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Original Article

Pharmacokinetics, toxicities, and tissue concentrations of belotecan sprayed by rotational intraperitoneal pressurized aerosol chemotherapy in a pig model

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ABSTRACT

Objective: We evaluated the pharmacokinetics, tissue concentrations, and toxicities of belotecan during rotational intraperitoneal pressurized aerosol chemotherapy (RIPAC) in pigs.
Methods: We sprayed belotecan in 10% and 30% of doses for intravenous chemotherapy in six pigs (cohort 1, n=3, 0.50 mg/m^2; cohort 2, n=3, 1.5 mg/m^2). We evaluated the time-dependent plasma concentrations of belotecan before RIPAC to 120 hours for the pharmacokinetics, tissue concentrations in twelve peritoneal regions, and hepatic and renal functions before RIPAC to 120 hours in the 2 cohorts.
Results: Mean values of the peak plasma concentration (C_{max}), the time to C_{max}, the time taken for C_{max} to drop in half, and the area under the curve from time zero to the time of last quantifiable concentration were 905 and 3,700 ng/mL, 1.42 and 1.50 hours, 3.64 and 5.60 hours, and 2,260 and 17,900 pg·hr/mL in cohorts 1 and 2, respectively. Mean values of tissue concentrations were 1.5 to 15.3 times higher in cohort 1 than in cohort 2 despite the similar ratio of tissue to plasma concentration, and tissue concentrations in the two cohorts were higher in the parietal peritoneum than in the visceral peritoneum. However, hepatic and renal functions were not different before RIPAC to 120 hours in the two cohorts.
Conclusion: RIPAC using belotecan of 0.5 mg/m^2 and 1.5 mg/m^2 may be feasible with fewer hepatic and renal toxicities in pigs. Thus, belotecan of 1.5 mg/m^2 may be considered as the starting dose for RIPAC in a phase 1 trial.

Keywords: Drug Therapy; Aerosols; Pharmacokinetics; Tissues; Swine

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Conflict of Interest
Hee Seung Kim is the Chief Executive Officer at Dreampac Corp. (Wonju, Republic of Korea). Moreover, Seungmee Lee and San-Hui Lee are the Chief Executive Officer and a director of Precision Medicine for Peritoneal Metastasis Corp. (Wonju, Republic of Korea). The other authors have no conflict of interest.

Author Contributions
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Synopsis
We evaluated the pharmacokinetics, tissue concentrations, and toxicities of belotecan during rotational intraperitoneal pressurized aerosol chemotherapy (RIPAC) in pigs. RIPAC using belotecan of 0.5 mg/m^2 and 1.5 mg/m^2 may be feasible with fewer toxicities. Belotecan of 1.5 mg/m^2 can be considered as the starting dose for RIPAC in a phase 1 trial.

INTRODUCTION

Recurrence of ovarian cancer with peritoneal metastasis (PM) is known to have a poor prognosis. In the era of targeted therapy, bevacizumab and poly (ADP-ribose) polymerase (PARP) inhibitors only improved progression-free survival (PFS) of 3 to 15 months despite no benefit of prolonged overall survival (OS) [1,2]. Although secondary cytoreductive surgery (SCS) has been introduced for better prognosis in platinum-sensitive disease [3,4], only different types of chemotherapeutic agents can be considered as palliative therapy in platinum-resistant disease, showing a low response rate of less than 10% because of multi-drug resistance [5]. Even though hyperthermic intraperitoneal chemotherapy reportedly showed improved PFS and OS in patients with advanced ovarian cancer who underwent interval debulking surgery followed by neoadjuvant chemotherapy [6], it failed to improve survival in those who received SCS for recurrent ovarian cancer [7].

To overcome these limitations in recurrent ovarian cancer with PM, pressurized intraperitoneal aerosol chemotherapy (PIPAC) is rapidly emerging in value as a palliative therapy. Even though PIPAC showed a high response rate of 17%–64% by spraying about 10% doses of chemo-resistant agents used in intravenous chemotherapy by a high-pressure injector in the abdominal cavity of patients with recurrent ovarian cancer [8], its disadvantage is that physicians' choice of anti-cancer agents is limited to a small number of anti-cancer agents including doxorubicin, cisplatin and oxaliplatin [9]. Thus, the expansion of types of anti-cancer agents used in PIPAC has the potential to increase treatment response and survival in patients with recurrent ovarian cancer.

Belotecan is a semi-synthetic camptothecin analogue with the water-solubilizing group at position 7, which inhibits the relegation of single-stranded DNA breaks by blocking topoisomerase I, and thereby disrupting DNA replication and inducing apoptosis like topotecan [10,11]. Even though intraperitoneal chemotherapy using topotecan has been used in ovarian cancer [12-17], there is no evidence of the efficacy and safety of topotecan or belotecan used in PIPAC. Considering the result of the phase II study that belotecan showed better survival than topotecan in recurrent platinum-resistant and non-high-grade serous carcinoma of the ovary [18], belotecan used in PIPAC has the potential to further improve treatment response and survival when compared with topotecan.

Recently, rotational intraperitoneal pressurized aerosol chemotherapy (RIPAC) has been developed by the KoRIA (Korean Rotational Intraperitoneal pressurized Aerosol chemotherapy) Trial Group, which increased tissue concentration and penetration depth of doxorubicin in the peritoneum by rotating the nozzle in preclinical studies [19,20]. Based on the result, RIPAC is on the verge of being marketed and used clinically. Thus, we performed a preclinical study to investigate the pharmacokinetics, tissue concentration, and toxicities of belotecan used during RIPAC in a pig model before conducting clinical trials on RIPAC using belotecan.

MATERIALS AND METHODS

1. Preparation

The Institutional Animal Care and Use Committee of CRONEX approved the study (No. 202210006). We bought a total of 13 female pigs weighing about 50 kg. Among them, one was used for providing normal peritoneal tissues greater than 50×50 cm^2 and the plasma over 150 mL as a control. Thus, we opened the abdomen, and the right and left peritoneal tissues from the diaphragm to the pelvis of 25×25 cm^2 were obtained. Moreover, 12 pigs received belotecan during RIPAC to evaluate the pharmacokinetics, tissue concentrations, and relevant toxicities.

Before RIPAC, capnoperitoneum was applied by CO_2 insufflation via a Veress needle to each pig. Then, we inserted two 12 mm bladeless trocars (Eagleport®; Dalim Medical Corp., Seoul, Korea) along the midline of the abdomen. The 2 trocar insertion sites were used for inserting the nebulizer (Dreampen®; Dreampac Corp., Wonju, Korea) and laparoscopic devices (Stryker Korea Co., Ltd., Seoul, Korea).

For determining the initial dose of belotecan, we hypothesized that weekly belotecan of 1.5 mg/m^2 for 3 consecutive weeks, which was 3 times belotecan of 0.5 mg/m^2 for 5 consecutive days every 3 weeks [21], was tolerable because weekly topotecan of 4 mg/m^2 for 3 consecutive weeks [22], which were about 3 times topotecan 1.2 to 1.5 mg/m^2/day for 5 consecutive days every 3 weeks [23,24], showed the efficacy with acceptable toxicities in recurrent ovarian cancer. Thus, belotecan of 4.5 mg/m^2 for three weeks could be considered as the hypothetical total dose for intravenous chemotherapy. Since more than 10% but less than 30% of the dose for intravenous chemotherapy was used for PIPAC for most anti-cancer agents with the exception of oxaliplatin and mitomycin-C [12], belotecan of 0.5 mg/m^2, about 10% dose of that, was determined as the initial dose of RIPAC in cohort 1, and belotecan of 1.5 mg/m^2, about 30% dose of the hypothetical maximal dose, was considered as the escalated dose for RIPAC in this study in cohort 2. After we converted the weight of the pig (kg) into the body surface area (mg/m^2) based on the previous criteria [25], we administered the initial dose (n=6) and the escalated dose (n=6) of belotecan used during RIPAC.

2. RIPAC

After general anesthesia, we inserted the nebulizer and camera through the two trocar insertion sites, and we equipped the conical pendulum motion device (Dreamcircle®; Dreampac Corp.) for rotating the nozzle during RIPAC. The spraying angle of the nozzle was about 70 degrees, and the angle between the nozzle and the vertical line was about 30 degrees. Thereafter, we covered the pig with the vinyl sheet (Dreamcover®; Dreampac Corp.) to prevent the leakage of aerosols during RIPAC into the operating room (**Fig. S1**).

According to our previous protocol, we sprayed about 30 μm droplets of belotecan through the rotating nozzle with the velocity of 0.6 mL/sec under the pressure of 130 to 140 psi made by the high-pressure injector (Illumena® Néo; Guerbet Korea Co., Ltd., Seoul, Korea). After spraying, the capnoperitoneum of 12 mmHg was maintained for 30 minutes by the laparoscopic system (Stryker Korea Co., Ltd.). Thereafter, CO_2 gas in the abdominal cavity was removed by negative suction (Plumesafe Turbo®; CONMED Corp., Seoul, Korea) [26].

3. Sampling for pharmacokinetics, toxicities, and tissue concentrations

For evaluating the pharmacokinetics of belotecan, plasma samples from the two cohorts (n=3 for each cohort; a total of 6 pigs) were obtained at the following time points:

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0 (before RIPAC), 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 4, 8, 24, 48, 72, 96, and 120 hours after RIPAC. Moreover, we collected serums of the same six pigs seven times as follows: 0 (before RIPAC), 0.5, 24, 48, 72, 96, 120 hours after RIPAC for investigating hepatic and renal functions using serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase, gamma-glutamyl transpeptidase (GGT), bilirubin, alkaline phosphatase (ALP), creatinine, and C-reactive protein (CRP).

In regard to the tissue concentration of belotecan sprayed during RIPAC, a separate group of pigs (n=3 for each cohort; a total of 6 pigs) from the plasma sampling group was used for peritoneal tissue sampling, as tissue sampling required sacrificing the animal 30 minutes after RIPAC. We obtained 2× 2 cm sized peritoneal tissues from nine regions of the parietal peritoneum (central, right upper, epigastrium, left upper, left flank, left lower, pelvis, right lower, and right flank) and three regions of the visceral peritoneum (ileum, jejunum, and stomach) depending on the division of abdominal regions used by the peritoneal cancer index (PCI) used in our previous study [26], and stored them at −80°C. The tissue samples were chopped and added 5 volumes (w/v) of ice-cold distilled water and homogenized using bead homogenizer (D2400; Benchmark Scientific, Sayreville, NJ, USA).

For evaluating the plasma and tissue concentrations of belotecan, 100 μL of 50% acetonitrile containing internal standard (belotecan-d7; 3 ng/mL for the plasma, 14 ng/mL for the tissue) were added to 200 μL of the plasma or 100 μL of the tissue homogenate. Three milliliters of ethyl acetate were subsequently added, followed by vortex mixing (15 minutes) and centrifugation (5 minutes at 4°C, 400 rpm). An aliquot (3 mL) of the mixture was then transferred into a clean tube and evaporated under nitrogen gas at 60°C. Dry contents were reconstituted with 80% acetonitrile containing 0.1% formic acid and transferred to an auto-sampler vial for injection onto liquid chromatography (LC) and tandem mass spectrometry (MS/MS).

4. LC and tandem mass spectrometry

We performed high-performance LC coupled with MS/MS using Agilent 1200 series (Agilent, Santa Clara, CA, USA) and API5500 (AB Sciex, Foster City, CA, USA) for investigating plasma and tissue concentrations of belotecan. HPLC-grade water and acetonitrile containing 0.1% formic acid (50:50, v/v) were used as a mobile phase with a flow rate of 0.25 mL/min. Luna C8(2) (2.0×100 mm, 5 μm) was used as the analytical column for the HPLC, with a column oven temperature of 35°C. The multiple reaction monitoring transitions used for analyte detection were as follows: m/z 434.2 to 363.1 for belotecan, and m/z 441.2 to 363.1 for belotecan-d7 in positive ionization mode. Limits of quantification were 10 pg/mL and 0.1 ng/mL in plasma and tissue homogenates, and linearity was demonstrated up to 10,000 pg/mL and 100 ng/mL, respectively.

5. Bioanalytical method validation

The bioanalytical methods for the quantitative determination of belotecan in plasma samples were validated in terms of carry-over, specificity, linearity, intra- and inter-day accuracy & precision, recovery, matrix effect, long-term stability, short-term stability, post-preparative stability, freeze & thaw stability, stock solution stability and working solution stability. For tissue homogenates, the bioanalytical method was validated in terms of carry-over, linearity, intra- and inter-day accuracy & precision, long-term stability, short-term stability, freeze & thaw stability, and dilution integrity (10-fold). The validations were conducted based on the guidance on bioanalytical method validation issued by the Ministry of Food and Drug Safety (MFDS) and Food and Drug Administration (FDA) [27,28].

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6. Statistical analysis

Pharmacokinetic analysis of belotecan was performed by non-compartmental analysis using Phoenix WinNonlin (version 8.3; Certara Inc., Princeton, NJ, USA). For characterizing the pharmacokinetic analysis, the maximum observed plasma concentration (C_{max}, pg/mL), the time to C_{max} (T_{max}, hr) and the apparent plasma terminal elimination half-life ($T_{1/2}$, hr) were identified. The area under the plasma concentration-time curve from time zero to the time of last quantifiable concentration (AUC_{last}, pg·hr/mL) and the area under the concentration-time curve from time zero extrapolated to infinity (AUC_{inf}, pg·hr/mL) were calculated using the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations. Friedman test for hepatic and renal functions was conducted with SPSS 22.0 (IBM, Armonk, NY, USA). All p-values were considered meaningful if less than 0.05.

RESULTS

Fig. 1 and **Table S1** show time-dependent plasma concentrations of belotecan sprayed by RIPAC. Mean values of C_{max} and AUC were about 4 and 8 times higher in cohort 2 than in cohort 1, whereas T_{max} and $T_{1/2}$ were similar between the two cohorts (**Table 1**).

Table S2 presents tissue concentrations of belotecan after RIPAC in 13 abdominal regions from the PCI. Mean values of tissue concentrations were 1.5 to 15.3 times higher in cohort 2 than in cohort 1, and tissue concentrations in the two cohorts were higher in the parietal peritoneum than in the visceral peritoneum. Moreover, tissue concentrations of the parietal and visceral peritoneum were 1.5 to 6.1 and 1.6 to 15.3 higher in cohort 2 than in cohort 1 (**Fig. 2**).

Table 1. Pharmacokinetic parameters of belotecan

Parameters	Cohort 1: belotecan of 0.5 mg/m²	Cohort 2: belotecan of 1.5 mg/m²
C_{max} (pg/mL)	905±288	3,700±1,060
AUC_{last} (pg·hr/mL)	2,260±610	17,900±6,300
AUC_{inf} (pg·hr/mL)	2,340±580	18,200±6,300
T_{max} (hr)	1.42±0.80	1.50±0.50
$T_{1/2}$ (hr)	3.64±1.42	5.60±2.14

AUC_{inf}, the area under the concentration-time curve extrapolated to infinity; AUC_{last}, the area under the plasma concentration-time curve from time zero to the time of last quantifiable concentration; C_{max}, the maximum observed plasma concentration; $T_{1/2}$, the apparent plasma terminal elimination half-life; T_{max}, the time to the maximum observed plasma concentration.

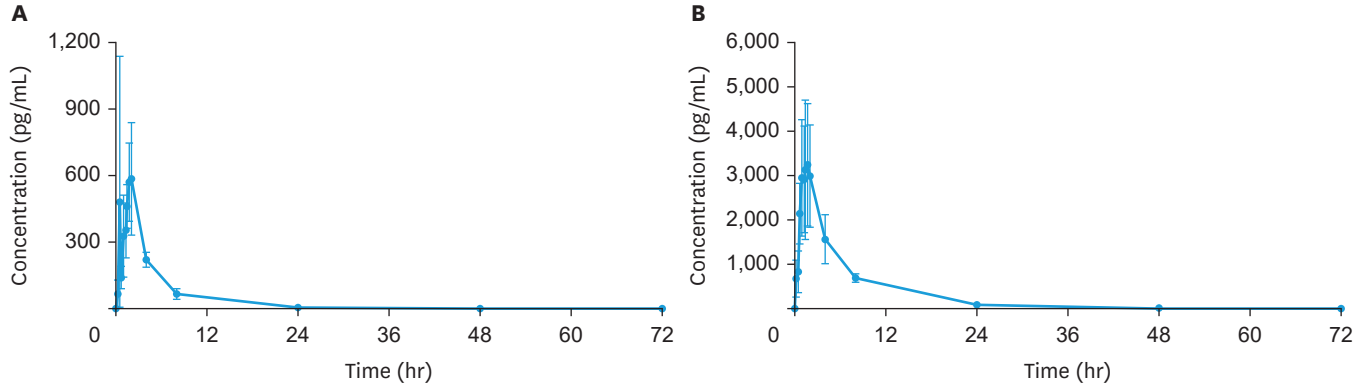

Fig. 1. The plasma concentration-time curve of belotecan used in rotational intraperitoneal pressurized aerosol chemotherapy: (A) belotecan of 0.5 mg/m²; (B) belotecan of 1.5 mg/m².

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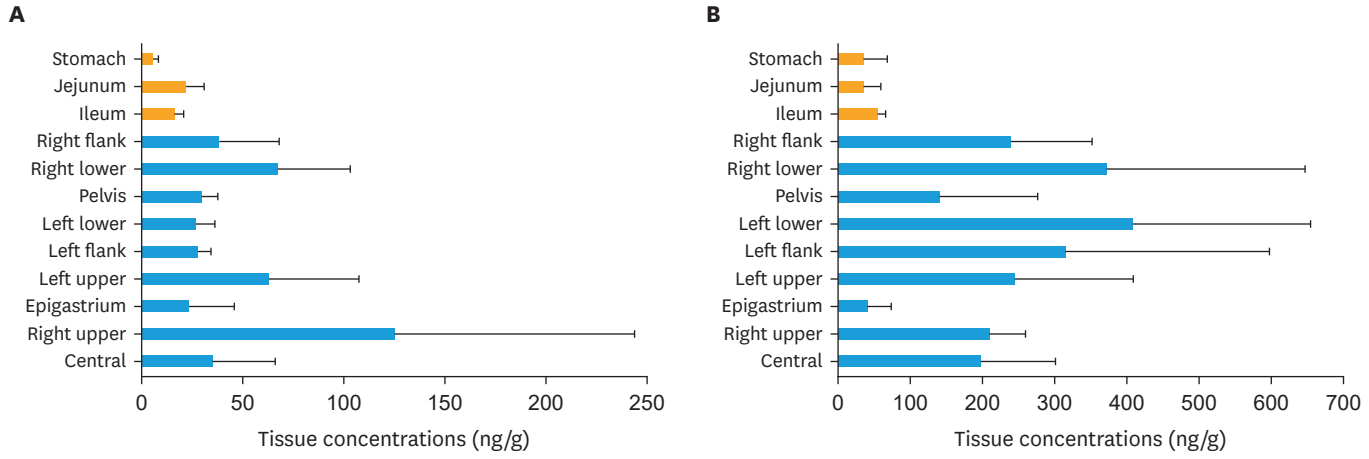

Fig. 2. Tissue concentrations of belotecan used in rotational intraperitoneal pressurized aerosol chemotherapy based on the modified peritoneal cancer index: (A) belotecan of 0.5 mg/m^2; (B) belotecan of 1.5 mg/m^2.

Table 2. Time-dependent changes in renal and hepatic functions related to rotational intraperitoneal pressurized aerosol chemotherapy using belotecan

Parameters	0 hr	0.5 hr	24 hr	48 hr	72 hr	96 hr	120 hr	p-value
Cohort 1: belotecan of 0.5 mg/m^2								
GGT	31.33±7.77	30.33±10.01	32.67±8.08	33.00±12.12	31.33±13.87	31.00±16.70	33.00±13.00	0.848
Bilirubin	0.03±0.01	0.03±0.03	0.02±0.02	0.03±0.02	0.03±0.02	0.05±0.01	0.17±0.05	0.114
AST	22.67±2.51	18.00±3.61	35.33±8.51	31.33±8.96	39.67±19.43	81.33±38.42	43.33±18.93	0.069
ALT	44.0±25.51	40.67±17.62	44.33±21.00	40.67±13.66	37.33±8.96	38.67±8.39	32.67±5.51	0.682
ALP	139.67±77.11	122.00±57.30	128.00±67.01	125.00±65.60	120.33±48.64	129.67±37.65	123.33±30.98	0.754
Creatinine	1.00±0.22	1.04±0.24	1.12±0.21	1.02±0.19	0.92±0.24	0.94±0.33	1.03±0.21	0.415
CRP	0.00±0.01	0.00±0.01	0.01±0.02	0.00±0.01	0.02±0.03	0.00	0.02±0.04	0.907
Cohort 2: belotecan of 1.5 mg/m^2								
GGT	20.00±7.54	21.33±4.51	24.00±6.00	22.33±7.37	15.00±3.46	19.67±6.67	16.67±4.93	0.078
Bilirubin	0.01±0.01	0.02±0.01	0.10±0.02	0.09±0.03	0.03±0.01	0.03±0.02	0.03±0.02	0.120
AST	18.00±2.65	17.67±0.58	46.33±12.66	26.67±3.51	21.67±17.62	23.33±6.66	17.33±5.51	0.112
ALT	20.33±4.93	22.33±5.51	25.00±6.00	20.00±2.00	18.00±8.72	18.00±1.73	15.67±3.78	0.376
ALP	95.00±40.70	98.67±23.16	112.00±46.03	103.33±45.44	74.00±24.76	88.00±52.85	76.33±50.95	0.189
Creatinine	0.83±0.13	0.91±0.23	1.11±0.47	0.99±0.22	0.84±0.23	0.98±0.25	0.80±0.26	0.108
CRP	0.03±0.05	0.04±0.08	0.05±0.08	0.08±0.07	0.00	0.17±0.03	0.00	0.477

All values were shown as mean ± standard deviation.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; GGT, gamma-glutamyl transferase.

Moreover, the ratio of tissue concentration to maximum observed plasma concentrations (C_{max}) was similar between cohorts 1 (range, 6 to 138.1) and 2 (range, 8.9 to 110.1).

In terms of bioanalytical method validation, the linear ranges for belotecan were 10–10,000 pg/mL and 0.1–100 ng/mL in the plasma and tissue homogenates, respectively. All validation results met the acceptance criteria defined by regulatory agencies, and the analytical methods were successfully applied for quantitative analysis of plasma and tissue concentrations.

Table 2 demonstrates renal and hepatic functions before and after RIPAC using belotecan. As a result, there were no differences in GGT, bilirubin, AST, ALP, ALP, creatinine, and CRP before RIPAC, after 0.5, 24, 48, 72, 96, and 120 hours in the 2 cohorts.

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DISCUSSION

Even though maximum tolerated doses (MTDs) of doxorubicin, cisplatin, and oxaliplatin used mainly in PIPAC have been evaluated through recent phase 1 trials [29-32], various types of anti-cancer agents with different doses based on the experience of surgeons are still being used for PIPAC [9]. Considering the potential of RIPAC to improve drug delivery such as tissue concentration and penetration depth [19], adequate doses of these agents used in RIPAC can be adjusted for improved treatment response with acceptable toxicities, and new agents sensitive to chemo-resistant tumor cells should be evaluated to determine MTDs for RIPAC through a phase 1 trial.

As a new agent, we selected belotecan in this study because it has already been used for treating recurrent ovarian cancer in a clinical setting. In particular, belotecan of 0.5 mg/m^2 showed overall response rates (ORRs) of 53.3% and 20% in platinum-sensitive and resistant tumors of the ovary, respectively [21], and an observational study reported that ORR was higher in belotecan-based chemotherapy than in topotecan-based chemotherapy with acceptable toxicities in recurrent ovarian cancer [33]. Furthermore, a relevant phase II trial also demonstrated that belotecan may have the OS benefit in platinum-resistant and non-high-grade carcinoma, especially, endometrioid or clear cell type, when compared with topotecan [19].

In the current study, we found that C_{max} and AUC of belotecan increased 4- and 8-fold when the dose used in RIPAC was raised from 0.5 mg/m^2 to 1.5 mg/m^2, with no significant change in T_{max} and $T_{1/2}$. This finding suggests that the dose escalation of belotecan for RIPAC leads to increased uptake of belotecan into the plasma. In the phase 1 study of belotecan, the intravenous administration of 0.5 mg/m^2 belotecan resulted in AUC_{inf} of 155.6 ng·hr/mL, C_{max} of 91.8 ng/mL, and $T_{1/2}$ of 8.55 hours [34]. On the other hand, the current study showed that when using RIPAC in pigs, belotecan of 1.5 mg/m^2 resulted in significantly lower plasma exposure with AUC_{inf} of 18.2 ng·hr/mL, and C_{max} of 3.70 ng/mL. Moreover, we could not find hepatic and renal toxicities till five days after RIPAC using belotecan of 0.5 and 1.5 mg/m^2 in pigs. Considering that belotecan is substantially excreted via the urinary route [34,35], these results suggest that belotecan of 1.5 mg/m^2 can be considered as the starting dose in a relevant phase 1 trial without relevant renal toxicities, despite being three times the dose used in intravenous chemotherapy.

Tissue concentrations of belotecan were higher in the parietal peritoneum than in the visceral peritoneum. This finding can confirm our previous results that tissue concentrations of paclitaxel, cisplatin, and doxorubicin used in RIPAC were lower in the visceral peritoneum than in the parietal peritoneum because it is difficult for these agents to penetrate into the hard muscularis layer beyond the visceral peritoneum when compared with the soft extraperitoneal fat tissues beyond the parietal peritoneum [19,26,36]. Since tissue concentrations in the visceral peritoneum increased 1.6 to 15.3-fold by raising from 0.5 mg/m^2 to 1.5 mg/m^2, we can expect the improved drug delivery into the visceral peritoneum after RIPAC using belotecan of 1.5 mg/m^2 instead of that of 0.5 mg/m^2 in an upcoming phase I trial.

Table 3 demonstrates the pharmacokinetic properties of anti-cancer agents used during PIPAC or RIPAC in pigs and humans [19,26,30,31,37,38]. T_{max} was similar among hydrophilic agents such as oxaliplatin, cisplatin, doxorubicin, and belotecan, whereas paclitaxel, a hydrophobic agent, was higher, reflecting its delayed absorption into the plasma [29]. On the other hand, AUC_{last} and C_{max} of belotecan were the lowest, suggesting that additional dose escalation can be considered in a relevant phase I trial if toxicities were acceptable.

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Table 3. Pharmacokinetic properties of anti-cancer agents sprayed during PIPAC or RIPAC in pigs and humans

Authors	Type	Target	Agent (mg/m^2)	AUC_{last} (ng·hr/mL)	C_{max} (ng/mL)	T_{max} (hr)
Dumont et al. [30]	PIPAC	Humans	O of 90/140	6,106/9,028	896/1,035	0.9/1.0
Ceelen et al. [37]	PIPAC	Humans	NP of 35, 70, 90, 112.5, 140	541.6–1,904.83	57.8–158.4	3–4
Kim et al. [31]	PIPAC	Humans	O of 45, 60, 90, 120	2,212.3–7,088.6	331.9–1,017.4	0.8–3.2
Lurvink et al. [38]	ePIPAC	Humans	O of 92 with IV L of 20 and F of 400	49,000–59,500	2,670–3,280	1.5–1.6
Park et al. [19]	RIPAC	Pigs	D of 3.5*	20.9	23.0	0.3
Park et al. [26]	RIPAC	Pigs	Paclitaxel of 25	162.2	4.7	24
			Cisplatin of 7.5	7,851.4	187.6	2
Current study	RIPAC	Pigs	B of 0.5/1.5	2.3/17.9	0.9/3.7	1.4/1.5

B, belotecan; C, cisplatin; D, doxorubicin; ePIPAC, electrostatic pressurized intraperitoneal aerosol chemotherapy; F, 5-fluorouracil; IV, intravenous; L, leucovorin; NP, nanoparticle albumin-based paclitaxel; O, oxaliplatin.
*Doxorubicin of 3.5 mg in 50 mL of 0.9% NaCl was sprayed.

This study has some limitations as follows: first, we did not investigate plasma and tissue concentrations of belotecan in a large number of pigs. Second, we did not compare the penetration depth of belotecan between the two cohorts despite being another important factor in drug delivery by RIPAC. Third, we used three pigs per cohort in this preclinical study because a minimum of three biological replicates selected on the basis of identity may be required for statistical analysis [39]. However, studies with larger numbers of pigs are needed to increase statistical power. Fourth, we believe that the comparison of tissue concentrations of belotecan in pigs between intravenous and intraperitoneal chemotherapy is of great value if the control group for intravenous chemotherapy using belotecan is added for this preclinical study, and it may be valuable as an indirect reference for comparing tissue concentrations of belotecan between intravenous and intraperitoneal chemotherapy in future clinical trials. Fifth, the treatment effect was not compared between the two cohorts in pigs with PM. In particular, tumors on the peritoneum and tissue adhesion can change the pharmacokinetic properties and tissue concentrations, which can act as a bias for evaluating the treatment effect. Thus, a preclinical study where the treatment effect of belotecan can be evaluated in pigs with PM made by our protocol may substantially strengthen the value of this preclinical study and be helpful in predicting that of belotecan in human clinical trials in the future [40]. Furthermore, we plan to conduct a phase I/IIA trial of RIPAC with belotecan of 1.5 mg/m^2 as the starting dose in 0.3 mg/m^2 increments up to a maximal dose of 2.7 mg/m^2 for patients with platinum-resistant ovarian cancer soon, which will help us predict the pharmacokinetics, therapeutic benefit and toxicity of belotecan used in RIPAC for humans.

In conclusion, RIPAC using belotecan of 0.5 mg/m^2 and 1.5 mg/m^2 may be feasible in pigs. Considering the relatively low AUC_{last} and C_{max}, an increase of drug delivery into the visceral peritoneum, and fewer toxicities, belotecan of 1.5 mg/m^2 for RIPAC may be considered as the starting dose for a phase I trial.

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SUPPLEMENTARY MATERIALS

Table S1

Time-dependent plasma concentrations of belotecan after rotational intraperitoneal pressurized aerosol chemotherapy in pigs

Table S2

Tissue concentrations of belotecan after rotational intraperitoneal pressurized aerosol chemotherapy in pigs

Fig. S1

RIPAC in pig: (A) the nozzle (Dreampen®; Dreampac Corp., Wonju, Korea); (B) the device for rotating the nozzle (Dreamcircle®; Dreampac Corp.); (C) the vinyl sheet to prevent the leakage of aerosols during RIPAC into the operating room (Dreamcover®; Dreampac Corp.).

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