Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Comparison of Pharmacokinetic Properties of Mitomycin C-Based Regimens for Hyperthermic Intraperitoneal Chemotherapy in a Rat Model

Sangho Joo
Asan Medical Center, University of Ulsan College of Medicine
Kyung-Sun Moon
Kangwon National University
Seunghee Baek
Asan Medical Center, University of Ulsan College of Medicine
Sung-Hoon Ahn
Kangwon National University
Eun Jung Park
kendoej@naver.com

Asan Medical Center, University of Ulsan College of Medicine

Article

Keywords: Hyperthermic intraperitoneal chemotherapy, Mitomycin C, HIPEC regimens, Colorectal cancer, Pharmacokinetics, Area under the curve

Posted Date: July 14th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9591837/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Mitomycin-C (MMC) is the most widely used agent for hyperthermic intraperitoneal chemotherapy (HIPEC) in colorectal peritoneal metastases, though dosing and intervals vary across institutions. This study aimed to compare the pharmacokinetic profiles of three MMC-based HIPEC regimens. Eighteen male Sprague–Dawley rats were randomized into three groups for 90 min of HIPEC at 41–42°C. Group 1 received 35 mg/m² in three divided doses at 0, 30, and 60 min; Group 2 received 40 mg/m² in two divided doses at 0 and 60 min; Group 3 received a single bolus dose of 35 mg/m² at HIPEC initiation. Peritoneal fluid and plasma concentrations were measured by liquid chromatography-tandem mass spectrometry. MMC concentrations differed significantly over time ($p < 0.001$). Group 1 exhibited significantly lower mean peritoneal concentrations (73.63 vs. 75.92 and 79.58 ng/mL; $p < 0.001$) and plasma concentrations (1.59 vs. 2.45 and 2.02 ng/mL; $p = 0.043$) than Groups 2 and 3. The peritoneal-to-plasma AUC ratios were comparable among groups (52.1 vs. 47.7 vs. 47.1, respectively; $p = 0.849$). In conclusion, the three MMC-based HIPEC protocols showed no significant differences in AUC ratios, while Group 1 demonstrated lower plasma MMC concentrations with a comparable peritoneal-to-plasma AUC ratio.

INTRODUCTION

Hyperthermic intraperitoneal chemotherapy (HIPEC), combined with cytoreductive surgery, is an established treatment for peritoneal metastases in patients with colorectal and appendiceal neoplasms.[1, 2] During HIPEC, hyperthermia enhances drug absorption and increases tumor cytotoxicity, facilitating the eradication of microscopic residual cancer cells by inducing apoptosis and inhibiting DNA repair mechanisms.[3, 4] Additionally, the peritoneal–plasma barrier plays a central pharmacologic role in HIPEC by maintaining high locoregional drug concentrations while limiting systemic absorption.[5, 6] To maximize the efficacy of intraperitoneal chemotherapy, optimal drug selection and administration protocols are essential.[6, 7]

Mitomycin-C (MMC) and oxaliplatin have been the most commonly used agents for HIPEC in patients with colorectal cancer and peritoneal metastases. However, following the negative results of the oxaliplatin-based HIPEC arm in the PRODIGE-7 trial,[8] clinical practice has increasingly favored MMC over oxaliplatin.[9] In the 2022 Delphi-based consensus on HIPEC regimens for colorectal cancer, high-dose oxaliplatin received a strong negative vote, whereas high-dose MMC (35 mg/m²) received a weak positive vote.[10] The high-dose MMC HIPEC regimen was identified as the most preferred protocol.

MMC has several pharmacologic advantages for HIPEC, including thermal stability, hydrophilicity, and enhanced cytotoxicity under hyperthermic conditions.[11] Nevertheless, no standardized HIPEC protocol has been universally accepted regarding the dose, timing, and frequency of MMC administration.[12] In the Dutch trial, a triple-dose HIPEC regimen using MMC at 35 mg/m² was administered in three fractions over 90 min.[2] In contrast, the American Society of Peritoneal Surface Malignancies (ASPSM) recommended an MMC-based HIPEC regimen of 40 mg/m², administered in split doses of 30 mg at time

0 and 10 mg at 60 min during a 90-min perfusion.[13] Additionally, a single administration of 30–35 mg/m^2 MMC during 90 min of HIPEC is also frequently employed. Despite the use of various MMC-based HIPEC regimens, evidence comparing the pharmacokinetic profiles of single, split, and fractionated MMC dosing under standardized HIPEC conditions remains limited, particularly in a controlled experimental model.

Therefore, this study aimed to compare the pharmacokinetic characteristics of three intraperitoneal MMC dosing strategies during HIPEC in a rat model and to identify the optimal MMC administration protocol.

RESULTS

Comparison of MMC Concentrations Among Groups During HIPEC

In the analysis of peritoneal fluid, the MMC concentration in Group 1 was 35.93 ± 10.33 ng/mL at 0 min, compared with 85.30 ± 15.84 ng/mL in Group 2 and 91.11 ± 12.27 ng/mL in Group 3. Over the 90-min HIPEC procedure, the MMC concentration in Group 1 reached 73.63 ± 13.34 ng/mL at 90 min, which was comparable to those observed in Group 2 (75.92 ± 27.45 ng/mL) and Group 3 (79.58 ± 17.43 ng/mL), respectively, as shown in Table 1 and Fig. 3a. Group 2, which received an additional 10 mg/m^2 of MMC at 60 min, showed a peak MMC concentration of 104.70 ± 22.53 ng/mL at 60 min. In contrast, Group 3 showed the highest MMC concentration of 91.11 ± 12.77 ng/mL at 0 min. MMC concentrations in peritoneal fluid differed significantly among the groups at 30, 60, and 90 min, respectively. The effect of time on peritoneal concentrations was significant ($p = 0.003$ for 0–30 min, $p < 0.001$ for 0–60 and 0–90 min), with a significant group × time interaction ($p < 0.001$ across all intervals).

Table 1
Time-dependent mitomycin-C concentrations in peritoneal fluid and plasma during HIPEC

Samples	Time (min)	Mitomycin-C concentration (ng/mL)					
		Group 1□	**Group 2**□	**Group 3**□	Overall *p* value (0–30 min)	Overall *p* value (0–60 min)	Overall *p* value (0–90 min)
Peritoneal fluid	0	35.93 ± 10.33	85.30 ± 15.84	91.11 ± 12.27	Group: <0.001 Time: 0.003 Group*Time: <0.001	Group: <0.001 Time: <0.001 Group*Time: <0.001	Group: <0.001 Time: <0.001 Group*Time: <0.001
	15	34.23 ± 5.01	82.11 ± 14.58	84.96 ± 13.39			
	30	49.21 ± 10.52	86.47 ± 13.44	81.16 ± 13.54			
	45	50.70 ± 6.37	74.62 ± 17.11	83.44 ± 12.60			
	60	63.96 ± 12.51	104.70 ± 22.53	78.26 ± 11.55			
	75	65.57 ± 14.22	96.44 ± 19.39	79.79 ± 11.24			
	90	73.63 ± 13.34	75.92 ± 27.45	79.58 ± 17.43			
Plasma	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Group: 0.786 Time: <0.001 Group*Time: <0.001	Group: 0.064 Time: <0.001 Group*Time: 0.083	Group: 0.011 Time: <0.001 Group*Time: 0.043
	15	0.45 ± 0.15	1.14 ± 0.37	1.39 ± 0.82			
	30	0.72 ± 0.34	1.72 ± 0.29	1.75 ± 0.46			
	45	1.10 ± 0.40	2.00 ± 0.24	1.90 ± 0.29			

□ Group 1: Mitomycin-C 35 mg/m² (17.5 mg/m² [0 min] + 8.75 mg/m² [30 min] + 8.75 mg/m² [60 min])

□ Group 2: Mitomycin-C 40 mg/m² (30 mg/m² [0 min] + 10 mg/m² [60 min])

□ Group 3: Mitomycin-C 35 mg/m² at 0 min

60	1.47 ± 0.37	2.20 ± 0.43	2.03 ± 0.35
75	1.64 ± 0.49	2.61 ± 0.30	2.04 ± 0.30
90	1.59 ± 0.37	2.45 ± 0.52	2.02 ± 0.64

- Group 1: Mitomycin-C 35 mg/m² (17.5 mg/m² [0 min] + 8.75 mg/m² [30 min] + 8.75 mg/m² [60 min])
- Group 2: Mitomycin-C 40 mg/m² (30 mg/m² [0 min] + 10 mg/m² [60 min])
- Group 3: Mitomycin-C 35 mg/m² at 0 min

In the plasma analysis, MMC concentrations increased gradually over time, with different time-dependent trends among the groups, as shown in Fig. 3b. In Group 1, plasma MMC concentration reached 0.72 ± 0.34 ng/mL at 30 min, 1.47 ± 0.37 ng/mL at 60 min, and 1.59 ± 0.37 ng/mL at 90 min. In Group 2, the MMC concentration increased gradually, peaking at 2.61 ± 0.30 ng/mL at 75 min after the additional administration of 10 mg/m^2 MMC at 60 min. In Group 3, plasma MMC concentrations increased steadily throughout HIPEC, reaching 2.02 ± 0.64 ng/mL at 90 min. MMC concentrations in plasma differed significantly among the groups at 30 and 90 min after HIPEC initiation.

Comparative Analysis of AUC Ratios Among Groups

The AUCs for Group 1 in plasma and peritoneal fluid were significantly lower than those for Groups 2 and 3 at 30, 60, and 90 min of HIPEC, respectively. From HIPEC initiation to 30 min, the plasma AUC was 10.9 in Group 1, which was lower than the values in Group 2 and Group 3 (29.4; p = 0.005). However, no significant difference observed in plasma AUC between Groups 2 and 3. The peritoneal fluid AUC in Group 1 was 1128.5, which was lower than that in Group 2 (2389.0) and Group 3 (2388.3) (p = 0.003). The peritoneal fluid AUC in Groups 2 and 3 was approximately twofold higher than that in Group 1 at 60 min after HIPEC initiation. The plasma AUC in Groups 2 and 3 was higher than that in Group 1 (Group 1 vs. 2 vs. 3; 42.8 vs. 88.9 vs. 83.3; *p* = 0.006). However, no significant difference observed between Groups 2 and 3 in either plasma or peritoneal fluid AUCs.

At 90 min of HIPEC, the peritoneal fluid AUC was 4608.7 in Group 1, which was lower than that in Group 2 (7674.0) and Group 3 (7110.0) (*p* = 0.004). The 90-min plasma AUC was 83.7 in Group 1, 164.7 in Group 2, and 142.5 in Group 3 (*p* = 0.009). However, no significant differences were observed between Groups 2 and 3 in the plasma and peritoneal fluid AUCs.

The AUC ratios at 30, 60, and 90 min of HIPEC did not differ significantly among the groups. At 30 min of HIPEC, the AUC ratio was 110.4 in Group 1, 79.3 in Group 2, and 80.9 in Group 3 (*p* = 0.692). At 90 min of HIPEC, the AUC ratio in Group 1 was 52.1, comparable to that in Group 2 (47.7) and Group 3 (47.1) (*p* = 0.849)

Notably, the AUC ratio decreased gradually as the HIPEC procedure progressed across all groups. In Group 1, the AUC ratio was 110.4 at 30 min, decreasing to 59.5 at 60 min and 52.1 at 90 min. Groups 2 and 3 showed a similar pattern of decline (Table 2).

Table 2
Comparison of mitomycin-C AUC values during HIPEC in a rat model

Time	Area under the curve (AUC)	Group 1	Group 2	Group 3	Overall	Post hoc analysis		
					p value	G1 vs. G2	G2 vs. G3	G3 vs. G1
0 min–30 min	**$AUC_{peritoneal\ fluid}$**	1128.5 (988.9–1213.0)	2389.0 (2299.5–2660.5)	2388.3 (2373.0–2712.7)	**0.003**	**0.003**	0.373	**0.001**
	AUC_{plasma}	10.9 (9.5–13.1)	29.4 (24.7–32.9)	29.4 (26.3–36.0)	**0.005**	**0.003**	0.478	**0.002**
	AUC ratio	110.4 (76.7–123.5)	79.3 (73.1–89.7)	80.9 (66.9–86.9)	0.692	0.258	0.436	0.209
0 min–60 min	**$AUC_{peritoneal\ fluid}$**	2640.3 (2494.9–2874.1)	4855.1 (4669.6–5573.4)	4801.6 (4560.6–5223.3)	**0.003**	**0.002**	0.457	**0.002**
	AUC_{plasma}	42.8 (34.6–47.6)	88.9 (82.5–99.5)	83.3 (77.4–101.5)	**0.006**	**0.004**	0.414	**0.002**
	AUC ratio	59.5 (52.3–73.5)	56.5 (51.3–59.3)	55.3 (46.9–62.2)	0.884	0.373	0.436	0.313
0 min–90 min	**$AUC_{peritoneal\ fluid}$**	4608.7 (4254.0–4893.3)	7674.0 (7101.8–8957.6)	7111.0 (6931.9–7583.3)	**0.004**	**0.001**	0.276	**0.006**
	AUC_{plasma}	83.7 (77.2–98.6)	164.7 (159.2–169.9)	142.5 (131.9–172.9)	**0.009**	**0.002**	0.258	**0.012**
	AUC ratio	52.1 (42.8–59.9)	47.7 (44.8–49.4)	47.1 (41.8–54.4)	0.849	0.333	0.457	0.294

Median (Interquartile); AUC, area under the curve; AUC ratio* = AUC_{fluid}/AUC_{plasma}

Comparison of Plasma Concentration Gradients

Table 3 presents a comparison of MMC plasma concentration gradients across groups at 30, 60, and 90 min. At 30 min, Groups 2 and 3 displayed steeper slopes than Group 1 (Group 1 vs. 2 vs. 3, 0.024 vs. 0.057 vs. 0.058), as illustrated in Fig. 3b. However, no significant differences were observed in plasma slopes among the groups at 60 and 90 min. Analysis of plasma MMC concentration slopes over the course of HIPEC revealed a gradual decline in all groups during the later stages. In Group 3, the plasma slope decreased from 0.058 at 30 min to 0.030 at 60 min and 0.018 at 90 min.

Table 3
Comparison of plasma mitomycin C concentration slopes during HIPEC

Time	Group	Slope	95% CI	*P* value	Post-hoc analysis		
					G1 vs. G2	G1 vs. G3	G2 vs. G3
0—30 min	**Group 1**	0.024	0.010–0.038	0.002	0.005	0.004	0.995
	Group 2	0.057	0.043–0.072	< 0.001			
	Group 3	0.058	0.044–0.073	< 0.001			
0—60 min	**Group 1**	0.024	0.017–0.031	< 0.001	0.075	0.392	0.637
	Group 2	0.035	0.028–0.042	< 0.001			
	Group 3	0.030	0.023–0.037	< 0.001			
0—90 min	**Group 1**	0.019	0.014–0.023	< 0.001	0.099	0.981	0.066
	Group 2	0.026	0.021–0.030	< 0.001			
	Group 3	0.018	0.014–0.023	< 0.001			

CI, confidence interval

DISCUSSION

Using a rat model, we compared three MMC-based HIPEC regimens and found comparable peritoneal-to-plasma AUC ratios over 90 min of HIPEC. Although the initial MMC concentrations differed among regimens, similar pharmacokinetic profiles were observed at 90 min. The triple-dose regimen (Group 1) showed lower plasma absorption while maintaining a comparable AUC ratio relative to the double-dose (Group 2) and single-dose (Group 3) regimens.

In the pharmacokinetic analysis of MMC across three distinct intraperitoneal dosing regimens during HIPEC, the initial low-dose MMC administration in Group 1 resulted in lower concentrations in both peritoneal fluid and plasma. The initial MMC dose was 17.5 mg/m^2 in Group 1, 30 mg/m^2 in Group 2, and 35 mg/m^2 in Group 3. Because MMC was administered every 30 min in Group 1, peritoneal fluid and plasma MMC concentrations increased gradually, as shown in Fig. 3. Although Group 3 showed a steep increase in MMC concentration from drug administration to 30 min of HIPEC, plasma MMC concentrations declined thereafter due to the absence of additional drug administration. Furthermore, although the total MMC dose administered in Groups 1 and 3 was identical (35 mg/m²), MMC concentrations in peritoneal fluid and plasma showed distinct patterns (Fig. 3). Nevertheless, the AUC ratios between Groups 1 and 3 were comparable (52.1 vs. 47.1; $p = 0.294$).

Importantly, the Group 2 regimen was based on the ASPSM-recommended HIPEC protocol,[13] which employs a total MMC dose of 40 mg/m², and is commonly used in clinical practice. In contrast, Group 1, which received a total dose of 35 mg/m² administered in three divided doses, exhibited the lowest plasma concentrations and highest AUC ratios across all perfusion intervals. These findings suggest that fractionated MMC administration may limit diffusion into the systemic circulation while maintaining adequate local drug exposure, possibly due to a more gradual increase in peritoneal drug concentration and sustained absorption within the peritoneal cavity.

Comparisons between Groups 2 and 3 revealed no significant differences in peritoneal fluid or plasma AUCs, or in AUC ratios, at 30, 60, and 90 minutes of HIPEC. These findings indicate that additional MMC administration during HIPEC increases drug concentrations in both compartments but does not necessarily improve the peritoneal-to-plasma AUC ratio. Therefore, HIPEC dosing strategies should account for the pharmacokinetic characteristics of the peritoneal–plasma barrier to optimize local drug exposure while minimizing systemic absorption.

The pharmacological advantage of HIPEC lies in the peritoneal–plasma barrier, which restricts the diffusion of drugs from the peritoneal cavity into the systemic circulation.[14] This peritoneal–plasma barrier helps maintain high local drug concentrations with relatively low plasma levels, thereby enhancing the efficacy of HIPEC while limiting systemic side effects.[7, 15–17] Given the slow transcapillary diffusion that characterizes the peritoneal–plasma barrier, chemotherapeutic agents administered intraperitoneally achieve markedly higher local concentrations relative to plasma levels.[16] Despite this barrier, MMC is partially absorbed into the bloodstream during HIPEC, raising concerns about systemic toxicity, particularly myelosuppression. Clinical data have demonstrated that greater systemic MMC absorption, as reflected by plasma AUC values, correlates with hematologic toxicity, including postoperative neutropenia.[2, 16] Such hematologic toxicity can prolong recovery, delay subsequent treatment, and increase susceptibility to infections.[15, 16] Accordingly, the triple-dose regimen, Group 1, may help mitigate systemic toxicity, as plasma concentrations remain lower throughout HIPEC.

In the 2022 Peritoneal Surface Oncology Group (PSOGI) International Consensus on HIPEC regimens for colorectal cancer, high-dose MMC-based HIPEC at 35 mg/m^2 was strongly recommended.[10] However, the timing and frequency of MMC administration during HIPEC are critical determinants of its pharmacologic behavior.[12, 18] Our study adds to this literature by providing direct comparative data in a controlled preclinical setting, reinforcing the potential benefits of tailored dosing strategies over conventional bolus administration. Furthermore, fractionated MMC administration may optimize the pharmacokinetic balance between local efficacy and systemic safety, independent of the total dose, as even the higher-dose split regimen in Group 2 did not significantly improve the AUC ratio compared with the triple-fractionated schedule in Group 1.

Although this study evaluated the pharmacokinetics of commonly used MMC-based HIPEC protocols, there are several limitations. First, although the rat model enabled detailed pharmacokinetic analysis, its translational applicability to human HIPEC procedures remains limited, and further validation in clinical trials is warranted. Second, toxicity outcomes were not assessed, precluding conclusions regarding the clinical safety of each regimen. Third, the sample size per group was relatively small (n = 6), which may have limited statistical power, particularly for AUC ratio comparisons. Fourth, the total administered dose differed between Group 2 at 40 mg/m² and the other groups at 35 mg/m², potentially confounding the interpretation of pharmacokinetic differences attributable to dosing schedule alone. Future studies should control for total dose to isolate the effects of administration timing and fractionation. Nevertheless, our findings suggest that fractionated MMC administration during HIPEC may confer pharmacokinetic advantages, supporting further evaluation in preclinical efficacy models and clinical trials. Additional studies should examine the impact of these regimens on antitumor efficacy, toxicity profiles, and long-term outcomes to better define optimal MMC dosing protocols for HIPEC.

CONCLUSION

In a rat model, three commonly used MMC-based HIPEC protocols demonstrated comparable peritoneum-to-plasma AUC ratios across all groups. These findings suggest that the triple-dose MMC regimen may provide a more favorable systemic toxicity profile by limiting drug exposure during the critical early perfusion period, while maintainingan intraperitoneal pharmacokinetic advantage comparable to that of bolus-based strategies.

METHODS

Animal Model and Group Allocation

A total of 18 male Sprague–Dawley rats (8 weeks old, weighing 290–320 g) were used in this study. Animals were acclimatized for one week under standard conditions (temperature: 21–24°C, humidity: 40–60%, 12-hour light/dark cycle) with free access to food and water. Rats were purchased from Orientbio Inc. (Kyunggi-do, Korea). The mean body surface area of the rats was calculated by using the Du Bois method.[19] They were allocated to three groups according to the intraperitoneal MMC

administration protocols used during the 90-min HIPEC procedure, as shown in Fig. 1. Group 1 (triple-dose regimen) received 35 mg/m² MMC in three divided doses: 17.5 mg/m² at HIPEC initiation, followed by 8.75 mg/m² at 30 and 60 min. In Group 2 (double-dose regimen), MMC was administered at 30 mg/m² at HIPEC initiation and 10 mg/m² at 60 min, for a total dose of 40 mg/m². Group 3 (single-dose regimen) received a single dose of 35 mg/m² MMC at the start of HIPEC. The experimental design was developed to replicate clinical high-dose MMC regimens in a rat model.[2, 10, 13]

Experimental Design and HIPEC Procedure

The HIPEC system was constructed using an open-circulation model with inflow and outflow peristaltic pumps that maintained constant fluid recirculation in the abdominal cavity and continuous heating using a heat reservoir, as shown in Fig. 2. The inflow and outflow Master C/L pumps (Bernant, USA; Models 77122-24 and 77200-50) were operated at a flow rate of 50 mL/min. A 2.5-mm-diameter silicone tube was immersed in a warm water bath maintained at 41–42°C by an electric heater during HIPEC. A total of 300 mL of carrier solution mixed with MMC was perfused and circulated. Temperature was monitored at three points: the abdominal cavity, the rat's rectum, and the warm water bath.

Rats were anesthetized with 3% isoflurane inhalation in a 1:1 mixture of oxygen and nitrous oxide. The limbs of the rats were immobilized with adhesive tape in the supine position. Following a 4–5cm midline laparotomy, the abdominal skin and muscle layers were retracted laterally. The edges of the abdominal wall were anchored to an external frame, positioned approximately 10 cm above the incision site at four points using stay sutures, maintaining constant tension and optimal exposure. The MMC–carrier solution mixture (Physioneal® PD-2 1.5% solution, Baxter, USA) was prepared prior to HIPEC initiation. To prevent hyperthermia-induced hypovolemia, 1 mL of normal saline was administered intramuscularly into the thigh before HIPEC and every 30 min thereafter. The HIPEC fluid and core temperatures were continuously monitored using intraperitoneal and rectal probes, respectively.

Sample Preparation and Analytic Method for MMC Concentration

During the HIPEC procedure, 1 mL of peritoneal fluid was collected from the abdominal cavity and transferred to a sterile tube for storage at − 80°C. A total of 0.5 mL of blood was collected into an anticoagulant-treated microhematocrit capillary tube and centrifuged at 3,000 rpm for 10 min at room temperature. The separated plasma supernatant was stored at − 80°C until drug concentration analysis. Peritoneal fluid and plasma samples were collected at 15-min intervals up to 90 min following drug administration (0 [baseline], 15, 30, 45, 60, 75, and 90 min).

MMC concentrations were measured using liquid chromatography–tandem mass spectrometry (LC–MS/MS) following solid-phase extraction, with carbamazepine as the internal standard.[20] Quantitative analysis was performed using an electrospray ionization (ESI) interface coupled to the API3200 mass

spectrometer (AB Sciex, Foster City, CA, USA). The method was validated over a calibration range of 10–4,000 ng/mL, demonstrating high selectivity and reproducibility for measuring MMC in rat plasma.

Statistical Analysis

Data were analyzed using linear mixed-effects models to account for time-dependent variation in MMC concentration across groups. The models included group, time, and their interaction as fixed effects, and were fitted separately for peritoneal fluid and plasma. To determine if concentration-time slopes differed significantly between groups, the group–time interaction was evaluated. Additionally, the area under the curve (AUC) for MMC in both peritoneal fluid and plasma was compared across groups. For pairwise comparisons, Dunn's test was employed following a significant Kruskal-Wallis test. The AUC ratio was defined as the AUC of the peritoneal fluid divided by that of the plasma. A p-value < 0.05 was deemed statistically significant. All statistical analyses were performed using R software (version 4.3.2; R Foundation for Statistical Computing).

Abbreviations

HIPEC, Hyperthermic intraperitoneal chemotherapy

MMC, Mitomycin-C

ASPSM, American Society of Peritoneal Surface Malignancies

AUC, Area under the curve

Declarations

Ethics approval and consent to participate

This study was approved and conducted by the Institutional Animal Care and Use Committee of Yonsei University College of Medicine, Seoul, Republic of Korea (IACUC approval No. 2022-0134). All experiments were performed and reported in accordance with the relevant guidelines and regulations, including the ARRIVE guidelines.[21]

Data availability

The dataset utilized and analyzed in the current research is accessible from the corresponding authors upon reasonable request.

ACKNOWLEDGMENTS

This study was presented as an oral presentation at the International Colorectal Research Summit 2025 Congress in Seoul, Republic of Korea. This study was supported by the Basic Science Research Program

through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (RS-2022-NR069827 and RS-2026-25498127), and grants (2024IF0030, 2025IP0041, 2025IP0028) from the Asan Institute for Life Sciences, Asan Medical Center, Seoul, Republic of Korea.

Authors' contributions

Conceptualisation, E.J.P.; methodology, software and formal analysis S.B, and E. J. P; investigation, K.M and S. A.; resources, E.J.P, K.M, and S.A.; data curation, S.J. and E.J.P; writing—original draft preparation and visualisation, J.S. and E.J.P.; writing—review and editing, S.B, S.A, and E.J.P; supervision, E.J.P; project administration, E.J.P.; funding acquisition, E.J.P.

Funding

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science and ICT (grant number RS-2022-NR069827 and RS-2026-25498127), and grants (2024IF0030, 2025IP0041 and 2025IP0028) from the Asan Institute for Life Sciences of Asan Medical Center, Seoul, Republic of Korea.

Competing Interest

The authors declare no competing conflicts.

References

1. Glehen O, Gilly FN, Boutitie F, Bereder JM, Quenet F, Sideris L, Mansvelt B, Lorimier G, Msika S, Elias D: Toward curative treatment of peritoneal carcinomatosis from nonovarian origin by cytoreductive surgery combined with perioperative intraperitoneal chemotherapy: a multi-institutional study of 1,290 patients. *Cancer* 2010, 116:5608-5618.
2. Verwaal VJ, van Ruth S, de Bree E, van Sloothen GW, van Tinteren H, Boot H, Zoetmulder FA: Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. *J Clin Oncol* 2003, 21:3737-3743.
3. Ceelen WP, Flessner MF: Intraperitoneal therapy for peritoneal tumors: biophysics and clinical evidence. *Nat Rev Clin Oncol* 2010, 7:108-115.
4. Van der Speeten K, Stuart OA, Mahteme H, Sugarbaker PH: A pharmacologic analysis of intraoperative intracavitary cancer chemotherapy with doxorubicin. *Cancer Chemother Pharmacol* 2009, 63:799-805.
5. Flessner MF: The transport barrier in intraperitoneal therapy. *Am J Physiol Renal Physiol* 2005, 288:F433-442.
6. Dedrick RL, Flessner MF: Pharmacokinetic problems in peritoneal drug administration: tissue penetration and surface exposure. *J Natl Cancer Inst* 1997, 89:480-487.

7. Sugarbaker PH, Van der Speeten K, Stuart OA: Pharmacologic rationale for treatments of peritoneal surface malignancy from colorectal cancer. *World J Gastrointest Oncol* 2010, 2:19-30.
8. Quénet F, Elias D, Roca L, Goéré D, Ghouti L, Pocard M, Facy O, Arvieux C, Lorimier G, Pezet D, et al: Cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy versus cytoreductive surgery alone for colorectal peritoneal metastases (PRODIGE 7): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol* 2021, 22:256-266.
9. van de Vlasakker VCJ, Lurvink RJ, Cashin PH, Ceelen W, Deraco M, Goéré D, González-Moreno S, Lehmann K, Li Y, Moran B, et al: The impact of PRODIGE 7 on the current worldwide practice of CRS-HIPEC for colorectal peritoneal metastases: A web-based survey and 2021 statement by Peritoneal Surface Oncology Group International (PSOGI). *Eur J Surg Oncol* 2021, 47:2888-2892.
10. Hübner M, van Der Speeten K, Govaerts K, de Hingh I, Villeneuve L, Kusamura S, Glehen O: 2022 Peritoneal Surface Oncology Group International Consensus on HIPEC Regimens for Peritoneal Malignancies: Colorectal Cancer. *Ann Surg Oncol* 2024, 31:567-576.
11. Sugarbaker PH: Laboratory and clinical basis for hyperthermia as a component of intracavitary chemotherapy. *Int J Hyperthermia* 2007, 23:431-442.
12. Van der Speeten K, Kusamura S, Villeneuve L, Piso P, Verwaal VJ, González-Moreno S, Glehen O: The 2022 PSOGI International Consensus on HIPEC Regimens for Peritoneal Malignancies: HIPEC Technologies. *Ann Surg Oncol* 2024, 31:7090-7110.
13. Turaga K, Levine E, Barone R, Sticca R, Petrelli N, Lambert L, Nash G, Morse M, Adbel-Misih R, Alexander HR, et al: Consensus guidelines from The American Society of Peritoneal Surface Malignancies on standardizing the delivery of hyperthermic intraperitoneal chemotherapy (HIPEC) in colorectal cancer patients in the United States. *Ann Surg Oncol* 2014, 21:1501-1505.
14. Jacquet P, Sugarbaker PH: Peritoneal-plasma barrier. *Cancer Treat Res* 1996, 82:53-63.
15. Kemmel V, Mercoli HA, Meyer N, Brumaru D, Romain B, Lessinger JM, Brigand C: Mitomycin C Pharmacokinetics as Predictor of Severe Neutropenia in Hyperthermic Intraperitoneal Therapy. *Ann Surg Oncol* 2015, 22 Suppl 3:S873-879.
16. Rietveld PCS, Guchelaar NAD, Sassen SDT, Koch BCP, Mathijssen RHJ, Koolen SLW: A Clinical Pharmacological Perspective on Intraperitoneal Chemotherapy. *Drugs* 2025, 85:931-943.
17. Sugarbaker PH, Stuart OA, Vidal-Jove J, Pessagno AM, DeBruijn EA: Pharmacokinetics of the peritoneal-plasma barrier after systemic mitomycin C administration. *Cancer Treat Res* 1996, 82:41-52.
18. Kusamura S, Delhorme JB, Taibi A, Villeneuve L, Deraco M, Dico RL, Glehen O, Moran B: The 2022 PSOGI International Consensus on HIPEC Regimens for Peritoneal Malignancies: Pseudomyxoma Peritonei. *Ann Surg Oncol* 2024, 31:6262-6273.
19. Du Bois D, Du Bois EF: A formula to estimate the approximate surface area if height and weight be known. 1916. *Nutrition* 1989, 5:303-311; discussion 312-303.
20. Moon KS, Song JM, Yi J, Pham QK, Ahn SH: Determination of mitomycin C in rat plasma by liquid chromatography-tandem mass spectrometry and its application for determining pharmacokinetics

in rat. *Heliyon* 2024, 10:e32927.

21. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG: The ARRIVE guidelines animal research: reporting in vivo experiments. *PLoS Biol* 2010, 8:e1000412.

Figures

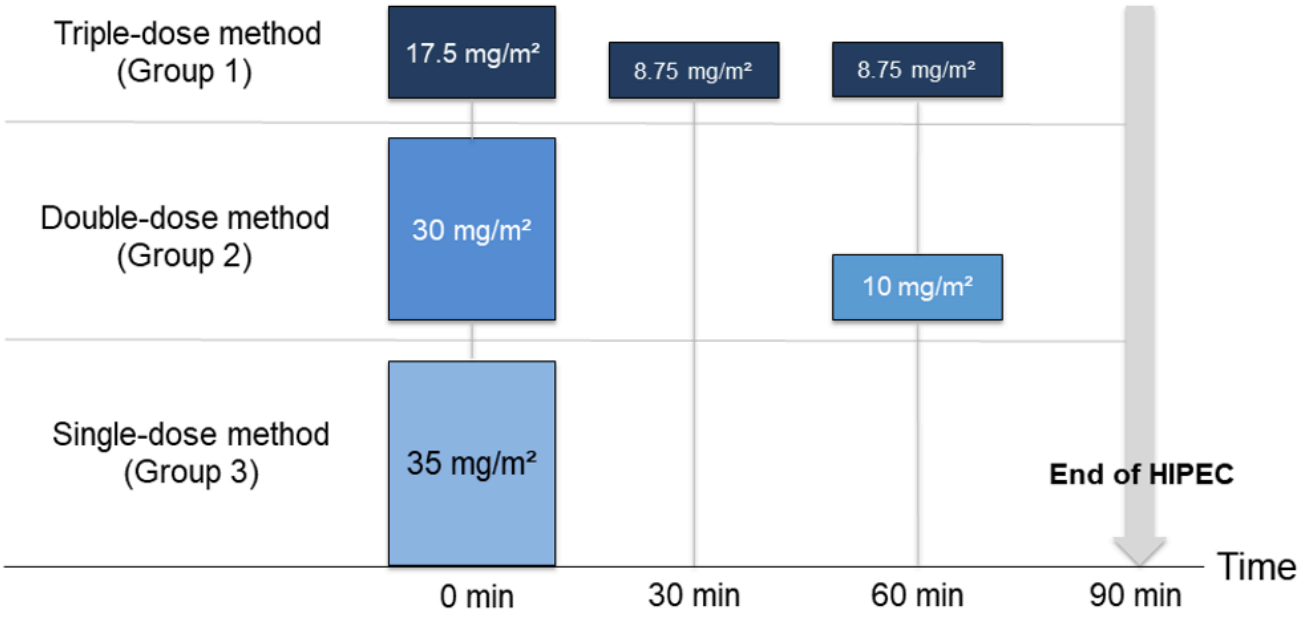


Figure 1

Mitomycin-C administration schedules by group

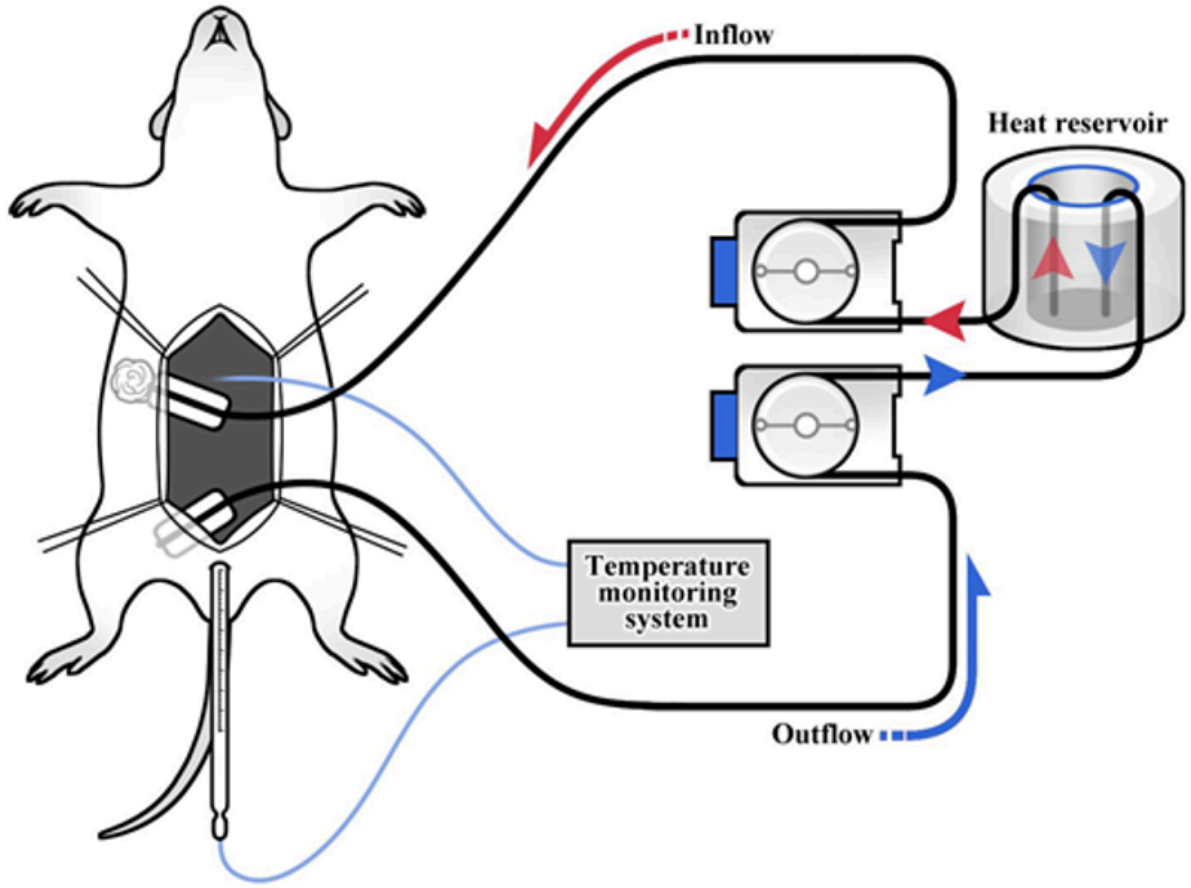


Figure 2

Schematic diagram illustrating the HIPEC system in a rat model

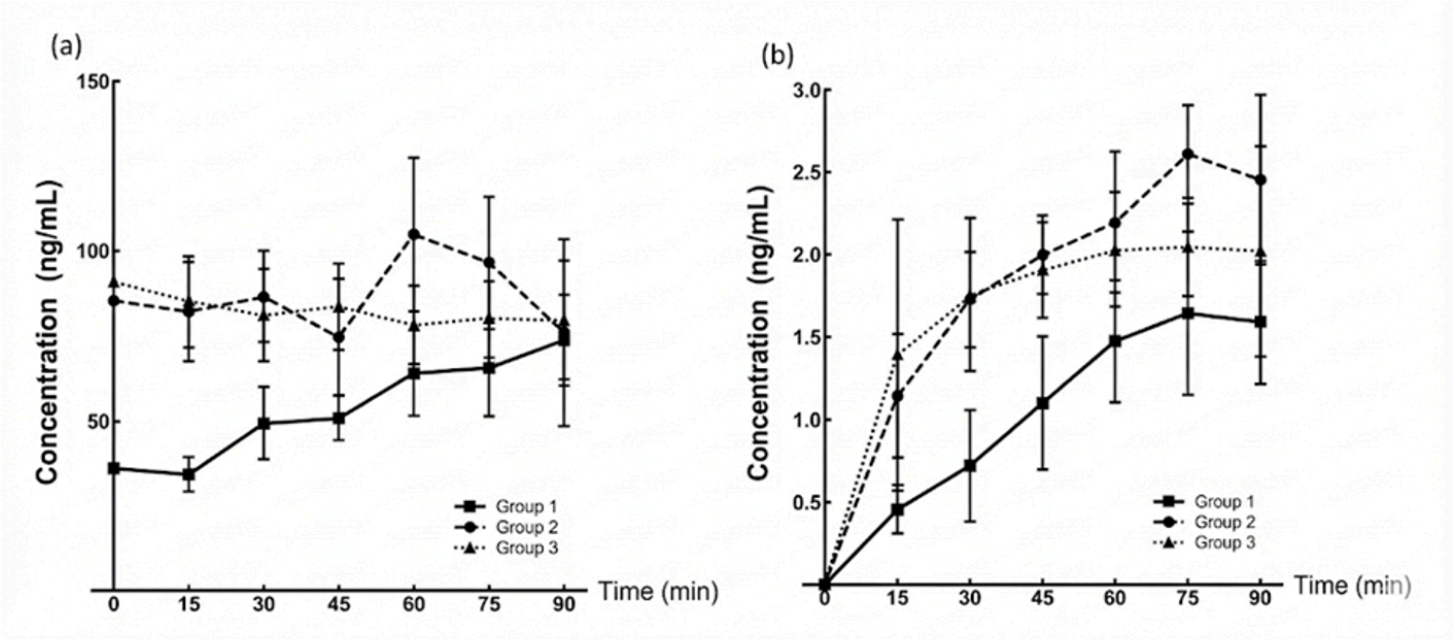


Figure 3

Mitomycin-C concentrations in peritoneal fluid and plasma during hyperthermic intraperitoneal chemotherapy. (**a**) Drug concentrations in peritoneal fluid. (**b**) Drug concentrations in plasma