

# Establishment of an Experimental System for Intraperitoneal Chemotherapy in a Rat Model

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**Abstract.** *Aim: To establish an experimental system for comparing different methods of intraperitoneal chemotherapy in a rat model. Materials and Methods: We used six-week-old Sprague–Dawley rats, and created an early postoperative intraperitoneal chemotherapy (EPIC) system using 18-gauge syringes and evacuators, and a hyperthermic intraperitoneal chemotherapy (HIPEC) system using two peristaltic pumps which controlled the flow rate and temperature. Pressurized intraperitoneal aerosol chemotherapy (PIPAC) was achieved using a nozzle for dispersing aerosols at a flow rate up to 41.5 ml/min. The distribution and intensity of 0.2% trypan blue dye was compared among three methods. Results: The distribution was limited and the intensity was weak after EPIC, and the dye stained moderately in gravity-dependent regions after*

*HIPEC. On the other hand, the distribution was the most comprehensive, and the intensity was the greatest after PIPAC. Conclusion: This experimental system in a rat model may reflect the comparative effect among EPIC, HIPEC and PIPAC in humans.*

Peritoneal metastasis (PM) represents disseminated and growing tumors on the peritoneal surface found in advanced or recurrent diseases with solid tumors (1). PM is found in 10-35% of patients with gastro-intestinal cancer, and in up to 50% of those with ovarian cancer (2, 3). Although various types of anticancer drugs for intravenous chemotherapy have been introduced to treat patients with peritoneal carcinomatosis, they still have poor prognosis, with median survival of less than 20 months (4). Intraperitoneal chemotherapy has been used as an option for overcoming drug resistance developed after intravenous chemotherapy because intraperitoneal chemotherapy acts *via* direct diffusion of drugs into tumors in the peritoneal cavity (5).

As the first-generation method of intraperitoneal chemotherapy, early postoperative intraperitoneal chemotherapy (EPIC) has been reported to improve survival in patients with colonic (6), and ovarian (7) cancer. However, an increase of chemotherapy-induced toxicity by the direct diffusion of drugs may not allow completion of a sufficient number of cycles of chemotherapy, and this may reduce survival. As a second-generation method, hyperthermic intraperitoneal chemotherapy (HIPEC) utilizes hyperthermia of 40-43°C and 30% of the dose of anticancer drugs used for intravenous chemotherapy (5). HIPEC is emerging as a new medical technology for improving survival because its addition to cytoreductive surgery was shown to improve survival compared with cytoreductive

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surgery alone in patients with ovarian cancer who received neoadjuvant chemotherapy (8). However, HIPEC requires careful attention because it is associated with renal or hepatic dysfunction in up to 23% of cases and treatment-related death by hyperthermia and drug-induced toxicity in up to 7% (9).

As a third-generation method, pressurized intraperitoneal aerosol chemotherapy (PIPAC) deliver anticancer drugs to the peritoneum in the form of aerosols under an abdominal pressure of 12 mmHg (10). It has some advantages such as a very low dose-equivalent, about 1% dose of anticancer drugs used for intravenous chemotherapy, and normothermia, which reduce treatment-related toxicity remarkably (11, 12). Although phase II trials showed the effect and safety of PIPAC in treating solid tumors, it has some disadvantages, including limited use of drugs including doxorubicin and cisplatin (13, 14).

Although these three methods of intraperitoneal chemotherapy are promising for treating patients with PM, there are few research studies on the most suitable conditions for performing intraperitoneal chemotherapy. For investigating the most appropriate methods and conditions of intraperitoneal chemotherapy, their utilisation in animal models is important because the effects can be compared among different methods and parameters of intraperitoneal chemotherapy. In this study, we established an experimental system using rats for comparing effect among EPIC, HIPEC and PIPAC.

## Materials and Methods

**Selection of animals.** Six-week-old female Sprague-Dawley rats (weighing from 160 to 180 g) were purchased from DBL (Chungcheong-do, Korea) and were allowed to acclimate for 1 week before the experiment. Two rats were used for EPIC, one for HIPEC and two for PIPAC. Rats were maintained with standard chow and housed under 12-h light on/off cycle. The animals were euthanized at the end of the experiments and immediately autopsied. This experiment was performed with ethical approval by Korea University (KUIACUC-2019-0055).

**EPIC setup.** An 18-gauge syringe and an evacuator were introduced into each side of the peritoneal cavity, and designed so that releasing the clamp of the evacuator ejected the solution 30 min after its injection.

**HIPEC setup.** Two peristaltic pumps were included in a HIPEC device. A pump served to deliver trypan blue from the drug reservoir to the peritoneal cavity of the animal while another was responsible for the return of the drug from the peritoneal cavity to the drug reservoir. Two stepper motors were used to drive the pumps. Tygon tubing (1.59 mm ID, 3.18 mm OD, Saint-Gobain Performance Plastics Co., Akron, OH, USA) was used as circuit lines. Intravenous catheters were connected to each end of the lines responsible for the inflow and outflow. The opposite ends of the lines were connected to the drug reservoir. A saline bag was used as the drug reservoir and was placed in a temperature-controlled water bath. All tube connections were secured using Luer Lock

Fittings (1/16" barb; KENT Systems, LLC., Loveland, CO, USA). An infrared thermometer was attached to the inflow line. Arduino Uno board was used to control the flow rate of the pumps and process the signals obtained from the thermometer. The flow rates for both inflow and outflow were synchronously controlled up to 7.5 ml/min. The flow rate (ml/min) and temperature (°C) were displayed via a 16x2 character dot-matrix LCD module.

**PIPAC setup.** A peristaltic pump was used to deliver trypan blue from the reservoir to the peritoneal cavity of the animal at a flow rate up to 41.5 ml/min. A stepper motor was used to drive the pump. A full cone spray nozzle (orifice diameter 0.8 mm; ISN, Republic of Korea) was selected for its ability to disperse drug droplets evenly and appropriately for the oval shape of the peritoneal cavity and a wide range of spray angles (maximum 40°). The spray angle was considered suitable for the low flow rate, less than 15 ml/min, for small-sized animals. The nozzle was connected to a ¼-inch tube fitting, which was then plugged into a Teflon tube (¼-inch; KITZ, Republic of Korea). Teflon tubing was selected for its stiffness to endure the pressure that Tygon tubing cannot provide. The opposite end of the Tygon tube was connected to a drug reservoir, which was made using a saline bag. All tube lines were secured with Luer Lock fittings. A pressure sensor (MS5412; TE Connectivity Ltd., Schaffhausen, Switzerland) was installed to monitor the pressure in the peritoneal cavity with CO<sub>2</sub> being supplied. A three-way stopcock was used to connect the pressure sensor to a CO<sub>2</sub> supply line and an intravenous catheter (BD Angiocath Plus; Becton, Dickinson and Company, NJ, USA). Arduino Uno board was used to control the flow rate of the pump and to present the flow rate (ml/min) and abdominal pressure (mmHg) values via a 16x2 character dot-matrix LCD module.

**Measurements.** Before intraperitoneal chemotherapy, Sprague-Dawley rats were anesthetized with 350 µl of a mixture of ketamine (100 mg/kg; Yuhan Corporation, Seoul, Republic of Korea) and Rompun® Inj. (10 mg/kg; Bayer, Suwon, Republic of Korea). Thereafter, equal volumes of 0.4% trypan blue dye (Sigma-Aldrich, St. Louis, MO, USA) and 0.9% NaCl were mixed, and injected into the peritoneal cavity during intraperitoneal chemotherapy for 30 min.

For EPIC, 50 ml of 0.2% trypan blue dye was injected into the peritoneal cavity of two rats for 30 min at room temperature of 22°C as shown in Supplementary Video S1. For HIPEC, 50 ml of 0.2% trypan blue dye was injected into the peritoneal cavity of two rats for 30 min (1.53±2 ml/min) as shown in Supplementary Video S2. The temperature of the water bath and inflow catheter was 52°C and 41-42°C, respectively (Figure 1). For PIPAC, CO<sub>2</sub> was injected into the peritoneal cavity of two rats through BD Angiocath Plus (BD, Singapore) generating 3±1 mmHg pressure in the peritoneal cavity. Thereafter, 50 ml of 0.2% trypan blue dye was dispersed through a nebulizer for 4 min at a room temperature of 22°C, and then the pressure was maintained for 30 min with a flow rate of 13.47 ml/min (Figure 2 and Supplementary Video S3). After 30 min, the dye was drained using the evacuator (Sewoon Medical Co., Ltd). The overall settings for each type of intraperitoneal chemotherapy are summarized in Table I.

After completion of the treatment, the peritoneum and gastrointestinal tract were washed with 50 ml of 0.9% NaCl for all rats. The abdomen of each rat was then opened immediately to evaluate the distribution and intensity of 0.2% trypan blue dye

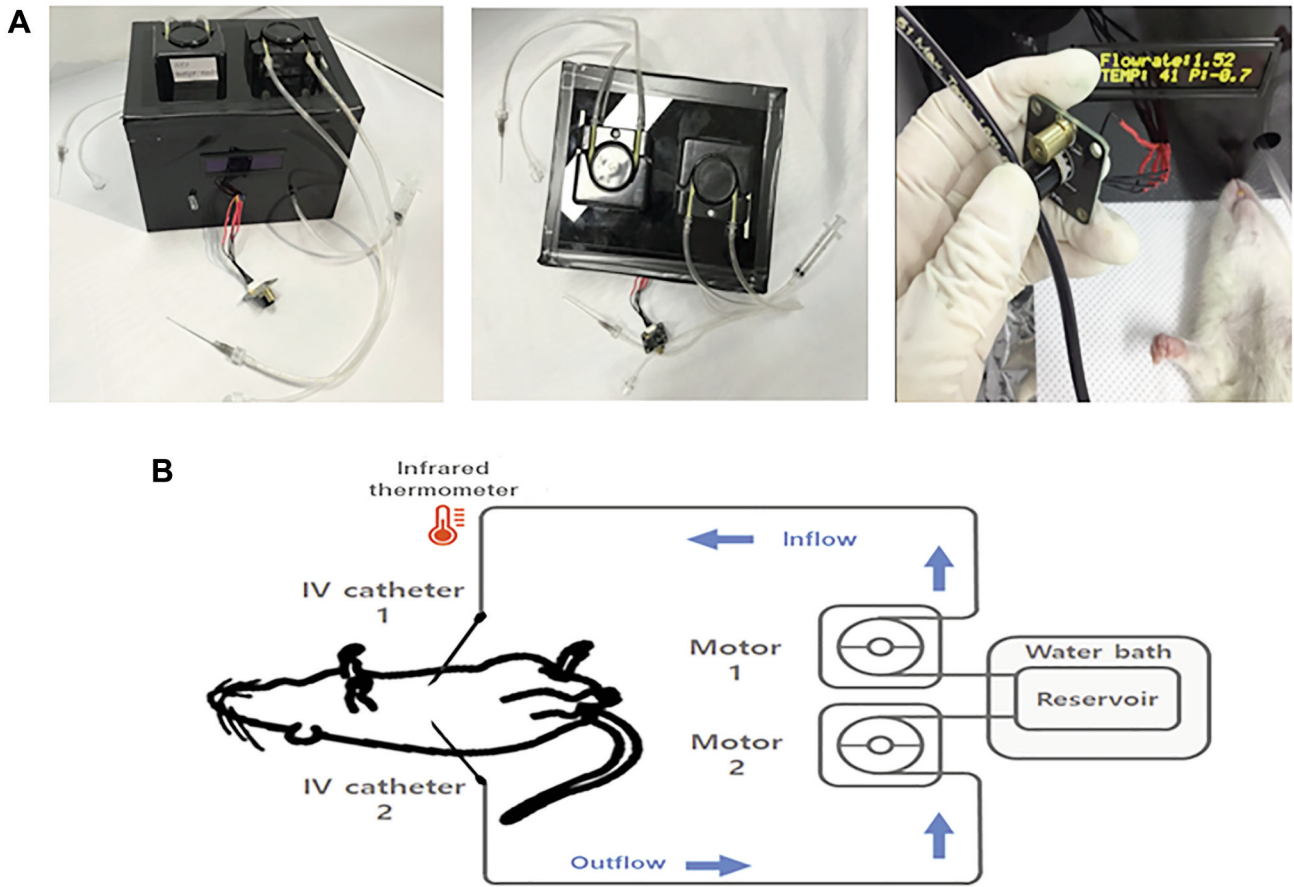


Figure 1. Equipment for hyperthermic intraperitoneal chemotherapy (HIPEC) in a rat model. A: The device with two stepper motors, an infrared thermometer, and a 16x2 character dot-matrix LCD module. B: Schematic diagram of the experimental setup for HIPEC, during which the flow rate was 1.53 ml/min and the inflow temperature was 41-42°C. IV: Intravenous.

staining. The naked eye staining of parietal (peritoneum) and visceral organs (small and large bowels) of rats was compared under EPIC, HIPEC and PIPAC. After the evaluation, the abdominal wall was closed with 4-0 surgifit suture, and the skin with 3-0 black silk. Thereafter, we checked for any side-effects and health of the rats after the surgery daily for 10 days.

## Results

When we compared the distribution and intensity of 0.2% trypan blue dye staining among EPIC, HIPEC and PIPAC, the distribution was limited and the intensity was weak after EPIC, whilst the dye moderately stained gravity-dependent regions of the visceral and parietal organs after HIPEC. On the other hand, the distribution was the most comprehensive without depending on gravity, and the intensity was the greatest in the visceral and parietal organs after PIPAC (Figure 3). All rats survived well for 10 days after EPIC, HIPEC and PIPAC.

Table I. Comparison of the experimental setup for early postoperative intraperitoneal chemotherapy (EPIC), hyperthermic intraperitoneal chemotherapy (HIPEC) and pressurized intraperitoneal aerosol chemotherapy (PIPAC) using a 1:1 volume ratio of 0.4% trypan blue:0.9% NaCl.

Type	EPIC	HIPEC	PIPAC
Total volume, ml	50	50	50
Temperature, °C	Room: 22	Water-bath: 52	Room: 22
Flow rate, ml/min	50	1.53	13.47
Pressure, mmHg	-	-	3
No. of catheters	2	2	3

## Discussion

Many relevant studies using small animals for evaluating the effect of different methods of intraperitoneal chemotherapy have been reported (Table II) (15-35). However, only one or

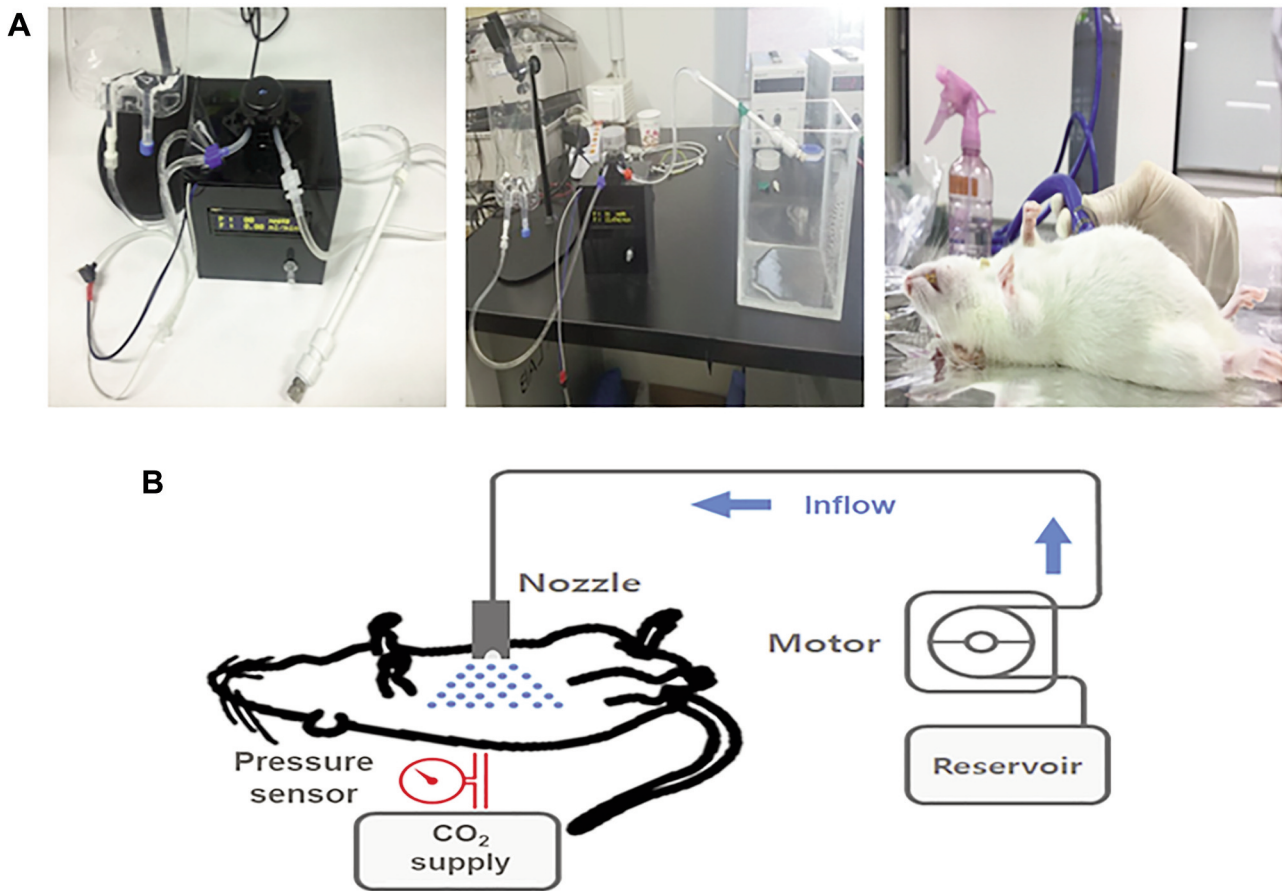


Figure 2. Equipment for pressurized intraperitoneal aerosol chemotherapy (PIPAC) in a rat model. A: The device with a peristaltic pump, a pressure sensor, and a full cone spray nozzle. B: Schematic diagram of the experimental setup for PIPAC, during which CO<sub>2</sub> was injected into the peritoneal cavity, and the flow rate and pressure in the peritoneal cavity were maintained at 13.47 ml/min and 3 mmHg at room temperature (22°C) for 30 min.

two methods of intraperitoneal chemotherapy have been utilized in most studies, and experimental conditions including temperature differ among them. In this study, we established an experimental system for comparing the effect among three types of intraperitoneal chemotherapy in a rat model, suggesting this may reflect the comparative effects among EPIC, HIPEC and PIPAC in humans.

This experimental system enables us to evaluate how modulation of experimental conditions can affect the efficacy of each intraperitoneal chemotherapy. For example, PIPAC reportedly achieves greater penetration of drugs than EPIC (36); deeper penetration by PIPAC is expected to have greater tumoricidal activity during the same cycles of intraperitoneal chemotherapy but experimental evidence for this is still needed. Moreover, the control of abdominal temperature or pressure has been suggested to change the effect during intraperitoneal chemotherapy in previous studies using cancer cell lines (37, 38). Thus, the effects of

modulating experimental conditions can be demonstrated in this experimental system using rats.

Synergistic effects achieved by combining the advantages of each method of intraperitoneal chemotherapy can also be investigated in this experimental system. HIPEC has two main pharmacokinetic problems, namely limited penetration into the peritoneum and limited distribution, whereas PIPAC lacks the advantage of hyperthermia. For overcoming these disadvantages of each method, the concept of hyperthermic PIPAC was introduced in a porcine model (39). Thus, the synergistic effect of hyperthermic PIPAC can be demonstrated by comparing effects among these different methods of intraperitoneal chemotherapy in this experimental system using rat models with PM.

Moreover, the safety of intraperitoneal chemotherapy can also be evaluated in this experimental system. Data on the maximal dose of drugs and temperature to ensure the safety of EPIC or HIPEC are lacking. Surgery is considered a

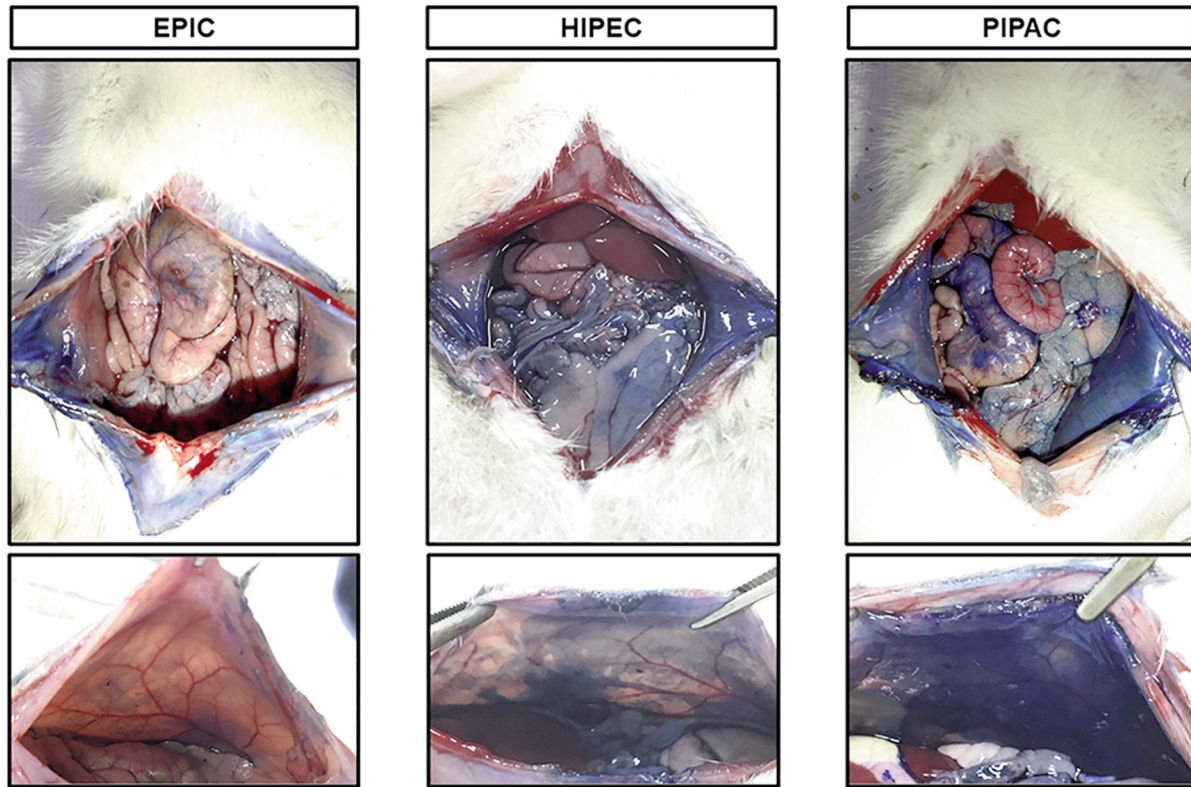


Figure 3. Comparison of the distribution and intensity of 0.2% trypan blue dye in the visceral organs (upper panel) and the parietal organ (lower panel) after early postoperative intraperitoneal chemotherapy (EPIC), hyperthermic intraperitoneal chemotherapy (HIPEC) and pressurized intraperitoneal aerosol chemotherapy (PIPAC).

Table II. Previous intraperitoneal chemotherapy studies in small animals.

Species	Type	Operative time (min)	Temperature (°C)	References
Sprague-Dawley Rat	EPIC	90	32.5-37	15-17
Sprague-Dawley rat	HIPEC	90	40-42.5	15-17
Sprague-Dawley rat	EPIC	60	36.4	18
Sprague-Dawley rat	HIPEC	60	42.5	18
Sprague-Dawley rat	EPIC	25	37	19
Sprague-Dawley rat	HIPEC	25	40, 43	19
WAG/Rij rat	EPIC	90	37	20
WAG/Rij rat	HIPEC	90	40-42	20-23
WAG/Rij rat	HIPEC	60	39-43.2	24, 25
WAG/Rij rat	EPIC	45	37	26
WAG/Rij rat	HIPEC	45	41	26
BDIX rat	HIPEC	60	41.2-42.3	27, 28
Wistar rat	EPIC	60 and 90	18-23	29
Wistar rat	HIPEC	60 and 90	42	29
Wistar rat	HIPEC	45	40.5-41.5	30
Athymic nude rat	HIPEC	60	41-42.5	31, 32
Athymic nude rat	HIPEC	45	41.5	33
C57BL/6, Mouse	HIPEC	12	43	34
Athymic nude rat	PIPAC	30	37	35

EPIC: Early postoperative intraperitoneal chemotherapy; HIPEC: hyperthermic intraperitoneal chemotherapy; PIPAC: pressurized intraperitoneal aerosol chemotherapy.

relative contraindication before PIPAC because a case of postoperative bowel perforation after surgery followed by PIPAC was reported (40). Although postoperative bowel perforation has been suggested to develop due to high concentration of drugs in tissues at the anastomosis site hindering wound healing, there are no further basic and clinical evidence as to why PIPAC cannot be performed immediately after surgery like HIPEC. Thus, we believe that this hypothesis can be investigated *via* this experimental system, using rats which underwent surgery before PIPAC.

In particular, a model using rats with peritoneal carcinomatosis is more realistic for evaluating the effect of intraperitoneal chemotherapy in this experimental system. Xenograft models using athymic mice or Fischer 344 rats have been reported (35, 41, 42), in which SKOV-3 Luc IP2 or NuTu-19 cells were injected intraperitoneally. Although xenograft models using nude mice are easy to develop, we were unable to equip the device of PIPAC in mice because the nozzle was too large to be inserted into the peritoneal cavity. Furthermore, capnoperitoneum was formed too quickly and CO<sub>2</sub> in the peritoneal cavity was also released too quickly due to the very small volume of the peritoneal cavity of the mice. Considering that rats are more tolerant of repetitive procedures than mice because the wound is relatively small for inserting the devices for intraperitoneal chemotherapy (35), we believe that this experimental system using rats is more appropriate for investigating the effect and safety of intraperitoneal chemotherapy. Furthermore, this experimental system using rats has an additional advantage that repetitive treatments are possible because most of the rats were healthy after intraperitoneal chemotherapy, as in a previous study (35).

However, this experimental system has some limitations as follows. Firstly, the size of aerosols during PIPAC was not measured in this experimental system. However, aerosols were injected at a flow rate of 13.47 ml/min, half of the value used in a previous study (0.5 ml/s), with maintenance of the pressure of capnoperitoneum in rats. Furthermore, PIPAC showed strong staining by 0.2% trypan blue dye in both the parietal and visceral organs similar to a previous study (35). *Ex vivo* or preclinical studies using porcine models showed that the depth of penetration of doxorubicin depended on the amount of doxorubicin distributed (43, 44), which suggests that the sizes of aerosols may not be important in increasing the depth of penetration in rats because most aerosols are well distributed, unlike swine models with their relatively large peritoneal cavity. Secondly, we did not validate this experimental system using rats with peritoneal carcinomatosis. This validation should be conducted in rats with peritoneal carcinomatosis from different types of tumor cells, considering the change of the dose of drugs used in the human body to that used in rats (45).

Although different types of intraperitoneal chemotherapy are used in the clinical setting, we found no evidence of the most appropriate method for patients with peritoneal carcinomatosis. Thus, this experimental system enables us comparison of the effect and safety among these three types of intraperitoneal chemotherapy in rats, and may be helpful in selecting the most appropriate method by modulating experimental conditions such as temperature.

## Conflicts of Interest

The Authors declare no competing interests.

## Authors' Contributions

HSK and GS conceived the study. SP, HSL, JK, JH, SR, EYL, SJP, SO, and AS performed the experiments. WL and JCL analyzed the data. SP, SJP, HSK and GS drew the figures, and wrote the article. All Authors read and approved the final article.

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Supplementary video 1. Early postoperative intraperitoneal chemotherapy in a rat model (available at: <https://youtu.be/Ukizu hUJFv8>).

Supplementary video 2. Hyperthermic intraperitoneal chemotherapy in a rat model (available at: <https://youtu.be/dxjySW BgRU4>).

Supplementary video 3. Pressurized intraperitoneal aerosol chemotherapy in a rat model (available at: <https://youtu.be/-Xi2F7i1 GDY>).

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