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Original Article

Positive Intrapleural Pressure with Carbon Dioxide May Limit Intraoperative Pulmonary Arterial Bleeding: Verification by Animal Model

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Purpose: Intraoperative complications, especially unexpected bleeding, are of great concern in the safety of thoracoscopic surgery. We investigated the hemostatic efficacy and safety of positive intrapleural pressure (PIP) with carbon dioxide insufflation by assessing the amount of blood loss in a pulmonary arterial hemorrhage model.
Methods: An ex vivo experimental model of saline flow into a swine vessel was created in a container simulating a chest cavity. From the results, in vivo experiments (swine model) were conducted to compare the pulmonary arterial bleeding volume while applying PIP.
Results: In the ex vivo experiment, regardless of the incision type, the outflow volumes did not significantly differ at flow pressures of 20, 30, and 40 mmHg. At each flow pressure, the outflow volumes at 10, 15, and 20 mmHg of positive pressure in the container were significantly smaller than those of the control ($p = 0.027$, $p = 0.002$, and $p = 0.005$, respectively). Similarly, the in vivo experiments showed that bleeding decreased as intrapleural pressure increased (slope = −0.22, $F = 55.13$, $p < 0.0001$).
Conclusion: It may be possible to temporarily suppress pulmonary arterial bleeding by increasing the intrapleural pressure to 10 to 20 mmHg using carbon dioxide insufflation. This method may be an adjunctive hemostatic maneuver for intraoperative bleeding.

Keywords: thoracoscopic surgery, intraoperative bleeding, carbon dioxide insufflation, intrapleural pressure

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Introduction

In recent years, the use of positive intrapleural pressure (PIP) with carbon dioxide (CO_2) has increased in video-assisted and robot-assisted thoracoscopic surgery for the purpose of obtaining a good view and large working field in the thoracic cavity. We speculated that using PIP during bleeding events as described above can temporarily control the bleeding speed and ultimately reduce the occurrence of fatal complications. We previously published in vivo data from swine that underwent CO_2 insufflation[1)] and showed that CO_2 insufflation can be safely introduced intraoperatively. Additionally, PIP suppressed bleeding from the injured site of the

pulmonary vein with no fatal changes, such as air embolisms, accompanying the PIP in the systemic condition. We also confirmed in another clinical study that PIP of up to 15 mmHg can be safely applied intraoperatively in humans.[2] However, the efficacy of PIP in treating pulmonary artery (PA) injury must be evaluated because PA laceration is a common cause of hemorrhage and is difficult to treat, as described in previous reports.[3,4] Because the PA is also a low-pressure circulatory system, PIP is expected to have a certain bleeding control effect. Therefore, we conducted this study to investigate the suppressive effect of PIP on bleeding from the PA.

In the field of general thoracic surgery, minimally invasive techniques such as video-assisted and robot-assisted thoracoscopic surgery are gaining popularity because of the increased detection rate of early stage lung tumors. These techniques allow surgeons to remove the lesions with decreased postoperative pain, a shorter air leak duration, shorter hospital stays, and lower overall complication rates with oncological results equivalent to those of traditional open thoracotomy.[5–7] However, the safety of thoracoscopic surgery has caused great concern because of reports of devastating intraoperative complications, especially bleeding.[3] A retrospective analysis of 8563 thoracoscopic lung surgeries listed in the United States national database in 2016 revealed that 9.2% of patients required blood transfusions for management of intraoperative bleeding.[8] Specifically, bleeding from the PA is also problematic because of secondary injury associated with surgical hemostasis. Several reports have described how to manage such severe intraoperative bleeding,[4,9–11] and the use of these techniques is very important. However, if the bleeding from the laceration site remains excessive, the probability of stopping it decreases, even with these techniques. Therefore, devices that will reduce the bleeding flow rate, even temporarily, are very useful.

Because proper use of laboratory animals is required to ensure animal welfare, we first collected detailed data from an ex vivo model using swine PAs immediately after slaughter. Subsequently, to validate these results, we applied the same procedure to in vivo models using a small number of experimental animals.

Materials and Methods

Ex vivo experiment

Figure 1A illustrates the ex vivo experiment. The right PA from the lung of a swine immediately after slaughter was harvested and cut to a length of approximately 5 cm. A polypropylene container resembling a chest cavity was prepared, with a hole on one side and attachment of a single-use retractor (Alnote-LAPSINGLE; Alfresa Pharma Corporation, Osaka, Japan). The two ends of the harvested PA were connected to the infusion route in a leak-proof manner and then led out of the container through a port. One side was connected to an infusion bag containing saline solution. On the other side, the infusion route was raised to the same height as the infusion bag. Saline solution was dripped from a height equivalent to the corresponding pressure. Measurements were taken when the water level in the opposite infusion route increased to the same level as that in the infusion bag, indicating that the designated pressure had been reached in the artery. First, different injury types, including 10-mm-long incisions and 5-Fr sheath placement, were prepared in the vessel. The amount of liquid outflow from the injury site was measured for 30 seconds under various conditions, including the inflow pressure and the pressure inside the container, using an AirSeal Intelligent Flow System (CONMED, Utica, NY, USA). No difference was found between the 10-mm incision and the 5-Fr sheath model as a PA injury model; therefore, the 5-Fr sheath model was used in the experiment with positive pressure in the container because of the reproducibility, measurement accuracy, and ease of the experiment.

In vivo experiments

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute for Laboratory Animal Research, published by the National Academies Press.[12]

The Azabu University Animal Experimentation Committee approved the in vivo experiments (approval number 200206-1), which were performed in accordance with institutional guidelines and with the National Institutes of Health guidelines regarding the principles of animal care. Two specific-pathogen-free, 50-day-old female swine weighing 32 to 35 kg each were fed a standard diet and allowed water ad libitum. Both swine underwent thoracoscopic surgery with target intrapleural pressures with CO_2 insufflation. Anesthesia was induced via intravenous injection of ketamine (10–15 mg/kg), xylazine (2 mg/kg), and propofol (2.5–3.5 mg/kg), and the animals were intubated with a 5.5-mm flexible silicone endotracheal tube (Univent; Fuji Systems Corporation, Tokyo, Japan) connected to a mechanical ventilator. The

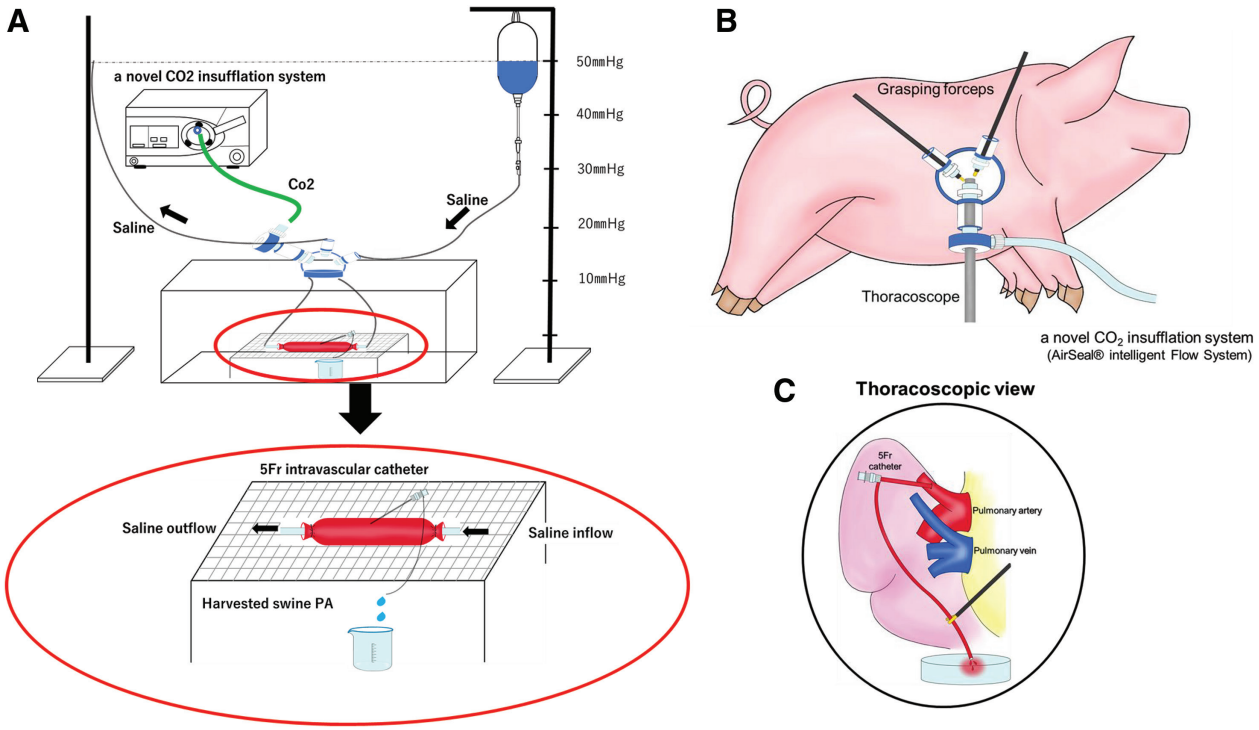

Fig. 1 Operative procedures. (**A**) Ex vivo experimental setting. The right PA from the lung of a swine was harvested. In a polypropylene container, the two ends of the harvested PA were connected to the infusion route in a leak-proof manner. One side was connected to an infusion bag containing saline solution, and on the other side, the infusion route was raised to the same height as that of the infusion bag. Saline solution was dripped from a height equivalent to the corresponding pressure. (**B**) In vivo experimental setting. The swine were placed in the left decubitus position. A single-use retractor (Alnote-LAPSINGLE) was placed through the incision in the fourth intercostal space at the anterior axillary line. A 10-mm-diameter 30-degree rigid scope was introduced through this incision. One of the four ports was connected to an AirSeal for CO_2 insufflation, and artificial pneumothorax was maintained at a designated pressure. (**C**) Intrathoracic view of the in vivo experiments. A 5-Fr intravascular catheter was inserted in the right pulmonary trunk. The intrapleural pressure was varied, and the amount of bleeding was measured. PA: pulmonary artery

animals inhaled 2% isoflurane/100% oxygen for the entire experiment. We cannulated the left femoral artery and vein with a 20-gauge needle intravascular catheter using a cutdown technique and monitored the central venous pressure (CVP). Biological parameters, including blood pressure from the left femoral artery and left main PA, were monitored and recorded during the operation. The swine were placed in the left decubitus position (**Fig. 1B**). The single-use retractor (Alnote-LAPSINGLE) was then placed through the incision in the fourth intercostal space at the anterior axillary line. A 10-mm-diameter, 30-degree rigid scope was introduced through this incision. One of the four ports was connected to an AirSeal for CO_2 insufflation, and artificial pneumothorax was maintained at a designated pressure. All surgical procedures were performed thoracoscopically. A 5-Fr intravascular catheter was inserted into the right pulmonary trunk (**Fig. 1C**). The intrapleural pressures varied between 0, 5, 10, 15, and 20 mmHg, and the amount of bleeding was measured for 10 seconds after applying pressure. The blood pressure of the left femoral artery, CVP, and heart rate were monitored throughout the experiment. The PA pressure before bleeding at each intrathoracic pressure (IP) was measured. However, it was technically impossible to evaluate the PA pressure at the end of bleeding in order to check the amount of bleeding.

Statistical analysis

GraphPad Prism, version 9.3.1 (GraphPad Prism Software Inc., San Diego, CA, USA) was used for the statistical analyses and to construct the figures. The results were assessed using the Kruskal–Wallis test and simple linear regression analysis to compare multiple groups, and the unpaired two-tailed Student's t-test with Welch's correction (Welch's t-test) to compare two selected groups. A p value of <0.05 was considered statistically significant.

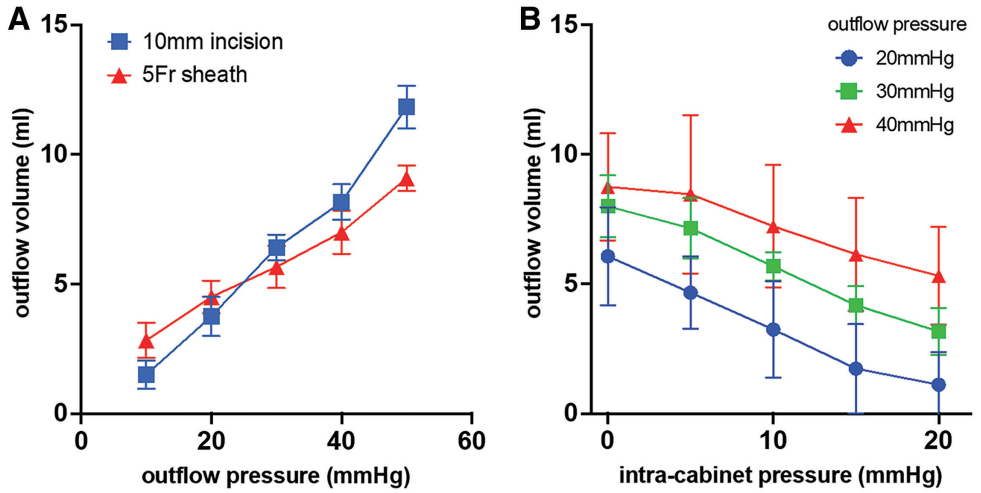

Fig. 2 Ex vivo experimental results. (**A**) Comparison of the outflow volumes for the two groups: the 10-mm group (10-mm-long incision) and the 5-Fr group (5-Fr sheath). For both wound types, the outflow volume increased with the inflow pressure. There was no significant difference between the two groups at outflow pressures of 20, 30, or 40 mmHg. (**B**) Experimental results of the 5-Fr model after varying the flow pressure to 20, 30, and 40 mmHg. For all fluid pressures, the outflow decreased as the container pressure increased.

Results

Ex vivo experiments with two different vascular injury models (5-Fr sheath insertion, 10-mm incision)

First, the ex vivo model was used to compare the outflow at different flow pressures for various wound types. Two groups were created: one with a 10-mm-long wound in the blood vessel (10-mm group) and the other with 5-Fr sheath placement (5-Fr group). **Figure 2A** shows the results of the outflow volume comparison for six samples per group. For both wound types, the outflow volume increased as the inflow pressure increased. Kruskal–Wallis nonparametric analysis showed significant differences between the two groups at outflow pressures of 10 and 50 mmHg but no significant differences between the two groups at outflow pressures of 20, 30, or 40 mmHg (**Table 1**). The results showed that 5-Fr sheath placement, which was easily applied during the experiment in the container, could be used as the representative wound type to measure the outflow volume at these outflow pressures.

Effect of pressure changes in the cabinet on vascular outflow in a series of 5-Fr sheath models

We then varied the pressure in the cabinet to 5, 10, 15, and 20 mmHg (PIP model) in the same experimental system with 5-Fr sheath placement and compared the outflow volumes. **Table 2** and **Fig. 2B** show the results of the experiments performed by changing the flow pressures to 20, 30, and 40 mmHg. For all flow pressures, the outflow decreased as the container pressure increased, and the F-test showed the validity of the model's approximation. The Welch's t-test showed that the outflow volumes at 10, 15, and 20 mmHg of container pressure were significantly smaller than those of the control ($p = 0.027$, $p = 0.002$, and $p = 0.005$, respectively).

Table 1 **Comparison of outflow volumes from different wound types**

Outflow pressure (mmHg)	10-mm incision (mL)	5-Fr sheath (mL)	p
50	11.8 ± 0.8	9.1 ± 0.5	0.008
40	8.2 ± 0.7	7.0 ± 0.8	0.102
30	6.4 ± 0.5	5.7 ± 0.8	0.245
20	3.8 ± 0.8	4.5 ± 0.6	0.344
10	1.5 ± 0.5	2.8 ± 0.7	0.044

Data are presented as mean ± standard deviation.

In vivo experiment with 5-Fr sheath model

To verify the results of the ex vivo experiment, we conducted in vivo experiments on two swine. **Figure 3A** shows the results of the bleeding volume measured by varying the intrapleural pressures between +5 mmHg and +20 mmHg. Although the results could not be fully validated because we used only two swine, simple linear regression analysis confirmed that the bleeding volume decreased in accordance with the increased intrapleural

Table 2 Comparison of outflow volumes under different IPs and linear regression analysis results

Outflow pressure (mmHg)	0 mmHg	5 mmHg	10 mmHg	15 mmHg	20 mmHg	Slope (95% CI)	F	p
20	6.1 ± 1.9	4.7 ± 1.4	3.3 ± 1.9	1.7 ± 1.7	1.1 ± 1.3	–0.26 (–0.34 to –0.17)	40.01	<0.0001
30	8.0 ± 1.2	7.2 ± 1.2	5.7 ± 0.5	4.2 ± 0.7	3.2 ± 0.9	–0.25 (–0.30 to –0.20)	116.5	<0.0001
40	8.8 ± 2.1	8.5 ± 3.1	7.2 ± 2.4	6.2 ± 2.2	5.3 ± 1.9	–0.18 (–0.30 to –0.07)	10.21	0.003

Data are presented as mean ± standard deviation unless otherwise noted. IP: intrathoracic pressure; CI: confidence interval

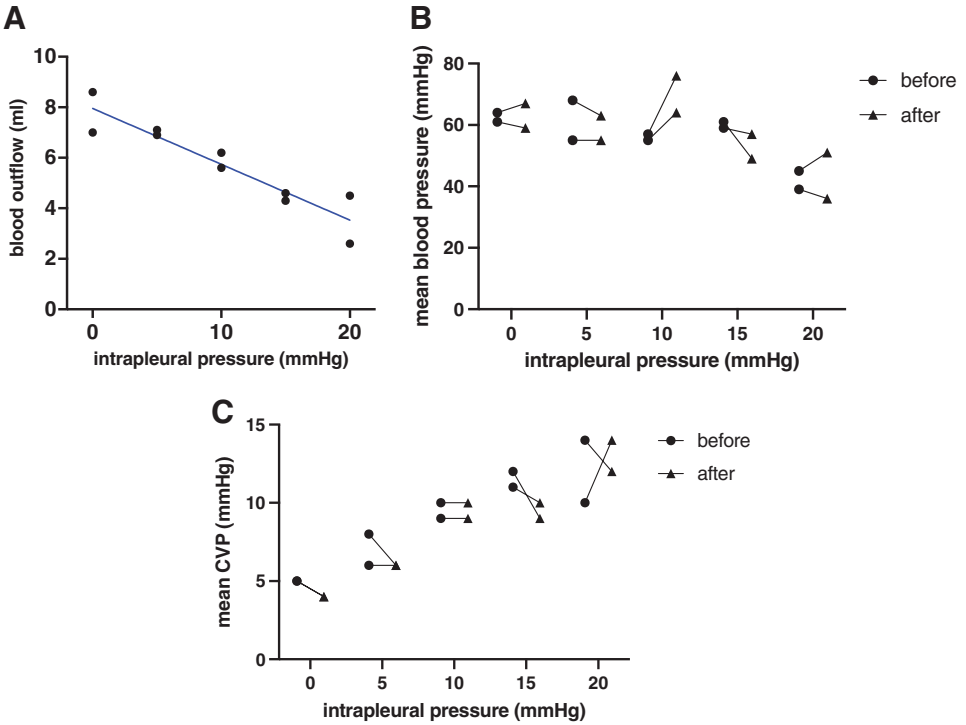

Fig. 3 In vivo experimental results of 5-Fr sheath model. (**A**) Bleeding volumes measured by varying the intrapleural pressure between 5 mmHg and 20 mmHg. The blue line shows a simple linear regression model. The bleeding volume decreased as the intrapleural pressure increased. (**B**) Arterial blood pressures measured before and after the experiment while varying the intrapleural pressure between 5 mmHg and 20 mmHg. (**C**) CVPs measured before and after the experiment while varying the intrapleural pressure between 5 and 20 mmHg. CVP: central venous pressure

pressure (slope = –0.22, F = 55.13, p <0.0001) (**Fig. 3A**). Additionally, recorded video confirmed that the momentum of bleeding was suppressed as the intrapleural pressure increased (**Supplementary Video 1**). No significant changes in arterial blood pressure, CVP, or heart rate were observed between the pre- and post-experiments. As the intrapleural pressure increased, the blood pressure decreased, and the CVP tended to increase in both the pre- and post experiments (**Figs. 3B** and **3C**, respectively). The mean PA pressure (average of two trials) before bleeding was 26 mmHg at an IP of 0 mmHg, 28 mmHg at an IP of 5 mmHg, 29 mmHg at an IP of 10 mmHg, 30 mmHg at an IP of 15 mmHg, and 24 mmHg at an IP of 20 mmHg.

Discussion

This study demonstrated that PIP with CO_2 insufflation can effectively control PA injury-induced bleeding during thoracoscopic surgery. We found that increasing

the intrapleural pressure to >15 mmHg should reduce the amount of bleeding by less than half compared with that of surgery without CO_2 insufflation. Moreover, the in vivo experimental results suggest that the mechanism of suppressing the bleeding volume involves both applying CO_2 pressure to the bleeding point and decreasing the cardiac output. To our knowledge, this is the first study to demonstrate that PIP may help control intraoperative bleeding from the PA.

To achieve reliable hemostasis, it is necessary to identify the bleeding point. However, the bleeding point is difficult to identify in the presence of excessive bleeding. In addition, the procedure used to identify the bleeding point may increase the amount of bleeding. Therefore, additional surgical procedures (e.g., securing the central side of the blood vessel) are performed to avoid increased bleeding when checking for bleeding points. However, performing these procedures under compression hemostasis often carries additional risks. Conversely, PIP can reduce the bleeding volume without additional surgical intervention and can help identify bleeding points. In other words, it may be regarded as a safer hemostasis procedure in the event of unexpected intraoperative PA bleeding.

The growing demand for minimally invasive surgery has led to widespread use of thoracoscopic surgery without definitive evidence. The expert opinions of many medical professionals on how to handle bleeding during surgery have been summarized.[13] This expert consensus clarified the following points. First, in the event of bleeding, surgeons must remain calm and use compression as the first step. Next, surgeons should convert the procedure to thoracotomy when the laceration site is large, bleeding is poorly controlled, no endoscopic view is available, or the laceration site has spread during repair. This expert consensus did not mention PIP with CO_2 insufflation. Our results suggest that additional application of PIP will temporarily suppress bleeding, making it easier to control bleeding by compression and thoracoscopically repair the laceration. Furthermore, we believe that even if surgeons convert the procedure to thoracotomy, reduced bleeding during the thoracotomy should reduce the surgical invasiveness and thus contribute to the safety of the thoracoscopic surgery. As shown in the results, the outflow and hemorrhage data at 0, 5, and 10 mmHg PIP in both the ex vivo and in vivo models decreased with increasing PIP. Therefore, even with PIP of less than 10 mmHg, a certain amount of bleeding control can be expected.

Here, the question regarding whether it is safe to introduce high intrapleural pressure during surgery may be raised. Two safety concerns may arise: the effect of PIP on cardiopulmonary function and the occurrence of an air embolism associated with CO_2 insufflation. The former has been difficult to evaluate in experimental animals. Scholars have concluded that PIP should not be applied because destabilization of cardiopulmonary function has been demonstrated in dogs,[14] swine,[15] and horses.[16] Conversely, studies have shown that gradually increasing the intrapleural pressure up to 14 mmHg has little effect on cardiopulmonary function in humans.[17,18] We used CO_2 insufflation in a previous study and found it to be stable up to 15 mmHg.[2] Thereafter, in our clinical practice, when a procedure requires applying PIP during general thoracic surgery, we set it at 10 to 15 mmHg using an AirSeal Intelligent Flow System, and patients' vital signs remain stable for several hours. This difference between humans and non-humans may be associated with the availability of isolated lung ventilation with a double-lumen tube because positive thoracic pressure has a minimal direct effect on ventilation. We used a maximum of 20 mmHg in the experimental setting for this study; however, the increase in PIP should be limited to 15 mmHg in actual clinical practice because the safety of PIP up to 15 mmHg has already been reported.[19] Additionally, hemodynamic changes may vary among individual patients; therefore, changes in hemodynamics should be carefully monitored during PIP application.

We found no clear reports of air embolisms caused by the use of thoracic CO_2 insufflation. However, various studies on air embolisms due to CO_2 insufflation were conducted in the field of laparoscopic surgery in the 1990s and 2000s, and we believe that these studies will help in evaluating the risks during thoracoscopic surgery. A meta-analysis showed that air embolisms occurred in only 7 of 489335 laparoscopic procedures (0.001%).[20] However, using transesophageal echocardiography to monitor for CO_2 embolism during laparoscopic surgery enabled the more frequent detection of bubbles.[21] Dion et al.[22] reported that a single 15-mL dose to the vena cava in dogs resulted in no intravascular bubbles, a single 100-mL dose resulted in only increased PA pressure, and a single 300-mL dose resulted in bubbles in the left ventricle and death. Mayer et al.[23] performed an in vivo experiment in which CO_2 was infused into the inferior vena cava at various rates for 2 hours in swine, resulting in air embolism and death in three of five animals after more than 50 minutes of infusion at 1.2 mL/kg/min. Graff et al.[24] concluded from experiments on dogs that the 50% lethal dose for transvenous CO_2 infusion in 70-kg humans

is 1750 mL. These results suggest that a rapid and massive influx of CO_2 is required to develop air embolisms with clinical symptoms because CO_2 is highly soluble in water. Because one death due to air embolization caused by artificial pneumothorax using non-CO_2 air has been documented,[25] a certain amount of gas entry should be expected during general thoracic surgery. When CO_2 is applied for insufflation, symptomatic air embolism is unlikely to occur because of the high solubility of CO_2 in water. Our research group previously performed an experiment involving a porcine model in which the pulmonary veins were injured under CO_2 insufflation, and no air embolisms occurred in the animal model.[1] During the present experiment, however, the development of air embolism was not investigated for three reasons. First, the PA pressure is higher than the pulmonary venous pressure. Second, CO_2 influx from the PA through the peripheral pulmonary parenchyma into the circulatory system is expected to be much less common than that from pulmonary vein. Third, the number of experimental animals was small, making evaluation difficult. Notably, however, during our experiments involving two pigs, we encountered neither instability of vital signs nor any findings that would suggest air embolism.

This study had some limitations. First, because of the small number of swine in both the in vivo and ex vivo experiments, especially in the in vivo experiments, the statistical data may be unreliable. However, the ultimate goal of this study was to establish a method for using CO_2 insufflation to safely stop intraoperative bleeding in humans. Therefore, we believe that a porcine experimental model should be established as soon as possible and that a small number of samples would provide a sufficient bridge to clinical practice. Moreover, constructing in vivo models would require sacrificing many animals, which should be avoided as much as possible to promote animal welfare. Second, anatomical differences exist between swine lungs and human lungs. However, obtaining the necessary length of human vessel to conduct the experiment is difficult; therefore, we chose swine as the experimental models. Third, regarding the wound type, we did not compare 5-Fr sheath models and numerous tiny wounds (e.g., 2- to 3-mm wounds), which we clinically experienced more often. The preliminary ex vivo experiments showed that measurement error was larger and less reproducible with smaller incisions. Therefore, we recognized that it would be very difficult to establish the experimental models with reliable tiny PA injuries (≤5 mm). Furthermore, we hypothesized that to validate the usefulness of PIP, we should evaluate PA injury models in which temporary hemostasis is not easy to achieve. For these reasons, we started the experiment with a 10-mm incision instead of a tiny one.

Conclusion

Ex vivo and swine in vivo experiments showed that increasing the intrapleural pressure (10–20 mmHg) via CO_2 insufflation may temporarily suppress bleeding from PA injury. This method may be useful as an adjunctive hemostatic maneuver for intraoperative bleeding if it is limited to bleeding from low-pressure systems such as the venous and pulmonary circulatory systems. Further investigation is needed to clarify the safety and efficacy of this method in clinical practice.

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Formal analysis: YY, YukSak
Funding acquisition: EK, MK, YukSak
Investigation: MA, YY, YukSak
Methodology: MA, EK, YukSak
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Visualization: MA, YY, YukSak
Writing – original draft: MA, YY, YukSak
Writing – review and editing: NM, EK, YuiSai, MK

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Disclosure Statement

All authors have no conflicts of interest.

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