduces hemorrhaging from the hepatic artery and portal vein [12, 16].

Therefore, hemorrhaging from the liver can be reduced by performing laparoscopic hepatectomy rather than laparotomic hepatectomy [27]. However, in dogs laparoscopic hepatectomy has not been sufficiently investigated. Thus, in this study, based on the fact that in laparotomic hepatectomy blood flow into the liver is blocked for 10-15 minutes, one group of dogs was subjected to three 10-minute periods of hepatic blood flow blockade, and another group was subjected to six 5-minute periods of hepatic blood flow blockade. In addition, the total blocking time was set at 30 minutes because the maximum duration of the blocking period has been reported to be 20 minutes.

We considered that, in dogs, if the total laparoscopic Pringle maneuver-based blocking time were the same, a greater number of short blocking periods would have less influence on hemodynamics and postoperative liver function than a lower number of longer blocking periods. Therefore, this study examined the hypothesis that hemodynamics, postoperative clinical symptoms, and blood test findings would be influenced less in the group subjected to six 5-minute periods of hepatic blood flow blockade than in the group subjected to three 10-minute periods of hepatic blood flow blockade.

Materials and experimental methods

1. Animals selection

Eight clinically healthy beagles (4 males and 4 females) were selected and divided into 2 groups; i.e., group 1, in which 4 dogs (2 males, 2 females; body weight: 11.3-12.4 kg) underwent a surgical procedure involving 3 cycles, in which the blood flow through the portal triad was blocked for 10 minutes and then restored for 5 minutes, and group 2, in which the other 4 dogs (2 males, 2 females; body weight: 9.8-11.0 kg) underwent a surgical procedure involving 6 cycles, in which the blow through the portal triad was blocked for 5 minutes and then restored for 5 minutes. The animals were fed once a day and allowed free access to water. However, they were fasted for 12 hours before the operation. All experimental animals used in this study were handled according to the experimental animal guidelines of Kitasato University.

2. Anesthesia and analgesic protocol

We used the same anesthesia protocol in both groups. Atropine sulfate (0.05 mg/kg, intravenously [IV], atropine; Mitsubishi Tanabe Pharma, Japan), midazolam (0.1 mg/kg, IV, Dormicum®; Astellas Pharma, Tokyo, Japan), and butorphanol tartrate (0.2 mg/kg, IV, Vetorphale®; Meiji Seika, Tokyo, Japan) were used to. Ampicillin sodium (20 mg/kg, IV, Viccillin®; Meiji Seika, Tokyo, Japan) and meloxicam (0.2 mg/kg, subcutaneously [SC], Metacam®; Vetmedica Japan CO., Ltd.) were administered at the time of induction as premedications. General anesthesia was induced with propofol (6 mg/kg, IV, animal propofol; Mylan Seiyaku, Tokyo, Japan), and then an endotracheal tube was inserted. The general anesthesia was maintained via the inhalation of 2.5% isoflurane (Isoflu®; Dainippon Sumitomo Pharma, Osaka, Japan). The animals were ventilated with 100% oxygen, and lactated ringer's

solution was infused (2 ml/kg/h, Lactec®; Otsuka Pharmaceutical Co; Tokyo, Japan) throughout the surgical procedure until the animals woke up. As for analgesia management, we administered buprenorphine (20 μg/kg, IV, Lepetan®; Otsuka Pharmaceutical Co; Tokyo, Japan) after surgery.

3. Hemodynamic parameters and evaluation methods

To perform the hemodynamic assessments, we placed the dog in the left lateral recumbence position. An incision was made in the right side of the neck, and the jugular vein and carotid artery were approached. A pulmonary arterial catheter (SG catheter 131H-7F; Nihon Kohden, Japan) was used to measure cardiac output (CO). It was inserted into the right jugular vein, and the tip was placed in the pulmonary artery. The catheter's position was confirmed by observing the characteristic pulmonary arterial pressure waves, and then the catheter was connected to a thermodilution CO meter (MTC-6100, Nihon Kohden, Japan). To measure arterial pressure, we used a blood pressure monitor (OMP-7201 K. Nihon Kohden, Japan), and a 7F catheter was inserted into the carotid artery, with its tip placed in the aorta.

a. Measurement of hemodynamic parameters

Heart rate (HR), aortic pressure (AP), pulmonary artery pressure (PAP), and CO were assessed as hemodynamic parameters in this study. All of the parameters were recorded before the Pringle maneuver was started to provide preoperative data (pre-blocking data). Then, all of the parameters, except CO, were recorded every minute, and CO was measured every 5 minutes. HR was measured with a monitor (BIO-SCOPE, AM 120, FUKUDA M·E). To measure AP and PAP, a vascular catheter and a Swan-Ganz catheter were placed in the carotid artery and pulmonary artery, respectively. These catheters were connected to a pressure transducer, and the

measurements were obtained using a bedside monitor (LifeScope 11, Nihon Kohden, Japan). To assess CO, 3 ml of saline with at temperature of 0°C was rapidly injected into the pulmonary artery via a thermodilution catheter, which was connected to a thermodilution CO monitor (MTC-6100, Nihon Kohden, Japan), and CO was calculated as the time until the temperature of the blood returned to normal. Mean arterial pressure (MAP), mean pulmonary artery pressure (MPAP), the cardiac index (CI), systemic vascular resistance (SVR), and pulmonary vascular resistance (PVR) were also assessed as hemodynamic parameters. The latter three parameters were calculated using the following formulas:

1. Cardiac index = $\frac{\text{Cardiac input}}{\text{Body surface area}}$ (l/min/m^2)
2. Systemic vascular resistance = $\frac{\text{Mean aortic pressure}}{\text{Cardiac index}} \times 80$ (dynes-sec-cm^{-5}/m^2)
3. Pulmonary vascular resistance = $\frac{\text{Mean pulmonary artery pressure}}{\text{Cardiac index}} \times 80$ (dynes-sec-cm^{-5}/m^2)

b. Clinical symptoms

The presence or absence of clinical symptoms, such as vomiting and diarrhea, was examined and recorded from day 1 to day 14 after the operation.

c. Assessment of blood cell counts and blood biochemistry

To assess blood cell counts and biochemical parameters, a sterile plastic syringe was used to collect blood from the cephalic vein, external saphenous vein, or left jugular vein and dispensed into an ethylenediaminetetraacetic acid (EDTA)-2Na microtiter blood collection tube and a heparinized blood collection tube. To assess blood biochemistry, the blood sample in the

heparinized blood collection tube was centrifuged at 3,000 rpm for 5 minutes, and then the plasma was separated and stored in a frozen state until the measurements were obtained.

Blood cell count measurements were obtained immediately after the blood collection procedure using an automated hemocytometer (Celltack-α, Nihon Kohden), and the blood biochemistry assessments (alanine transaminase [ALT], alkaline phosphatase [ALP], and aspartate aminotransferase [AST], and C-reactive protein [CRP] levels) were conducted using a rapid automatic analyzer (Dimension® R×L Max™). The blood collection was performed during the preoperative period to provide preoperative baseline data and on days 1, 2, 3, 5, 7, 10, and 14 of the postoperative period.

4. Procedure for the laparoscopic Pringle maneuver

Pneumoperitoneum (intraperitoneal pressure: 10 mmHg) was induced with carbon dioxide gas using a pneumoperitoneum device (STORZ), after insufflation needles had been inserted on the caudal side and upper median side of the xiphoid process in the supine position. Thereafter, trocars of 5 mm in diameter (CORE) were inserted into the umbilicus (the umbilical port) and points located approximately 1 cm caudal to the 13th rib on both the right (the right umbilical port) and left (the left umbilical port) sides. After inserting a 5-mm rigid endoscope (OLYMPUS) into the umbilical port and confirming the location of the liver, laparoscopic forceps were inserted into the right and left umbilical ports in order to approach the portal vein and hepatic artery. Then, nylon thread was used to encircle the portal vein and hepatic artery, before both ends of the nylon thread were taken out of the abdominal cavity through the trocar and passed into a rubber tube. To block blood flow into the liver, the tube was pushed forward and clamped with artery forceps to maintain the blockade. The portal vein and hepatic artery were declamped by

loosening the artery forceps, pulling the rubber tube out of the abdominal cavity, and loosening the nylon thread.

Hemodynamic measurements were obtained for 10 minutes in the preoperative period. Next, the portal vein and hepatic artery were blocked for 10 minutes or 5 minutes, and then released for 5 minutes. The total blocking time was set at 30 minutes. In group I, 3 cycles of blocking for 10 minutes followed by release for 5 minutes were performed. In group II, 6 cycles of blocking for 5 minutes followed by release for 5 minutes were performed. After the completion of the last blocking procedure, measurements were taken for 10 minutes in order to confirm whether there had been any changes in circulatory dynamics.

5. Postoperative management

Enrofloxacin (5 mg/kg, SC, Baytril®; Bayer Pharmaceutical) was given once a day for 5 consecutive days. Food consumption was restarted from the first day after the operation, and the surgical wound was kept clean. On day 10 after the operation, the stiches were removed.

6. Statistical analysis

Two-way repeated-measures ANOVA and Bonferroni's post-hoc multiple comparisons test were performed, and P-values of <0.05 were regarded as significant. For hemodynamic data, only significant differences from the values obtained before each blocking period are presented.

Results

1. Hemodynamic parameters

a. Heart rate (HR)

Compared with the pre-blocking level, the dogs' HR had a tendency to increase immediately after the initiation of the blocking procedure in both groups and then returned to the pre-blocking level after blood flow was restored. In addition, compared with that seen in the first blocking period, the dogs' HR was lower in every blocking period from the second blocking period onwards. There were no significant differences in HR between the groups (Figs. 4-1 and 4-2). No arrhythmia was observed in this experiment.

b. Aortic pressure (AP)

In both groups, the mean AP increased in the first minute of the blocking period and then decreased below the pre-blocking level during the first blocking period. It returned to the pre-blocking level after blood flow was restored. The mean AP was lower in the second blocking period than in the first blocking period. However, there were no significant differences in the mean AP between the blocking periods or between the groups (Figs. 4-3 and 4-4).

c. Pulmonary artery pressure (PAP)

An immediate reduction in the mean PAP was observed after the initiation of blocking in both groups, and the mean PAP remained lower than the pre-blocking level throughout the blocking procedure. After blood flow was restored, the mean PAP returned to the pre-blocking

level. However, no significant differences were detected between the groups (Figures 4-5 and 4-6).

d. Cardiac output (CO)

A thermodilution CO monitor (MTC - 6100, Nihon Kohden, Japan) was used to measure CO. However, it was unable to measure CO levels of <0.5 L/min. In the 10-minute blocking group, dogs exhibited CO levels of <0.5 L/min in the second blocking period, and one more dog displayed such CO levels in the third blocking period. In the 5-minute blocking group, two dogs exhibited CO levels of <0.5 L/min in the first blocking period, and three dogs displayed such CO levels in the second and subsequent blocking periods. In this study, CO levels of <0.5 L/min were recorded as 0.5 L/min. In the 5-minute blocking group, CO decreased markedly after the initiation of the blocking procedure (compared with the pre-blocking level) and remained at a lower level throughout the blocking period. After blood flow was restored, CO increased to the pre-blocking level. In the 10-minute blocking group, CO was lower in the second blocking period than in the first blocking period. On the other hand, no significant difference in CO was observed between the first and second blocking periods in the 5-minute blocking group. CO in the first blocking period was lower in the 5-minute blocking group than in the 10-minute blocking group (Figure 4-7 and 4-8). In addition, similar results were obtained for the cardiac index.

e. Systemic vascular resistance (SVR)

Immediate increases in SVR were observed after the initiation of blocking in both groups, and elevated SVR levels persisted throughout the blocking procedure. After blood flow was restored, SVR returned to the pre-blocking level. There was almost no difference in SVR between the groups (Figure 4-9 and 4-10).

f. Pulmonary vascular resistance (PVR)

PVR gradually increased after the initiation of blocking in both groups and remained elevated throughout the blocking period. Then, after blood flow was restored PVR tended to decrease to the pre-blocking level. There was almost no difference in PVR between the groups (Figure 4-11 and 4-12).

2. Clinical symptoms

On the first day after the operation, vomiting was observed in one dog in each group. No other abnormal clinical symptoms were observed.

3. Total white blood cell count

The total leukocyte count increased on days 1 and 2 after the operation in both groups, and after that it tended to decrease. The 10-minute blocking group had a significantly lower mean total leukocyte count ($P<0.05$) than the 5-minute blocking group on day 14 after the operation (Figure 4-13).

4. Liver enzyme levels

a. Alanine transaminase (ALT)

The ALT level peaked on day 1 after the operation in both groups. However, it tended to decrease on day 2 after the operation and reached the preoperative level on day 14 after the operation. In the 5-minute blocking group, the ALT levels seen on day 1 after the operation were significantly higher than the preoperative levels. No significant differences in the AST level were detected between the groups (Table 4-1).

b. Alkaline phosphatase (ALP)

The ALP level peaked on day 1 after the operation in the 5-minute blocking group and on day 2 after the operation in the 10-minute blocking group. However, it tended to decrease on day 3 after the operation in the 10-minute blocking group, whereas in the 5-minute blocking group a similar tendency was observed from postoperative day 2 onwards. There was no significant difference in the ALP level between the groups (Table 4-1).

c. Aspartate aminotransferase (AST)

The AST level peaked on day 1 after the operation in both groups. However, it tended to decrease to the preoperative baseline level on day 2 after the operation, and the baseline level was reached on day 10 after the operation. Moreover, no significant difference in the AST level was seen between the groups (Table 4-1).

5. C-reactive protein (CRP) level

The CRP level peaked on days 1 and 2 after the operation in the 5- and 10-minute blocking groups, respectively. However, it tended to decrease to the preoperative baseline on day 3 after the operation, and the preoperative level was reached on day 7 after the operation in both groups. There was no significant difference in the CRP level between the groups (Figure 4-13).

Discussion

The risk of massive intraoperative or postoperative hemorrhaging is a concern when hepatectomy is performed. Therefore, methods that can be used to reduce blood loss, for instance, pharmacological methods for reducing central venous pressure [20, 29] or procedures for inducing vascular occlusion, should be considered during hepatectomy [10]. The Pringle maneuver, in which blood flow into the liver is blocked, has been widely used for such purposes. In humans, performing the Pringle maneuver during laparotomic hepatectomy has been shown to reduce bleeding from the liver. In addition, minimally invasive surgery, such as laparoscopic hepatectomy, minimizes incisional wounds and suppresses retrograde bleeding from the hepatic vein via the use of high pneumoperitoneum pressure (18-20 mmHg) [3, 12]. Therefore, performing laparoscopic hepatectomy in combination with the Pringle maneuver is a feasible way of reducing blood loss and surgical trauma during hepatectomy. Despite the fact that this approach might reduce bleeding from the liver, its use in dogs has not been examined.

In the current study, we performed the Pringle maneuver laparoscopically, and blood flow into the liver was blocked intermittently for 5 or 10 minutes and then restored for 5 minutes in both groups. The total blocking time was set at 30 minutes in both groups. Therefore, 6 and 3 cycles of blocking and release were performed in the 5-minute and 10-minute blocking groups, respectively. The effects of these procedures on intraoperative hemodynamics and their postoperative effects on the living body, such as on hepatic enzyme and CRP levels, were examined and compared.

Increases in HR, SVR, and PVR were seen immediately after the initiation of blocking in both groups, but they tended to decrease and return to the pre-blocking level after blood flow was restored. Typically, during hepatectomy in humans blocking of the portal triad leads to increases in HR, SVR, PVR, and MAP [6, 8, 9]. Conversely, in the latter studies CO only decreased slightly,

whereas in our study CO fell by >60%. The increases in the former parameters might have been due to stimulation with catecholamines, especially epinephrine or norepinephrine, which are secreted by the adrenal gland after the occurrence of intestinal ischemia [14], or blood redistribution from the spleen, intestine, and aorta to prevent hypotension [28]. More importantly, the pneumoperitoneum employed in laparoscopic surgery can cause an increase in intra-abdominal pressure (IAP), which has effects on venous return and SVR. For example, increases in IAP can result in greater SVR [23]. However, in the current study the blocking period was short in both groups; therefore, we suggest that the increase in SVR might have counteracted the reduction in CO. Moreover, marked reductions in MAP and CO could stimulate cardiopulmonary baroreceptors to increase SVR. Occlusion of the portal vein causes congestion in the portal system (the splenic vein, inferior mesenteric vein, and superior mesenteric vein) and portal vein hypertension, leading to peripheral vasodilation [18]. Furthermore, reductions in venous return and CO might lead to reductions in MAP. In addition, the hemodynamic changes observed in the present study were similar to those induced by the Pringle maneuver in pigs [15]. In contrast, in human studies [6, 8, 9] MAP increased by 6-18% from the pre-blocking level after the portal triad was blocked, whereas in our study MAP decreased to 25% lower than the pre-blocking level. However, all of the hemodynamic parameters returned to their pre-blocking levels after the release of the vascular clamps. In dogs, acute ligation of the portal triad can lead to rapid cardiovascular collapse subsequent to shock caused by a sudden reduction in MAP, which can result in death, whereas humans have adequate collateral circulatory systems. Thus, in humans after the blockade of the portal vein, portal venous volume increases, portosystemic collateral blood vessels develop, and blood is returned to the systemic circulation via pelvic collateral blood vessels [4].

Hence, blocking of the portal vein and the hepatic artery during the Pringle maneuver can cause hepatic ischemia-reperfusion (I/R) injuries, which can lead to hepatic necrosis and/or

increases in hepatic enzyme levels. Therefore, the levels of hepatic enzymes, such as AST, ALT, and ALP, were assessed as hepatic function indicators in the current study. The levels of AST and ALT increased on day 1 after the operation and then tended to decrease to their preoperative levels on day 2. However, the increases in hepatic enzyme levels seen in this study were temporary and relatively mild, which agrees with the results of a previous study [11]. However, since slightly higher ALP levels tended to be seen in the 10-minute blocking group, it remains possible that the effects of hepatic blood flow blockade on the liver might increase as the duration of the blocking period gets longer. The effects of the repeated blocking and restoration of blood flow on the liver were compared with those of continuous blood flow blocking in a previous study [2]. The standard deviation values for the AST and ALT level data were large in both groups. This was due to the fact that large increases were seen in one animal, and small changes sometimes resulted in large variations in the data. In addition, the time at which the level of each enzyme peaked varied slightly among the animals. Moreover, only 4 animals were used in each group (to reduce the number of animals used), which increased the standard deviation. Therefore, shortening the duration of blood flow blocking periods as much as possible during surgery might reduce postoperative complications. On the other hand, the current study only investigated the effects of the Pringle maneuver on normal livers; i.e., the livers were not resected.

The total white blood cell count and CRP level are widely used as indicators of systemic inflammation and to predict postoperative complications [7]. Our results demonstrated that the total white blood cell count and CRP level peaked on days 1 and 2 after the operation in the 10- and 5-minute blocking groups, respectively, but they tended to decrease to their preoperative levels on postoperative day 3 in both groups. However, the total white blood cell counts of both groups were within the normal range on postoperative day 3. Thus, these changes represented normal responses to postoperative inflammatory conditions.

Our results showed that HR, SVR, and PVR increased in both groups after the portal triad was blocked and tended to return to their pre-blocking levels after blood flow was restored. On the other hand, reductions in MAP, MPAP, CO, and the CI were observed after blood flow was blocked, and these parameters tended to return to their pre-blocking levels after the restoration of blood flow. There were almost no differences in these parameters between the two groups. The levels of AST, ALT, and ALP tended to increase on day 1 after the operation, but then tended to decrease on day 2 after the operation. Increases in the total white blood cell and CRP level were seen after the operation, but these represented normal responses to inflammation or surgical trauma. As for clinical symptoms, vomiting was occurred on day 1 after the operation in one dog from each group.

This study involved healthy dogs, and it is unclear whether similar findings would have been acquired in animals with liver disorders. Moreover, it remains unclear whether hepatic blood flow can be safely blocked in animals with heart disease because the effects of such a procedure on the circulatory system would be marked. Furthermore, it will be necessary to investigate whether lobectomy combined with the concomitant laparoscopic Pringle maneuver causes less blood loss than laparotomic hepatectomy, as has been found in humans.

No differences in the hemodynamic effects of the laparoscopic Pringle maneuver were noted between the dogs subjected to blocking times of 5 and 10 minutes. In addition, it was suggested that blocking blood flow into the liver for a total of 30 minutes using either method does not have a marked influence on postoperative liver function.

Summary

In this study, we aimed to confirm the effects of combining laparoscopic surgery with various surgical techniques on intraoperative blood loss and the risk of postoperative complications in dogs. Laparoscopic surgery is used to reduce tissue invasion around surgical sites, and it can be combined with various other surgical techniques to improve its effectiveness.

Gastric dilatation-volvulus (GDV) syndrome is a life-threatening condition caused by sudden stomach dilation or torsion, which often occurs in large dog breeds, such as Dobermans, German Shepherds, Great Danes, and Saint Bernards. Gastropexy is a surgical method that can be used to prevent the occurrence or recurrence of GDV. Several open gastropexy techniques for preventing GDV, including incisional, circumcostal, and belt-loop GDV methods, and gastrocolopexy, have been used for more than 3 decades. Laparoscopic gastropexy has advanced and gained acceptance in recent years, and it results in a lower morbidity rate and fewer postoperative complications than open gastropexy. To further reduce intraoperative blood loss during such procedures and the duration of such procedures, a total laparoscopic gastropexy procedure involving the use of knotless sutures was developed. Furthermore, prophylactic gastropexy can be performed in combination with elective neutering.

Several neutering procedures were examined in this study, laparotomic ovariohysterectomy, laparoscopic-assisted ovariohysterectomy, and laparoscopic ovariectomy. Usually, conventional ovariectomy (OVE) takes less time and causes smaller surgical wounds than ovariohysterectomy (OVH). In turn, laparoscopic surgery involves less tissue invasion and takes less time than OVE. Although the frequency of long-term complications, such as urinary incontinence and obesity, do not differ significantly between animals that undergo OVH and OVE, other complications, such as vaginal bleeding, ureteral ligation, and intraabdominal bleeding are more common after OVH than after OVE. However, both neutering procedures can reduce the incidence of cystic endometrial hyperplasia, endometritis, pyometra, and mammary gland tumors,

if they are carried out correctly. Therefore, combining laparoscopic ovariectomy with prophylactic gastropexy using knotless sutures results in a smaller surgical wound, shorter operation time, less postoperative pain, and faster recovery compared with the other surgical techniques.

Splenectomy can be used to treat splenic conditions, such as splenic tumors or hematological disorders. In patients with hematological disorders, extraction of the spleen can cause massive hemorrhaging. Stimulating the sympathetic nervous system using endogenous catecholamines, such as epinephrine, can cause splenic contraction, which results in a reduction in splenic size and increased venous hematocrit levels. Therefore, reducing the size of the spleen during splenectomy might reduce intraoperative blood loss and ameliorate any post-splenectomy anemia. However, epinephrine has strong effects on the hemodynamic system.

In this study, it was confirmed that directly administering 10 μg/kg of epinephrine onto the splenic surface had minimal adverse hemodynamic effects, while it reduced the volume of the spleen by an average of 46.1%. To further reduce tissue invasion, laparoscopic surgery can be employed. A comparison between conventional surgery and laparoscopic splenectomy demonstrated that laparoscopic splenectomy involved less tissue invasion. However, combining this technique with the direct administration of 10 μg/kg epinephrine during the splenectomy procedure resulted in reductions in intraoperative blood loss; surgical stress; tissue invasion; and the risk of postoperative complications, such as anemia, especially in patients with hematological disorders.

Several surgical techniques have been used to reduce bleeding during hepatectomy, including the induction of hepatic ischemia (the Pringle maneuver). However, blocking the portal vein and hepatic artery can cause sudden death in dogs, and hepatic ischemia can damage hepatocytes. Therefore, this study aimed to investigate the effects of performing the Pringle maneuver during laparoscopic surgery on intraoperative hemodynamics and postoperative hepatic

function. The duration of the ischemic period was set at 5 or 10 minutes, and each ischemic period was followed by a 5-minute period of reperfusion. We found that performing the Pringle maneuver during laparoscopic surgery is feasible. There were no significant differences in hemodynamic parameters or postoperative hepatic function between the animals subjected to 5-minute and 10-minute periods of ischemia (the total duration of ischemia was 30 minutes in both groups). However, this study involved healthy dogs, and so it remains unclear whether the Pringle maneuver can be conducted in patients with hepatic disorders or heart disease. It is necessary to investigate this issue further in patients with hepatic disorders. Furthermore, intraoperative bleeding should be compared between hepatectomy procedures in which the Pringle maneuver is combined with laparotomy and hepatectomy procedures in which the Pringle maneuver is combined with laparoscopic surgery.

The most common complication of surgery is hemorrhaging, and massive hemorrhaging due to tissue invasion can cause serious postoperative complications. In laparoscopic surgery, iatrogenic hemorrhaging due to visceral organ laceration is the most common type of hemorrhaging. It can occur during the initial insertion of a Veress needle (which is used to insufflate CO_2 into the abdominal cavity), which most commonly results in splenic laceration. In some cases, conversion to open surgery is necessary to correct the cause of the bleeding. The risk factors for conversion to open surgery include obesity and a surgeon with insufficient laparoscopic experience. Although hemorrhaging is a primary complication of laparoscopic surgery, combining laparoscopic surgery with the use of topical hemostatic agents or a blood vessel-sealing device can reduce bleeding from surgical sites. In this study, none of the animals required conversion to open surgery.

In the current study, we concluded that epinephrine can be used to reduce intraoperative blood loss and prevent postoperative complications, such as anemia, in cases involving splenectomy, as it causes the spleen to contract and expel its contents into the systemic circulation,

resulting in an increase in venous hematocrit levels. In the experiment investigating the effects of performing the Pringle maneuver during laparoscopic surgery, we confirmed that this procedure can be conducted during hepatectomy. However, comparisons of intraoperative bleeding between laparoscopic and open hepatectomy should be carried out in future. Furthermore, the laparoscopic technique examined in this study was effective; i.e., it minimized tissue invasion, caused less postoperative pain, and resulted in weaker inflammatory reactions and lower surgical stress than open surgery. This study involved experimental animals, but in future we expect that this technique will be improved and become applicable to clinical cases.

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Table 1-1. University of Melbourne pain scale (UMPS).

Category	Descriptor	Score
Physiological data		
a)	Physiological data within reference range	0
b)	Dilated pupil	2
c) C*hoose only one :*	Percentage increase in heart rate relative to baseline	
	>20%	1
	>50%	2
	>100%	3
d) C*hoose only one :*	Percentage increase in respiratory rate relative to baseline	
	>20%	1
	>50%	2
	>100%	3
e) Rectal temperature exceeds reference range		1
d) Salivation		2
Response to palpation		
a) C*hoose only one :*	No change from preprocedural behavior	0
	Guards/reacts[a] when touched	2
	Guards/reacts[a] before touched	3
Activity		
a) C*hoose only one :*	At rest – sleeping or semiconscious	0
	At rest – awake	1
	Eating	0
	Restless (pacing/getting up and down)	2
	Rolling, thrashing	3
Posture		
a)	Guarding or protecting affected area (includes fetal position)	2
b) *Choose only one :*	Lateral recumbency	0
	Sternal recumbency	1
	Sitting/standing, head up	1
	Standing, head hanging down	2
	Moving	0
	Abnormal posture (prayer position, hunched)	2
Vocalization[b]		
a) *Choose only one :*	Not vocalizing	0
	Vocalizing when touched	2
	Intermittent vocalization	2
	Continuous vocalization	3
Mental status		
a) *Choose only one :*	Submissive	0
	Overtly friendly	1
	Wary	2
	Aggressive	3
	Melbourne score	**27**

[a] Turning head toward affected area, biting, licking, scratching at the wound; snapping at handler; or tense muscles and a protective (guarding) posture.
[b] Does not include alert barking.

Table 1-2. Operation time (min)

	Time (min)
OOVHG	75.2 ± 6.7
LAOVHG	55.2 ± 8.2*
LOVEG	43.2 ± 10.1*

*Statistically significant difference, * = $P < 0.05$ vs OOVHG.
OOVHG = Laparotomy ovariohysterectomy with circumcostral gastropexy.
LAOVHG = Laparoscopic ovariohysterectomy with incision gastropexy.
LOVEG = Laparoscopic ovariectomy with gastropexy.

Table 1-3. Incision length (cm)

	Incision length (cm)
OOVHG	19.7 ± 1.8
LAOVHG	5.8 ± 1.7*
LOVEG	2.9 ± 0.4*

*Statistically significant difference, * = $P < 0.05$ vs OOVHG.
OOVHG = Laparotomy ovariohysterectomy with circumcostral gastropexy.
LAOVHG = Laparoscopic ovariohysterectomy with incision gastropexy.
LOVEG = Laparoscopic ovariectomy with gastropexy.

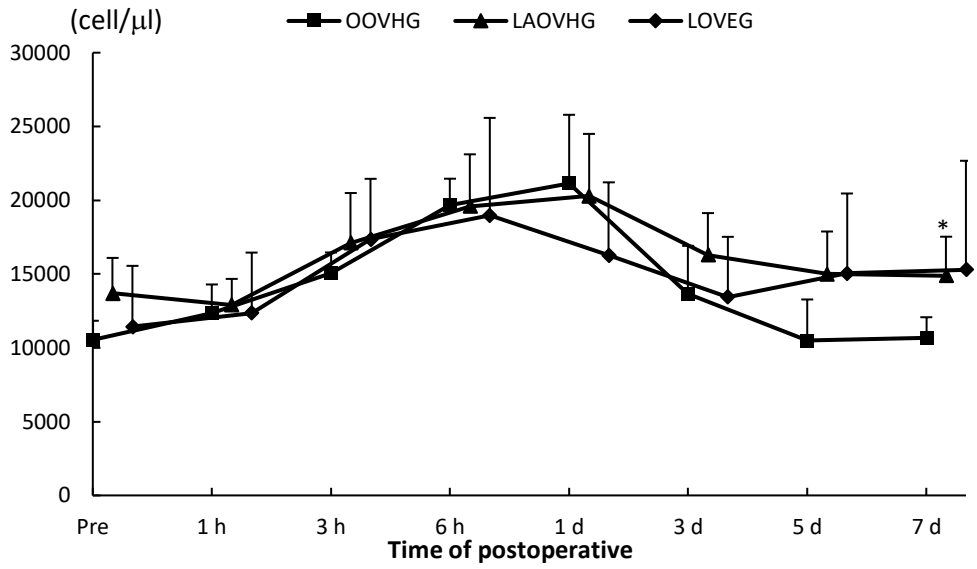

Figure 1-1. Changes in white blood cell count (WBC) in preoperative and postoperative period in laparotomy ovariohysterectomy with circumcostral gastropexy group (OOVHG), laparoscopic-assisted ovariohysterectomy with incision gastropexy group (LAOVHG) and total laparoscopic ovariectomy with gastropexy group (LOVEG). The results are presented as means ± SD. * = P<0.05 vs OOVHG.

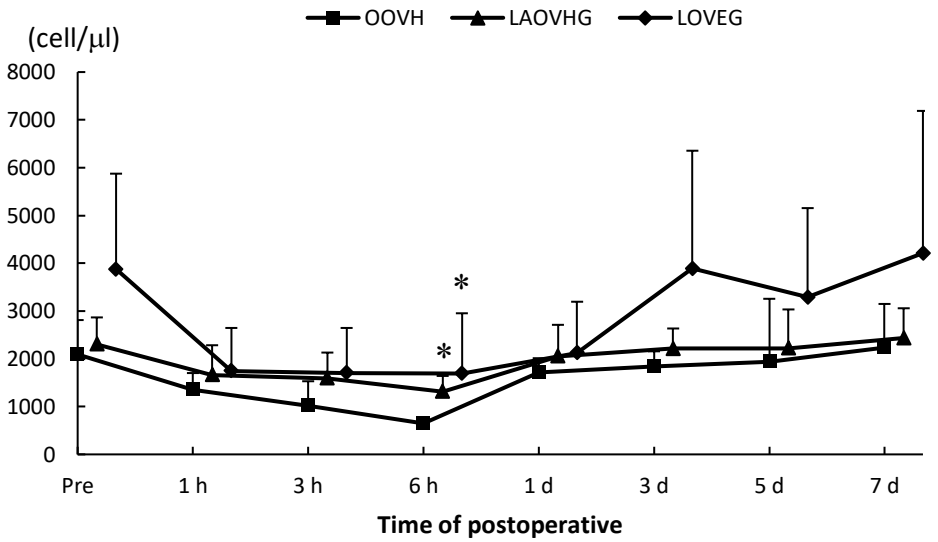

Figure 1-2. Changes in lymphocyte count in preoperative and postoperative period in laparotomy ovariohysterectomy with circumcostral gastropexy group (OOVHG), laparoscopic-assisted ovariohysterectomy with incision gastropexy group (LAOVHG) and total laparoscopic ovariectomy with gastropexy group (LOVEG). The results are presented as means±SD. * = P<0.05 vs OOVH

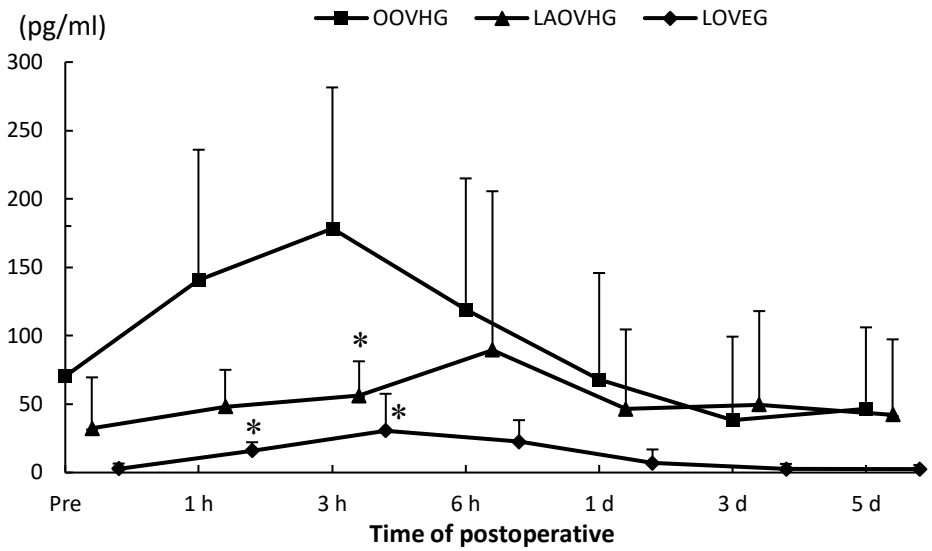

Figure 1-3. Changes in interleukin-6 (IL-6) level at preoperative and postoperative period in laparotomy ovariohysterectomy with circumcostral gastropexy group (OOVHG), laparoscopic-assisted ovariohysterectomy with incision gastropexy group (LAOVHG) and total laparoscopic ovariectomy with gastropexy group (LOVEG). The results are presented as means ± SD. * = P<0.05 vs OOVHG.

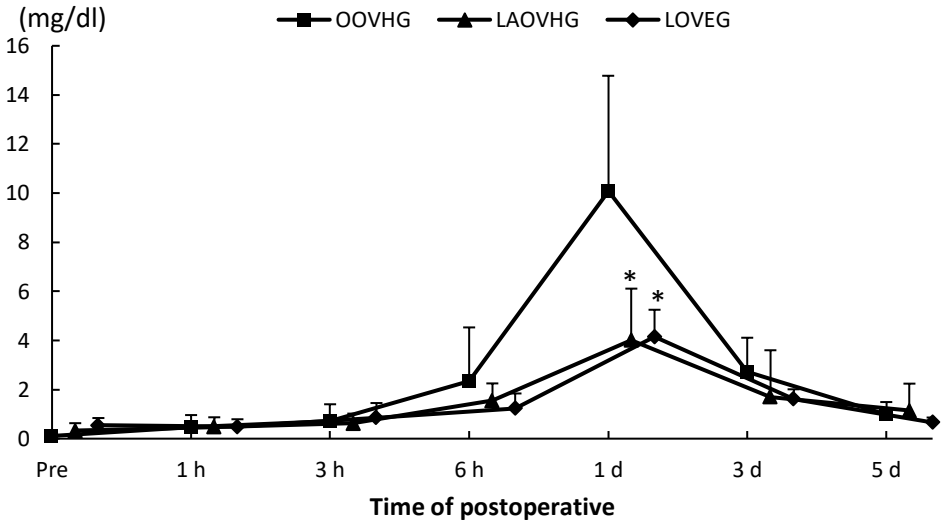

Figure 1-4. Changes in C-reactive protein (CRP) level at preoperative and postoperative period in laparotomy ovariohysterectomy with circumcostral gastropexy group (OOVHG), laparoscopic-assisted ovariohysterectomy with incision gastropexy group (LAOVHG) and total laparoscopic ovariectomy with gastropexy group (LOVEG). The results are presented as means ± SD. * = P<0.05 vs OOVHG.

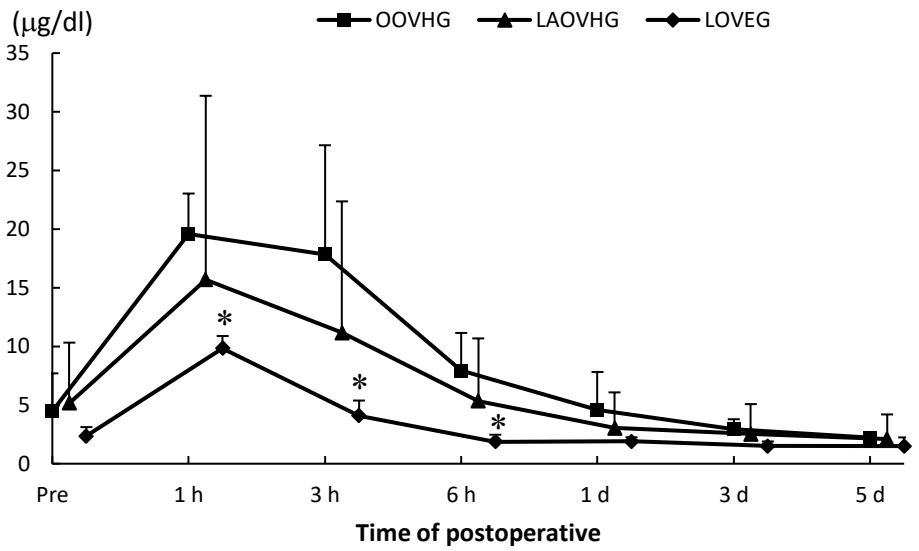

Figure 1-5. Changes in cortisol concentration level at preoperative and postoperative period in laparotomy ovariohysterectomy with gastropexy group (OOVHG), laparoscopic-assisted ovariohysterectomy with gastropexy group (LAOVHG) and laparoscopic ovariectomy with gastropexy group (LOVEG). The results are presented as means ± SD. * = P<0.05 vs OOVHG.

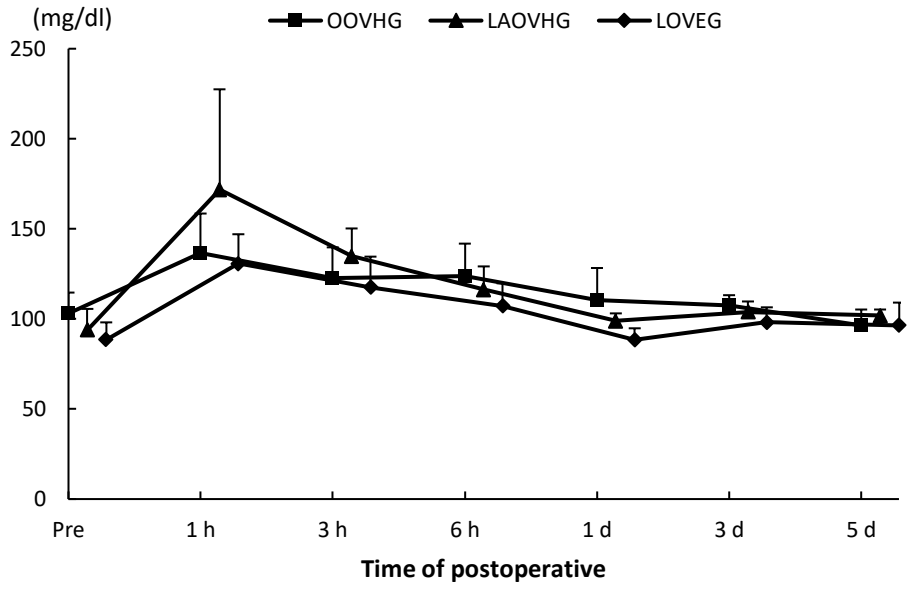

Figure 1-6. Changes in glucose concentration level at preoperative and postoperative period in laparotomy ovariohysterectomy with gastropexy group (OOVHG), laparoscopic-assisted ovariohysterectomy with gastropexy group (LAOVHG) and laparoscopic ovariectomy with gastropexy group (LOVEG). The results are presented as means ± SD.

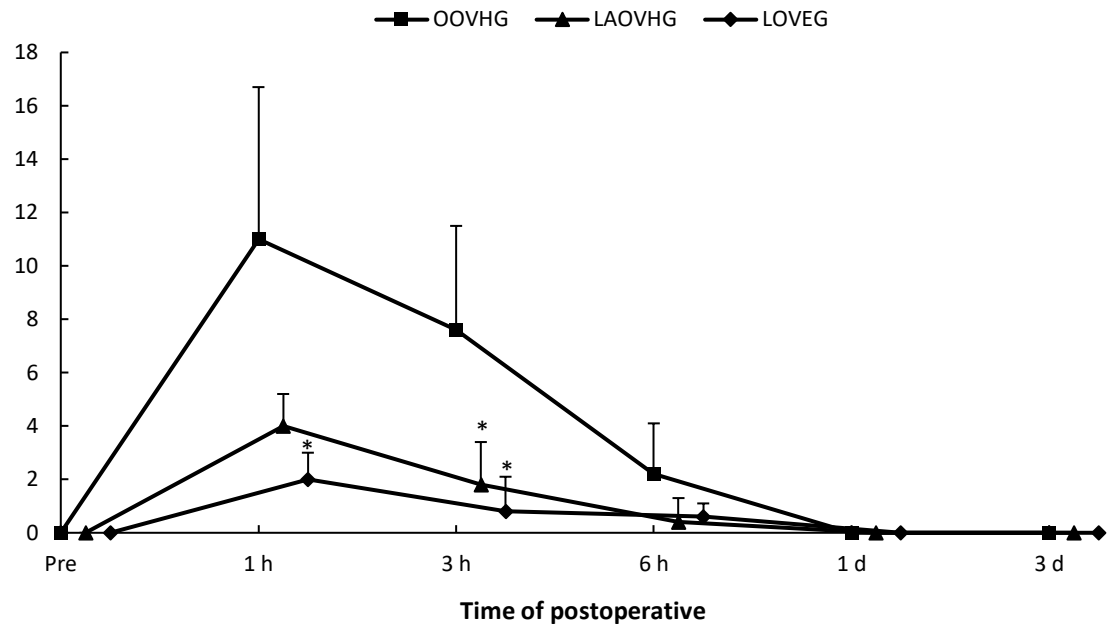

Figure 1-7. Pain scores using with UMPS score to measure postoperative pain and compared between laparotomy ovariohysterectomy with circumcostral gastropexy group (OOVHG), laparoscopic-assisted ovariohysterectomy with incision gastropexy group (LAOVHG) and total laparoscopic ovariectomy with gastropexy group (LOVEG). The results are presented as mean ± SD. * = $P<0.05$ vs OOVHG.

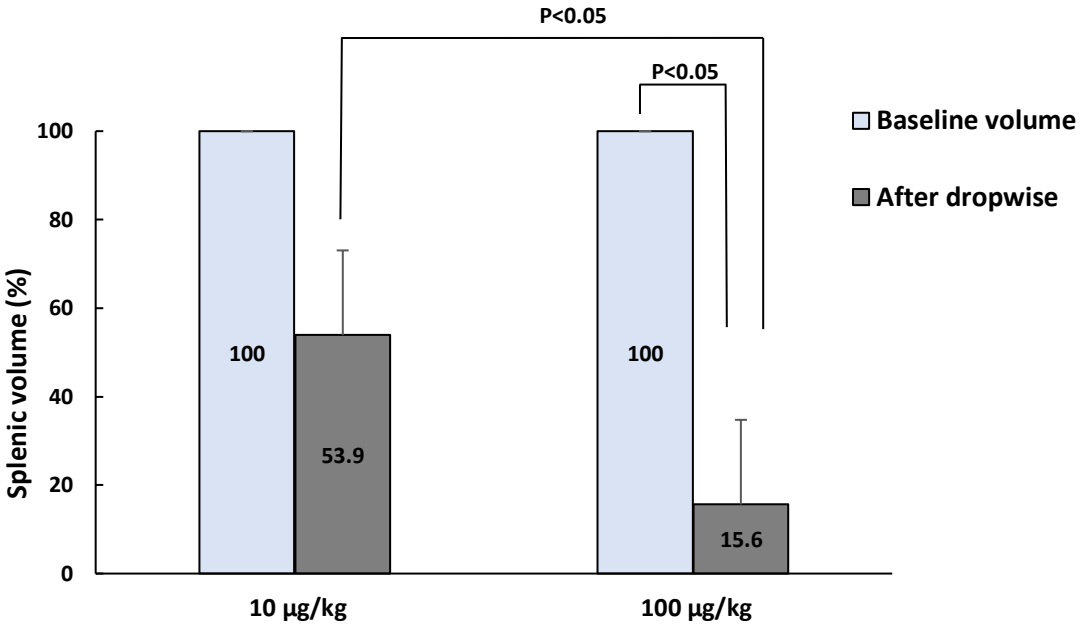

Figure 2-1. The splenic volume changes after dropwise of epinephrine 10 µg/kg and 100 µg/kg. The results are express as percentage and comparing with baseline volume.

Table. 2-1 Changes in heart rate (HR), mean arterial pressure (MAP), mean pulmonary artery pressure (MPAP), renal blood flow (RBF), cardiac index (CI), stroke volume (SV), systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) as express in percentage change from baseline levels. T_1= baseline level before dropped saline, T_2= 5 minutes after dropped saline, T_3= 10 minutes after dropped saline, T_4= 15 minutes after dropped saline, T_5= baseline level before dropped epinephrine, T_6= 5 minutes after dropped epinephrine, T_7= 10 minutes after dropped epinephrine, T_8= 15 minutes after dropped epinephrine, and (-) = Not measurement. Values are presented as means ± SE. a) $P<0.05$ vs T_1. b) $P<0.05$ vs T_5.

		T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
HR	EP10	100	102.3 ± 4.3	100.7 ± 4.1	100.3 ± 2.9	100	104.6 ± 3.6	101.6 ± 2.2	101.3 ± 2.5
	EP100	100	98.7 ± 2.1	98.4 ± 2.7	99.1 ± 3.6	100	110.4 ± 4.3	112.6 ± 5.1	113.9 ± 5.1
MAP	EP10	100	97.0 ± 3.7	93.3 ± 4.5	92.0 ± 4.6	100	128.7 ± 4.4 b)	117.4 ± 2.0 b)	115.5 ± 1.3 b)
	EP100	100	96.0 ± 5.3	93.7 ± 6.6	92.7 ± 7.4	100	105.9 ± 10.3	106.9 ± 7.7	108.6 ± 7.6
MPAP	EP10	100	101.5 ± 1.5	100.3 ± 3.6	100.0 ± 4.2	100	139.7 ± 4.6 b)	126.9 ± 3.1 b)	117.1 ± 4.6 b)
	EP100	100	92.9 ± 4.8	86.6 ± 6.2	91.1 ± 4.6	100	160.2 ± 23.8 b)	157.9 ± 11.7 b)	162.8 ± 12.5 b)
RBF	EP10	100	102.1 ± 2.9	106.1 ± 1.8 a)	107.7 ± 2.4 a)	100	104.9 ± 6.3	103.9 ± 7.5	104.7 ± 8.1
	EP100	100	102.1 ± 1.7	103.8 ± 5.8	103.6 ± 6.3	100	75.2 ± 24.6	86.0 ± 27.2	85.6 ± 26.9
CI	EP10	100	-	-	109.8 ± 4.8	100	-	-	106.9 ± 8.4
	EP100	100	-	-	112.3 ± 5.1 a)	100	-	-	157.8 ± 10.5 b)
SV	EP10	100	-	-	109.5 ± 4.0	100	-	-	105.1 ± 6.6
	EP100	100	-	-	111.5 ± 2.3 a)	100	-	-	138.3 ± 7.0 b)
SVR	EP10	100	-	-	84.5 ± 5.7 a)	100	-	-	111.1 ± 10.1
	EP100	100	-	-	89.1 ± 7.4	100	-	-	69.8 ± 5.7
PVR	EP10	100	-	-	91.9 ± 6.1	100	-	-	111.5 ± 6.4
	EP100	100	-	-	81.6 ± 5.1	100	-	-	106.1 ± 13.2

Table 3-1. Comparison in splenic weight, splenic volume, operation time and incision length between laparotomy group and laparoscopic group.

	Splenic weight (g)	Splenic volume (cm^3)	Operation time (min)	Incision length (cm)
Laparotomic group	199.0 ± 66.7	202.2 ± 44.4	42.0 ± 3.8	9.6 ± 0.4
Laparoscopic group	162.0 ± 17.5	138.2 ± 26.5*	53.8 ± 18.3	5.7 ± 0.8**

* = P<0.05, ** = P<0.01 vs Laparotomic group

Table 3-2. Changes in hematocrit (Hct), platelet (PLT) and white blood cell count (WBC) at pre and postoperative period in laparotomy group (OS) and laparoscopic group (LS). Pre-ope = Pre-operative. Values are presented as means ± SD. a) = P<0.05 vs laparotomy group. b) = P<0.05 vs Pre-ope.

		Pre-ope	Postoperative						
			1 h	3h	6 h	1 d	3 d	5 d	7 d
Hct (%)	OS	52.0 ± 4.6	43.4 ± 8.9[b)]	42.6 ± 7.5[b)]	43.0 ± 9.7[b)]	40.2 ± 9.4[b)]	45.0 ± 8.1[b)]	40.8 ± 8.6[b)]	43.4 ± 8.3[b)]
	LS	50.4 ± 5.1	46.0 ± 2.6	45.6 ± 3.1	45.8 ± 1.9	46.4 ± 4.3	47.8 ± 2.8	47.0 ± 3.9	46.4 ± 3.9
PLT (10^4/μl)	OS	36.2 ± 11.3	31.1 ± 8.5	21.8 ± 13.3[b)]	30.7 ± 8.6	30.6 ± 10.7	42.2 ± 10.0	46.2 ± 20.3	75.7 ± 12.9[b)]
	LS	34.3 ± 9.2	20.0 ± 8.0	22.5 ± 11.2	32.9 ± 9.7	32.2 ± 11.8	52.3 ± 16.4[b)]	64.1 ± 10.2[b)]	73.4 ± 15.5[b)]
WBC (10^2/μl)	OS	97.2 ± 21.2	98.0 ± 16.6	131.0 ± 7.1[b)]	160.4 ± 21.1[b)]	199.2 ± 49.7[b)]	120.6 ± 51.7	127.0 ± 54.1	137.3 ± 27.9
	LS	111.8 ± 29.3	111.8 ± 37.5	173.4 ± 41.2[b)]	205.2 ± 34.4[a,b)]	221.2 ± 30.6[b)]	179.2 ± 57.8[b)]	163.4 ± 39.1[b)]	156.6 ± 48.1[b)]

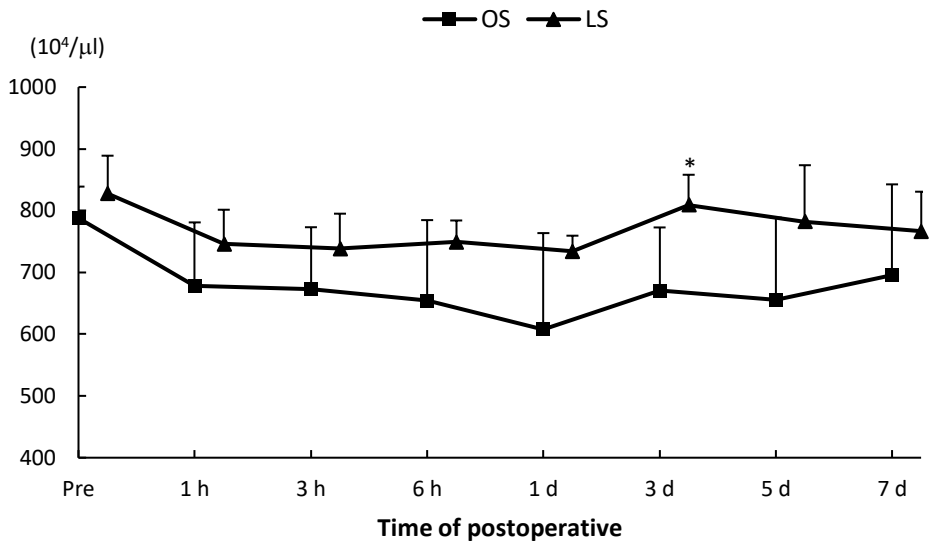

Figure 3-1. Changes in red blood cell concentration following the splenectomy in the laparotomy group (OS) and the laparoscopic surgery (LS) group. Values are presented as means±SD. * = P<0.05 vs OS.

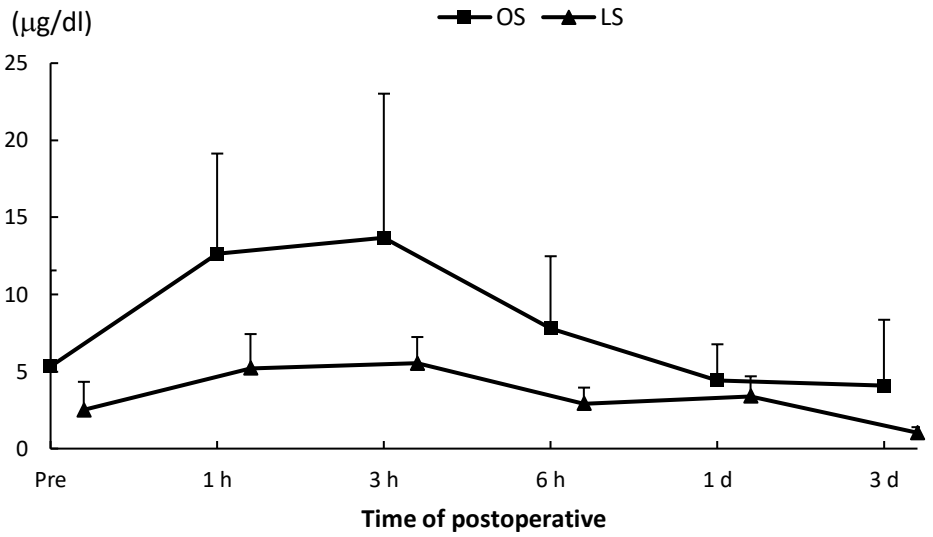

Figure 3-2. Changes in serum cortisol concentration following the splenectomy in the laparotomy group (OS) and the laparoscopic surgery (LS) group. Values are presented as means±SD.

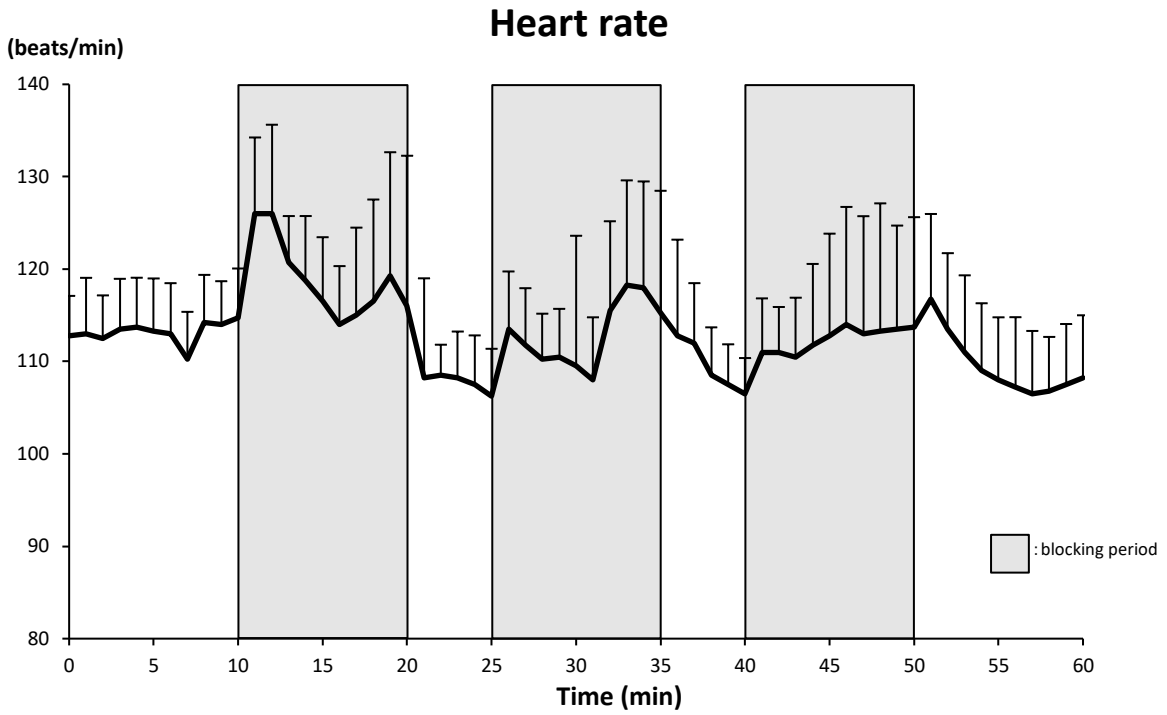

Figure 4-1. Changes in heart rate in the 10-minute blocking group during blocking the portal vein and hepatic artery.

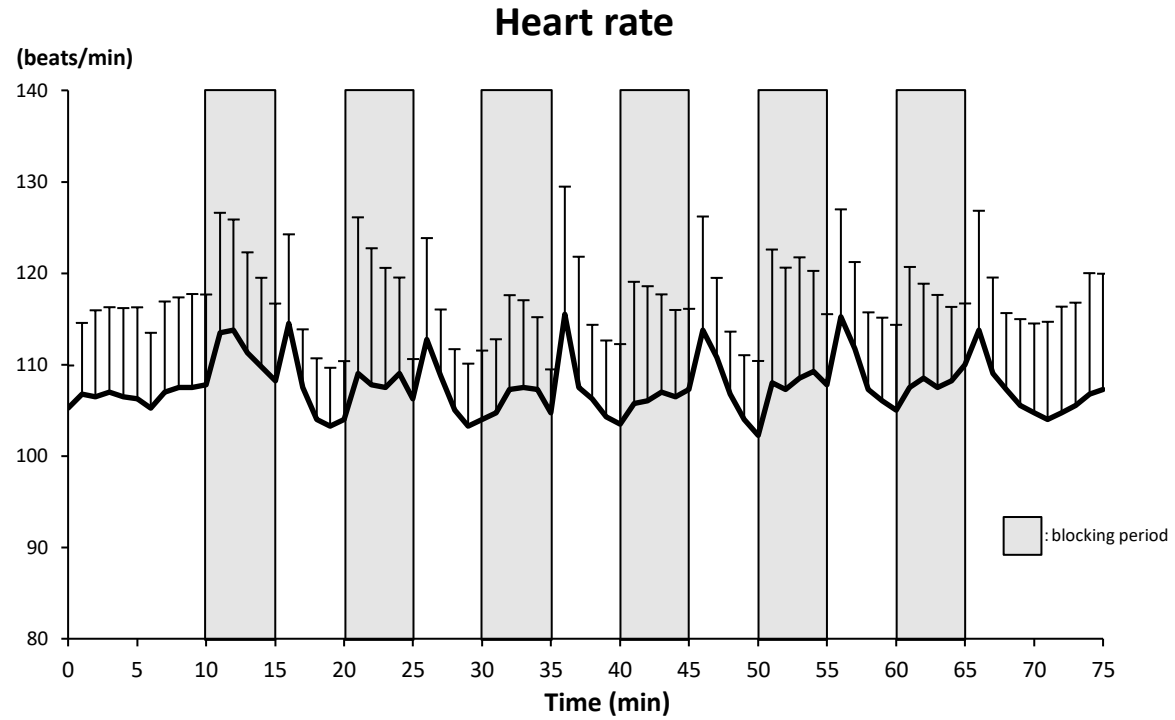

Figure 4-2. Changes in heart rate in the 5-minute blocking group during blocking the portal vein and hepatic artery.

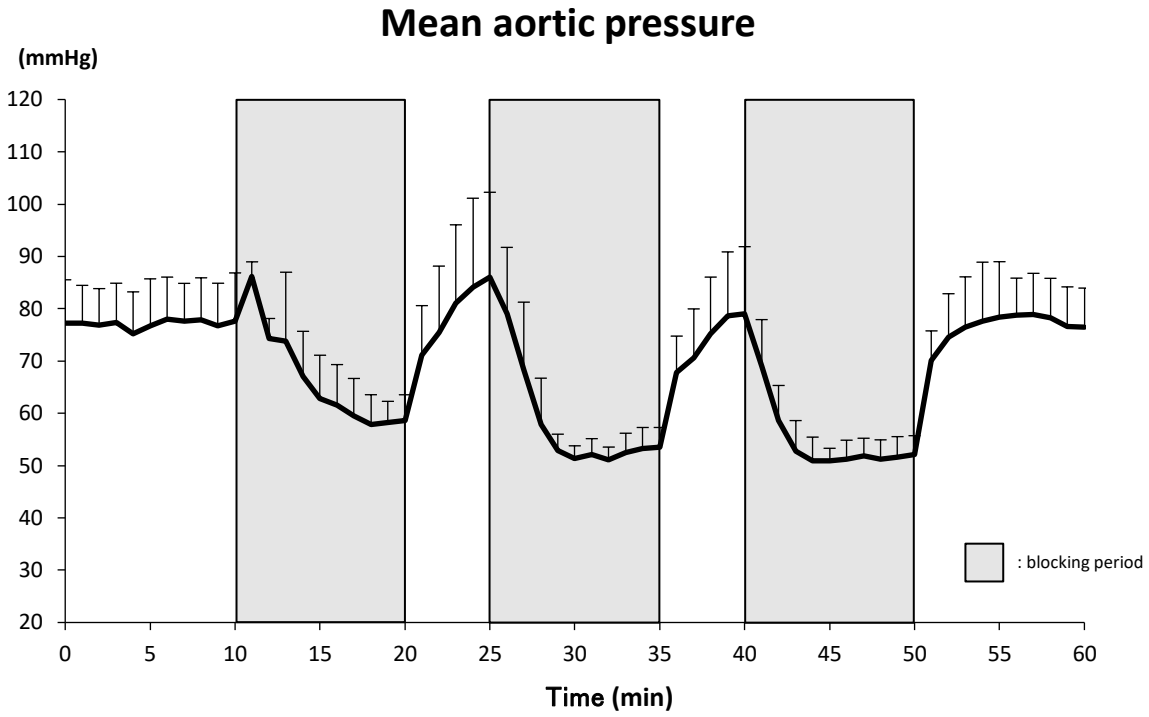

Figure 4-3. Changes in mean aortic pressure in the 10-minute blocking group during blocking the portal vein and hepatic artery.

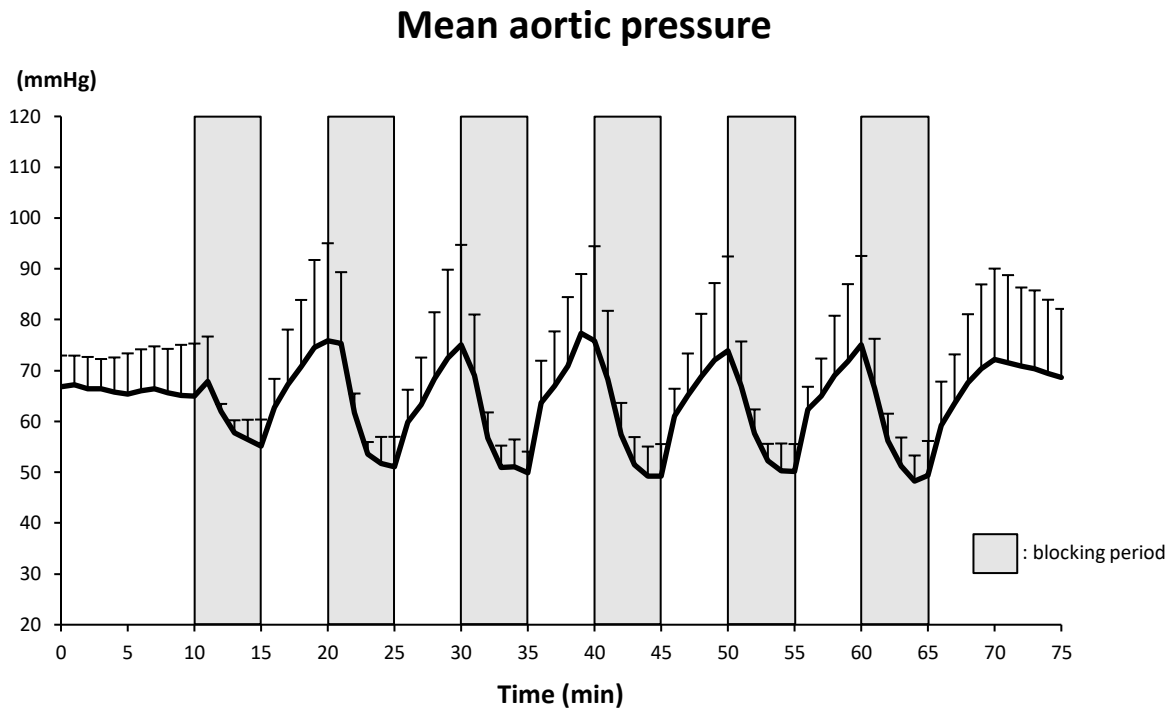

Figure 4-4. Changes in mean aortic pressure in the 5-minute blocking group during blocking the portal vein and hepatic artery.

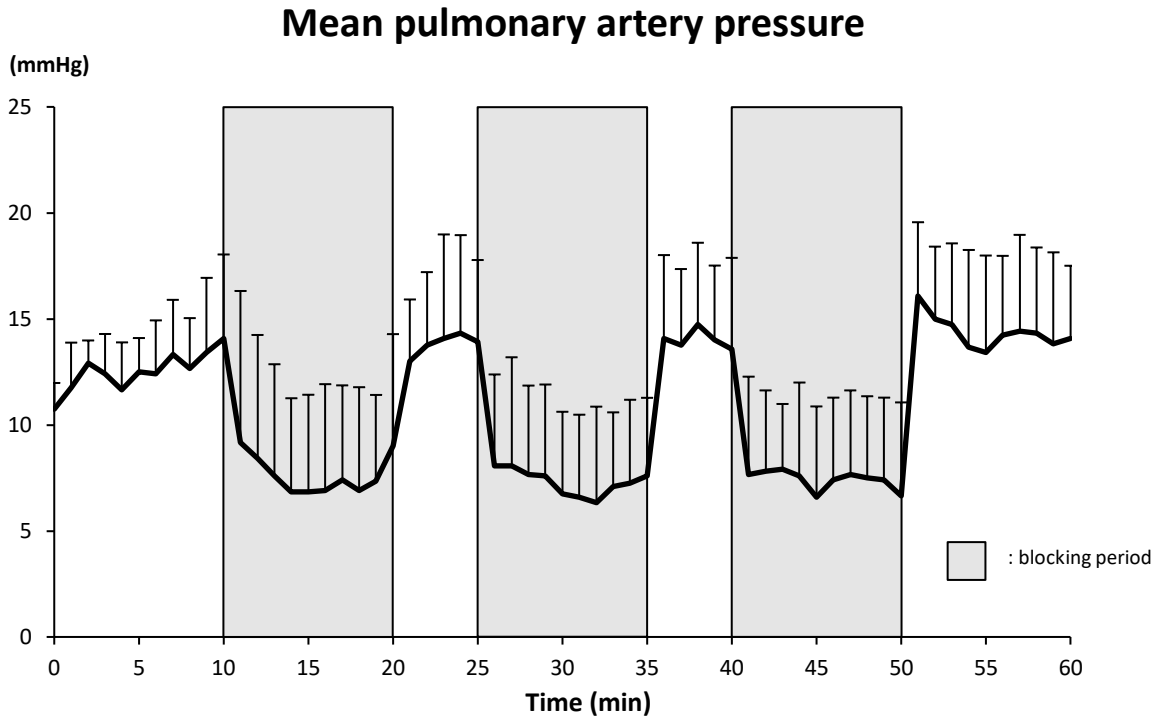

Figure 4-5. Changes in mean pulmonary arterial pressure in the 10-minute blocking group during blocking the portal vein and hepatic artery.

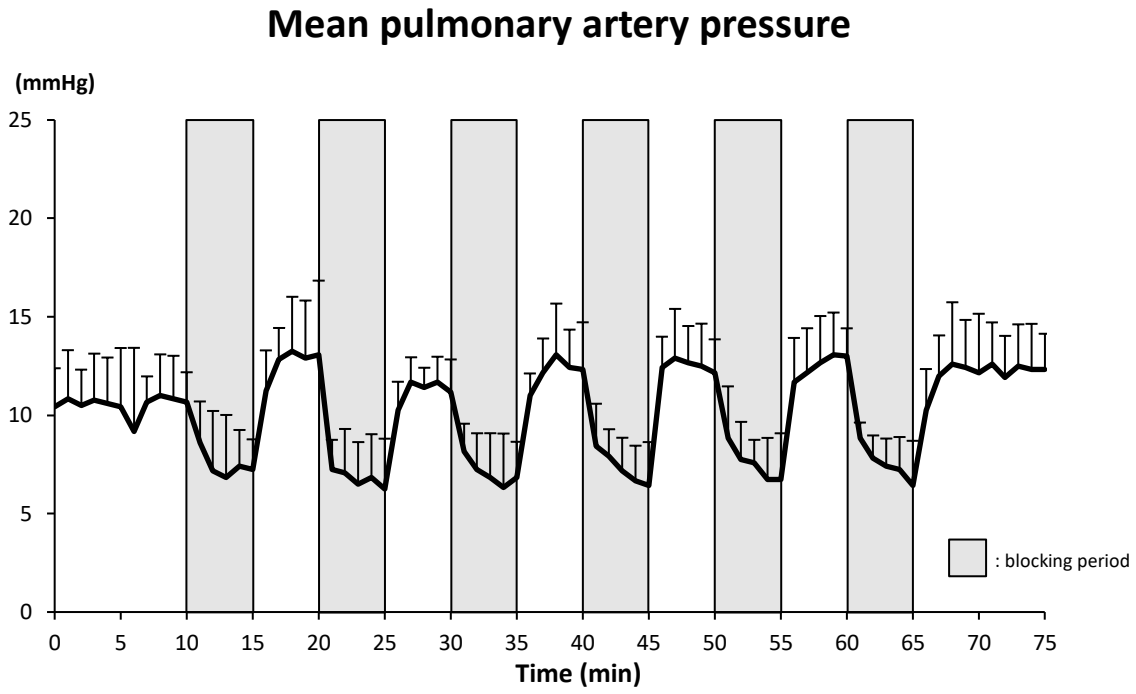

Figure 4-6. Changes in mean pulmonary artery pressure in the 5-minute blocking group during blocking the portal vein and hepatic artery.

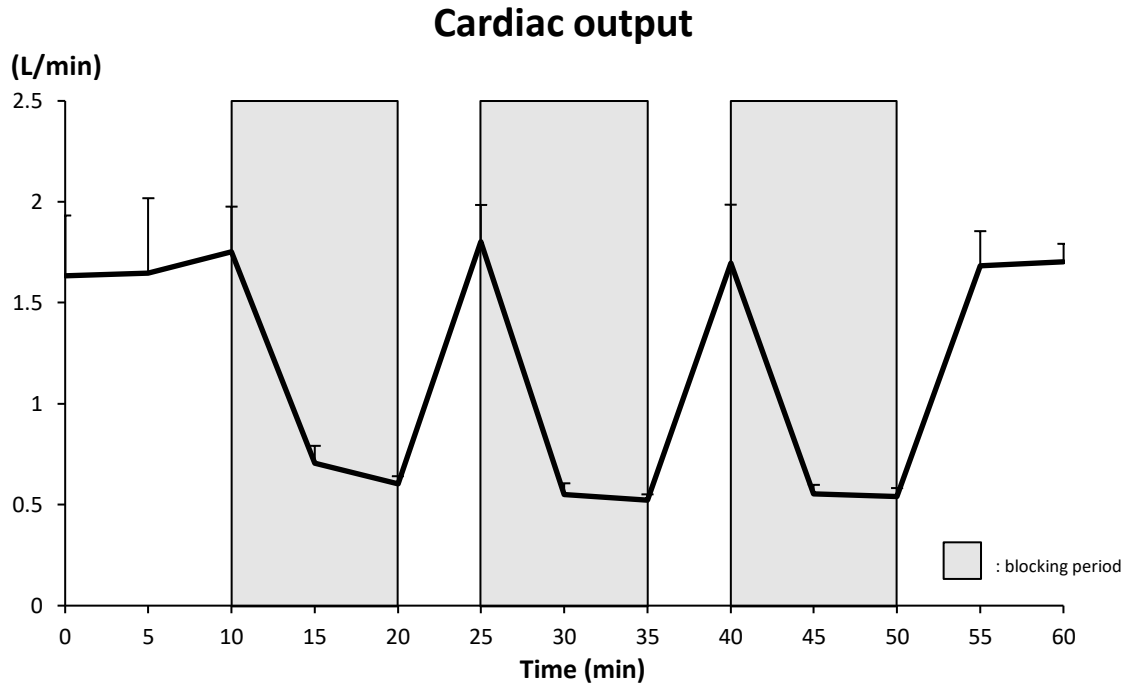

Figure 4-7. Changes in cardiac output in the 10-minute blocking group during blocking the portal vein and hepatic artery.

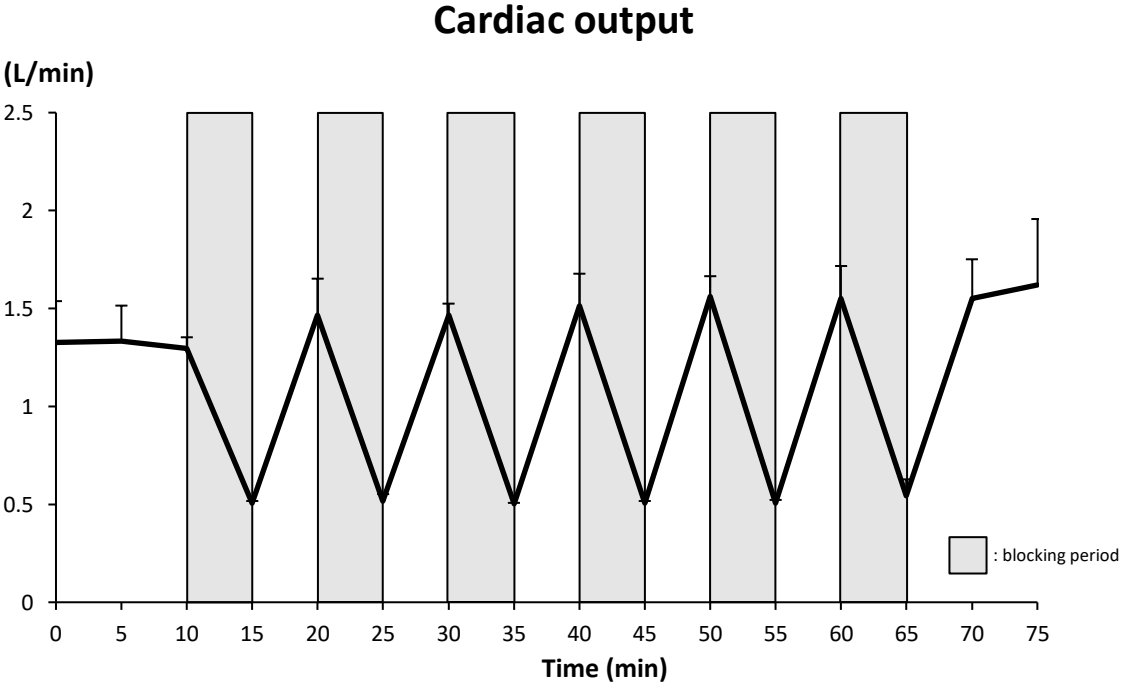

Figure 4-8. Changes in cardiac output in the 5-minute blocking group during blocking the portal vein and hepatic artery.

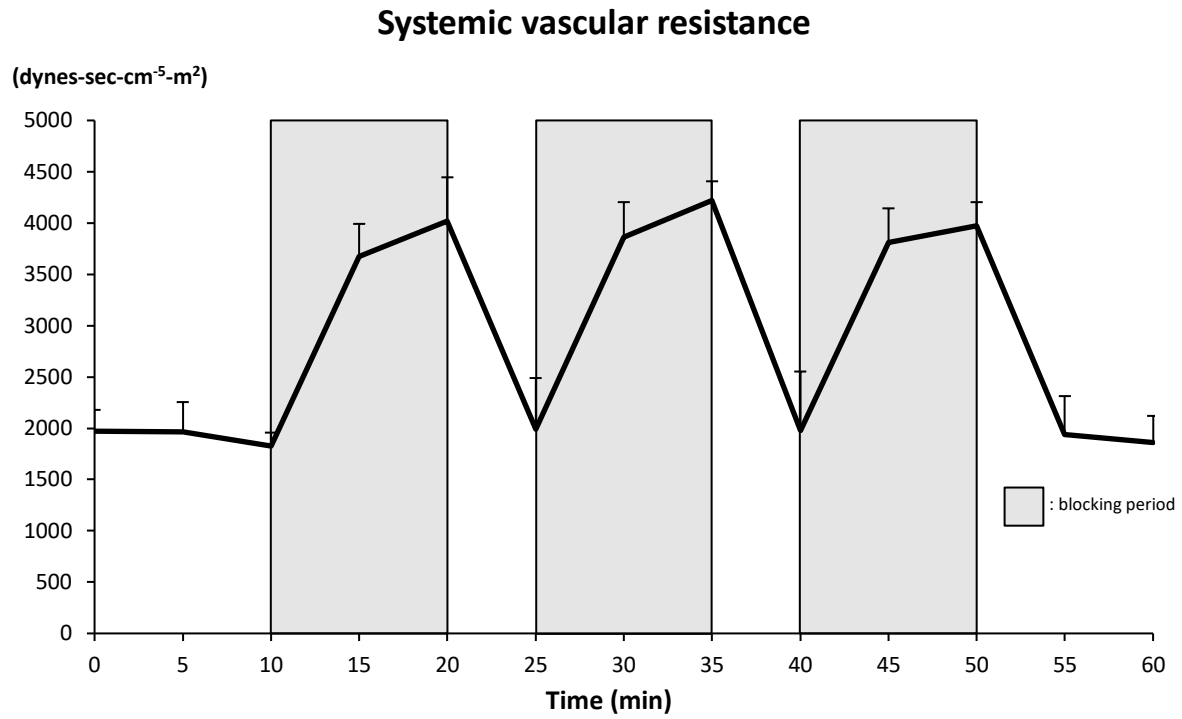

Figure 4-9. Changes in systemic vascular resistance in the 10-minute blocking group during blocking the portal vein and hepatic artery.

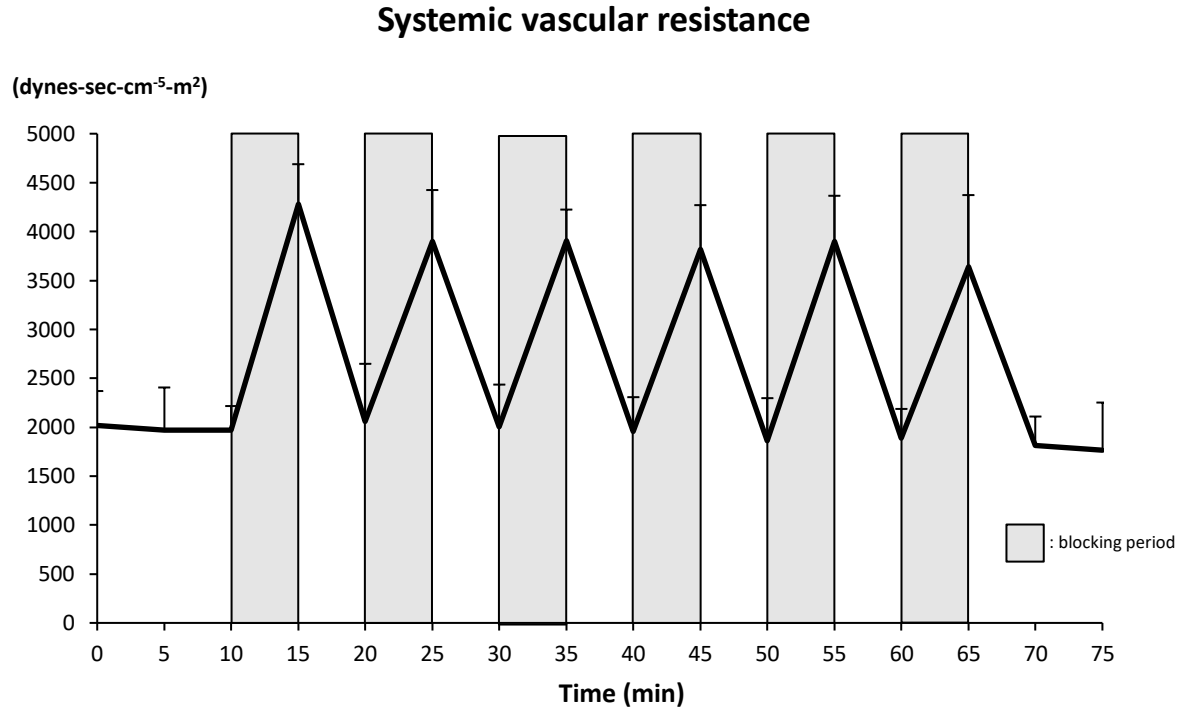

Figure 4-10. Changes in systemic vascular resistance in the 5-minute blocking group during blocking the portal vein and hepatic artery.

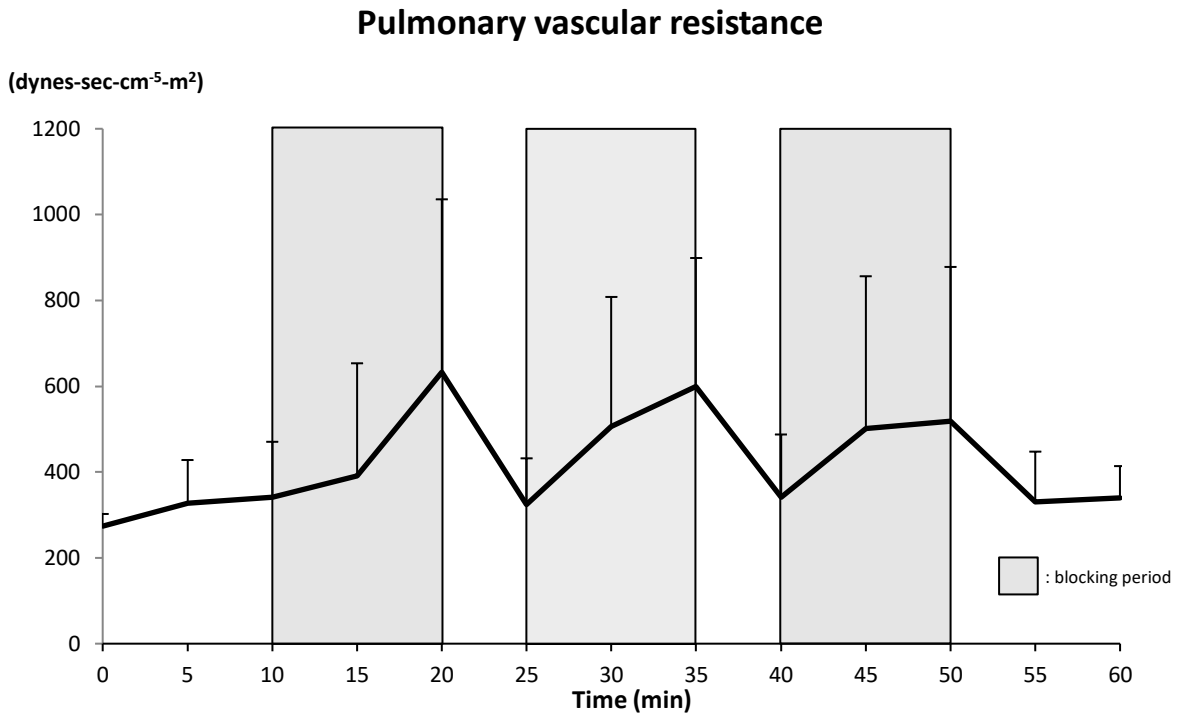

Figure 4-11. Changes in pulmonary vascular resistance in the 10-minute blocking group during blocking the portal vein and hepatic artery.

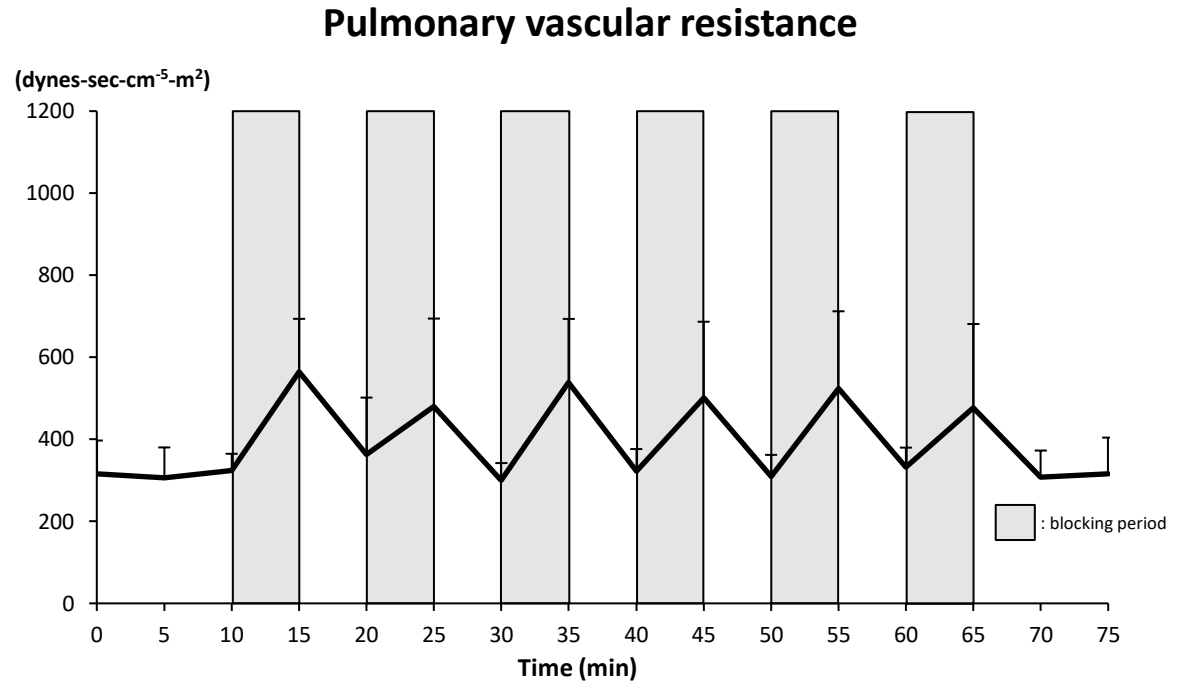

Figure 4-12. Changes in pulmonary vascular resistance in the 5-minute blocking group during blocking the portal vein and hepatic artery.

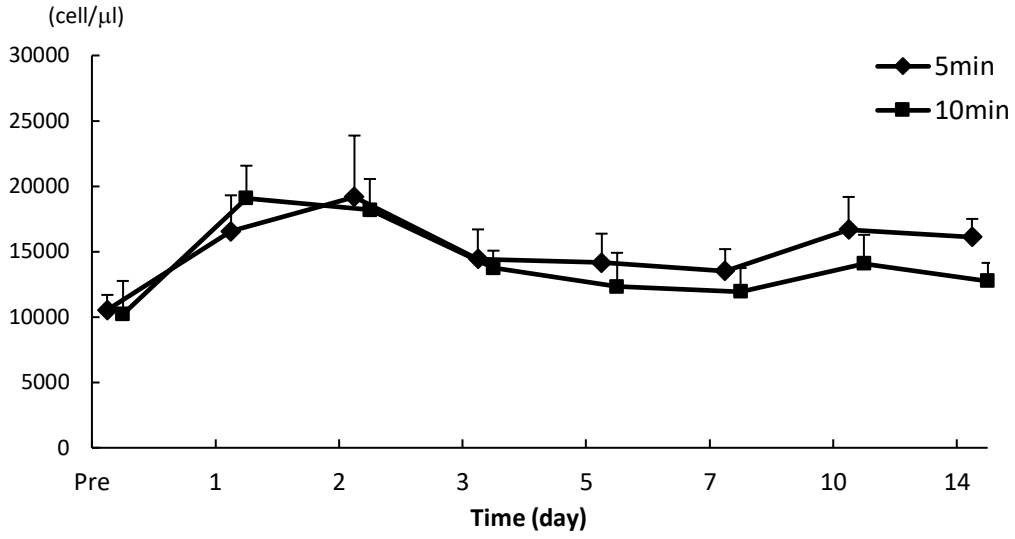

Figure 4-13. Changes in total white blood cell count in the 5-minute and 10- minute blocking group after operation.

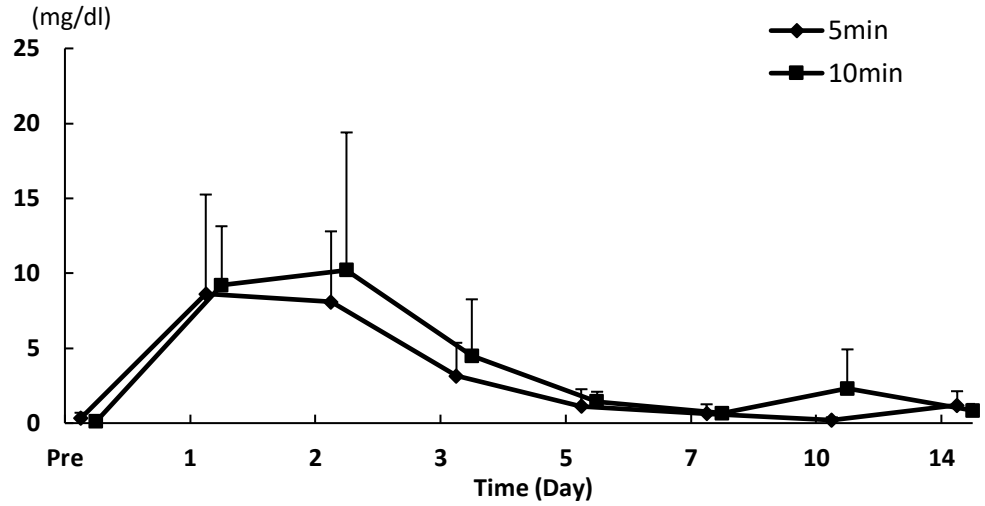

Figure 4-14. Changes in C-reactive protein (CRP) in the 5-minute and 10- minute blocking group after operation

Table 4-1. Changes in alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) after operation. Pre-ope = level at preoperative time, D_1 = 1st day postoperative, D_2 = 2nd day postoperative, D_3 = 3rd day postoperative, D_5 = 5th day postoperative, D_7 = 7th day postoperative, D_{10} = 10th day postoperative and D_{14} = 14th day postoperative. The results are presented as means ± SD. a): $p<0.05$ vs Pre-ope, b): $p<0.05$ vs D_1, c): $p<0.05$ vs D_2, d): $p<0.05$ vs D_3.

Time (Day)		Pre-ope	D_1	D_2	D_3	D_4	D_7	D_{10}	D_{14}
ALT	5-minute	23.3 ± 6.7	164.0 ± 199.9 [a)]	148.8 ± 183.6	104.8 ± 111.7	69.3 ± 62.1	53.8 ± 42.2	45.3 ± 39.6	49.0 ± 44.7
(IU/L)	10-minute	34.5 ± 3.7	156.0 ± 101.1	130.3 ± 70.7	116.0 ± 56.4	119.0 ± 80.0	90.3 ± 48.8	53.8 ± 41.1	38.8 ± 11.6
ALP	5-minute	173.8 ± 41.5	491.3 ± 87.7 [a)]	433.5 ± 30.6 [a)]	362.0 ± 51.1 [a)]	272.3 ± 18.0 [b)]	228.8 ± 27.4 [b,c)]	201.5 ± 16.9 [b,c)]	210.0 ± 36.3 [b,c)]
(IU/L)	10-minute	149.3 ± 22.7	480.8 ± 79.0 [a)]	485.8 ± 152.4	423.5 ± 151.3 [a)]	380.0 ± 144.2 [a)]	296.0 ± 109.3 [b,c)]	201.8 ± 129.1 [b,c)]	164.8 ± 57.3 [b,c,d)]
AST	5-minute	23.3 ± 2.1	136.0 ± 178.3	95.3 ± 87.4	41.3 ± 6.9	27.3 ± 5.4	28.5 ± 5.8	25.8 ± 9.6	28.8 ± 6.2
(IU/L)	10-minute	31.3 ± 4.2[a)]	128.8 ± 63.4	53.8 ± 19.2	58.5 ± 25.4	61.3 ± 42.9	40.8 ± 9.6	23.3 ±5.3	37.8 ± 9.5