Disinfecting Effects of Decontamination Solutions and Ultraviolet Irradiation on Exposure to Cyclophosphamide or 5-Fluorouracil Inside Biological Safety Cabinets

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ABSTRACT— ***To ensure safe decontamination in Biological Safety Cabinets (BSCs), we evaluated various solutions and ultraviolet (UV) irradiation against anticancer drugs. Cyclophosphamide (CPA) and 5-fluorouracil (5-FU) were tested. After 16 h of UV exposure, CPA degraded to 42–55% of its initial amount, while 5-FU showed minimal reduction. A single wipe with purified water effectively decreased drug residues, and the combination of purified water and UV irradiation was most effective for CPA. In contrast, a 0.1% benzalkonium chloride solution showed poor performance with or without UV. Overall, physical cleaning with purified water followed by UV irradiation offers a practical and reliable decontamination method in routine hospital practice.***

Keywords— antineoplastic drugs, cyclophosphamide, decontamination, UV irradiation, 5-fluorouracil

1. INTRODUCTION

Anticancer drugs are internationally defined as medications that pose a risk to healthcare professionals; they are termed hazardous drugs (HDs), particularly based on carcinogenicity, teratogenicity, and mutagenicity. In response, guidelines for exposure management that outline procedures for chemotherapeutic drug preparation, transportation, storage, administration, and disposal have been established in Japan, the United States, European countries, and Australia. Each institution implements its facilities and systems according to these guidelines. Using biological safety cabinets (BSCs) is strongly recommended to minimize drug exposure and environmental contamination. The United States National Institute for Occupational Safety and Health (NIOSH) warnings regarding the health impacts of chemotherapy drug exposure in the 2000s recommends adhering to guidelines and using closed drug transfer devices during the preparation and administration of these drugs to prevent leaks. In 1999, combining closed drug transfer devices with BSCs during preparation was demonstrated to significantly reduce exposure to chemotherapy drugs[1]. However, despite these measures, anticancer drug traces are still detectable in BSCs after cleaning.

In Japan, the "Guidelines for Aseptic Preparation of Injectable and Anticancer Drugs" published in 2008 by the Japanese Society of Hospital Pharmacists recommended that during anticancer drug preparation, BSC interiors were to be

disinfected with ethanol prior to operation and wiped with water at least twice after use, using a 0.3 M sodium hydroxide solution. Depending on the contamination type, neutralization with 1% sodium thiosulfate after disabling with 2% sodium hypochlorite (NaClO) was strongly recommended. However, ethanol disinfection is used after neutralization because of possible reverse HD inhalation[2]. The 2015 Joint Guidelines for Exposure Control in Cancer Drug Therapy recommend regularly cleaning BSCs and other preparation environments, alcohol disinfection before work, and wiping with water after use; depending on the anticancer drug type, 2% NaClO or 1% sodium thiosulfate is recommended. As a countermeasure against HD spills, they are recommended to be wiped with absorbent sheets or cotton swabs, followed by repeated washing with detergent and water. Disabling HD with NaClO is considered a weak recommendation because it is limited to be effective against few HD and raise concerns regarding equipment corrosion[3]. The latest 2019 Occupational Exposure Control Guidelines for Oncology Drug Therapy state that using NaClO and sodium hydroxide for routine BSC cleaning is weakly recommended because of concerns regarding human health effects and equipment corrosion[4]. The US USP800 guidelines state that water wiping and surfactant wiping have no statistically significant differences for periodic BSC cleaning; however, using surfactant solutions is weakly recommended[5]. As described, cleaning BSC interiors with an appropriate decontaminant after anticancer drug preparation is necessary; however, a decontaminant that is effective for all anticancer drugs does not exist currently. Therefore, in many clinical practices, BSCs are cleaned with alcohol disinfectants and wiped with water or surfactants after use.

While each medical institution conducts cleaning procedures empirically, numerous reports indicate HD detection in BSCs after anticancer drug preparation[6-9]. Conversely, as an alternative to the less recommended NaClO and sodium hydroxide, research is underway to apply the anticancer drug inactivation effects of ozone water and photocatalysts for decontamination in BSCs[10-17]. However, its effectiveness is limited to certain HDs and its widespread use in medical institutions is hindered by equipment problems. Considering this background, a simple cleaning method that can be performed effectively and safely for daily cleaning as a preventive measure against anticancer drug exposure has not been established. From a cost-benefit perspective, selecting a safe, inexpensive, and highly effective decontamination method is desirable for daily operations.

In contrast, ultraviolet (UV) lamps associated with BSCs use the UV-C wavelength range, which can significantly reduce bacterial infection risk via destroying bacterial and viral DNA and RNA, while also having air purification and surface disinfection effects. When combined with photocatalysts, they can be used to decompose organic substances in the environment. The UV lamps equipped with the BSC can be turned on after preparing the anticancer drugs until the next usage, to maintain BSC interior cleanliness. However, the decomposition effect of UV lamps on anticancer drugs has not been verified, and guidelines in Japan, Europe, the United States, and Australia do not mention this effect.

In this study, we examined the decontamination effects of basic liquids, such as purified water, aqueous detergent solutions, and ethanol for disinfection. Our objective was to establish a simple decontamination method for cleaning BSCs using cyclophosphamide (CPA) and 5-fluorouracil (5-FU) as HDs.

2. METHODS

2.1 Materials:

CPA (pKa = 3.0, Log P = 0.478) and 5-FU (pKa = 8.01, Log P = -1.000) formulations were purchased and used as commercial injectable formulations of Endoxan® for injection (Lot: 5057; Shionogi & Co., Ltd., Osaka, Japan) and 5-FU Injection® (Lot: 22501SF Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan), respectively. CPA and 5-FU bulk powders were purchased from Nacalai Tesque Inc. (Kyoto, Japan) as monohydrate (Lot: M1G0379) and anhydride (Lot: M2E8226), respectively. The chemical structures of the studied anticancer drugs are shown in Fig. 1.

For the benzalkonium chloride (BKC) solution, Osban S® 10% (Lot: S416) was purchased from Nippon Pharmaceutical Co., Ltd. (Kyoto, Japan), and diluted to 0.1 v/v% with purified water for testing. The 0.03 M sodium hydroxide solution (Lot: WEL4365; FUJIFILM Wako Pure Chemical Industries, Ltd., Tokyo, Japan) was diluted with purified water and used for the experiments. Ion-exchanged water was used as purified water. Soft microwiper S220 (PROWIPE, Elleair Paper Co., Ltd., Shizuoka, Japan) was used as the nonwoven paper for the wiping. Other reagents were commercially available special grade reagents. Class II type A (manufactured by SANYO Electric Biomedica Co., Ltd., Kyoto, Japan) was used for the BSC. The BSC was equipped with two ozone-less low-pressure mercury lamps (GL15 sterilizing lamps: UV output: 4.9 W, UV irradiance: 51 μW/cm²; made by Toshiba Corporation, Tokyo, Japan). Stainless steel sheets (0.1 mm × 2 cm × 2 cm and 1 mm × 10 cm × 10 cm), which were similar materials to the BSC working surface, were purchased and used for the anticancer drug loading recovery tests.

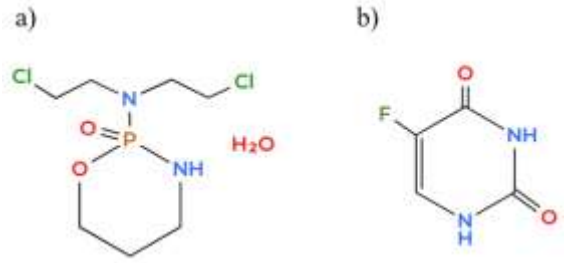

Figure 1: Chemical structures of antineoplastic drugs tested
a) Cyclophosphamide (CPA) b) 5-Fluorouracil (5-FU)

2.2 Effect of the BSC-attached UV lamp on anticancer drug degradation:

CPA or 5-FU bulk powder was dissolved in ion-exchanged water to prepare 0.5 mg/mL, 1.0 mg/mL, and 5.0 mg/mL solutions. For each 2 cm × 2 cm stainless-steel sheet, 4 µL of each solution was dried on it at room temperature via dropping (20 µL total) with a microsyringe in five spots. Four 2 cm × 2 cm stainless-steel sheets with anticancer drugs dripped and dried on them were placed at the BSC center, and two UV lamps (GL15 sterilizing lamps) attached to the BSC top were illuminated to irradiate the UV. UV lamp irradiation times were 0 min, 30 min, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, and 16 h. After removal following an irradiation period, a stainless-steel sheet was placed in a covered test tube, 2 mL purified water was added, was stirred with a stirrer for 10 min, and anticancer drugs were extracted. The residual CPA or 5-FU from each supernatant was quantified using the high-performance liquid chromatography (HPLC) method.

2.3 Single cleaning effect of CPA and 5-FU with various decontamination solutions:

The experiment was conducted following the procedure outlined by Lamerie et al. [18]. Injectable endoxane (15 µL, equivalent to 300 µg CPA and 750 µg 5-FU) at concentrations of 20 and 50 mg/mL for injectable 5-FU were left at room temperature for 10 min via dropping on a stainless-steel sheet (1 mm × 10 cm × 10 cm thickness) using a micropipette at a single spot. PROWIPE was saturated with 500 µL decontamination solution (purified water, 0.1 v/v% BKC solution, disinfecting ethanol, 0.02 v/v% NaClO solution, 0.2 v/v% NaClO solution, or 2 v/v% NaClO solution), and a single cleaning procedure was performed using each decontamination solution. Subsequently, the stainless-steel plate was wiped with PROWIPE that was moistened with 500 µL of 0.03 M sodium hydroxide solution to collect any remaining anticancer drugs on the stainless-steel plate. After cleaning with 0.03 M sodium hydroxide, PROWIPE was placed in a 50 mL polyethylene centrifuge tube, 30 mL purified water was added, and the mixture was subjected to ultrasonic extraction (1 h). The supernatant liquid (2 mL) in the centrifuge tube was separated and filtered through a membrane filter (0.2 µm) to serve as measurement data. The CPA and 5-FU residual quantities in the samples were measured using HPLC-tandem quadrupole mass spectrometry (LC-MS/MS).

2.4 Effects of a single wiping with decontamination solution and the combination with a BSC-attached UV lamp irradiation on anticancer drugs:

The experiments were performed according to the method proposed by Lamerie et al.[18]. For each endoxane injection formulation at 20 mg/mL or 5-FU at 50 mg/mL, 15 µL (equivalent to 300 µg CPA and 750 µg 5-FU, respectively) was dropped onto a stainless-steel sheet (1 mm thick × 10 cm × 10 cm) using a micropipette and left at room temperature for 10 min. PROWIPE was soaked with either purified water or a 0.1% BKC solution (500 µL) as a decontamination solution, which was used for wiping once. Two stainless steel plate sets wiped with the decontamination solution were prepared; one set was placed in the BSC center for 16 h under UV lamp irradiation, and the other set was wiped with a PROWIPE soaked in 0.03 M sodium hydroxide solution (500 µL) without UV lamp irradiation and it collected the remaining anticancer drugs on the stainless-steel plate. After wiping with a 0.03 M sodium hydroxide solution, PROWIPE was placed in a 50 mL polyethylene centrifuge tube, and 30 mL purified water was added for ultrasonic extraction (1 h). The supernatant (2 mL) from the centrifuge tube was taken, filtered through a membrane filter (0.2 µm, ADVANTEC Co., Ltd., Tokyo, Japan), and used as the measurement sample. Residual CPA and 5-FU quantities in the samples were measured using LC-MS/MS.

2.5 Measuring residual CPA and 5-FU levels after UV lamp irradiation using HPLC:

The CPA or 5-FU concentration in the samples was measured using reversed-phase UV-HPLC. LC-20AD (Shimadzu Corporation, Kyoto, Japan) was used for the HPLC pump. The SPD-20A (Shimadzu Corporation) UV detector, SIL-

20AC (Shimadzu Corporation) autosampler, and CTO-20A (Shimadzu Corporation) column thermostat were used. Quantitative analyses were performed using the absolute calibration curve method. Detailed HPLC measurement conditions for CPA and 5-FU are shown in Table 1. The calibration curve for CPA showed good linearity through the origin ($R^2 > 0.999$), with a minimum detection limit of 0.5 µg/mL. The calibration curve for 5-FU also showed good linearity ($R^2 > 0.999$) for both compounds, with a minimum detection limit of 0.025 µg/mL.

Table1. HPLC and LC-MS/MS method for cyclophosphamide (CPA) and 5-fluorouracil (5-FU)

HPLC	cyclophosphamide (CPA)	5-fluorouracil (5-FU)
Column	Cosmosil®C18 （5µm, 4.6 i.d. × 150 mm）	Cosmosil®C18 （5µm, 4.6 i.d. × 150 mm）
Wavelength	195 nm	254 nm
Temperature	40 ℃	40 ℃
Mobile phase	Acetonitrile: distilled water 25: 75	40 mM phosphate buffer (pH7.4)
Flow rate	1.0 mL/min	1.0 mL/min
Analytical time	15 min	15 min
LC-MS/MS	cyclophosphamide (CPA)	5-fluorouracil (5-FU)
Detection mode	ESI (＋)	ESI (－)
Temperature	40 ℃	40 ℃
Mobile phase	0.1% formic acid: acetonitrile 50: 50	0.1% formic acid: acetonitrile 95: 5
Flow rate	0.6 mL/min	0.6 mL/min
Analytical time	10 min	10 min
m/z	261/140	129/42

The detection limits of CPA and 5-FU by HPLC were 0.1 and 0.025µg/mL, respectively. The LC-MS/MS system was constructed with LC system and 8040 mass spectrometers (Shimadzu Ltd. Kyoto). The detection limits of CPA and 5-FU were 0.1 and 0.05µg/mL, respectively.

2.6 Measuring residual CPA and 5-FU levels after one-time cleaning with various decontamination solutions:

The residual CPA and 5-FU quantity after one-time cleaning with various decontamination solutions was measured using the quadrupole LC-MS/MS method. The HPLC pump (LC-20AD, Shimadzu Corporation), MS detector (LCMS-8040 mass spectrometer, Shimadzu Corporation), autosampler (SIL-20ACHT, Shimadzu Corporation), and column insulation tank (CTO-20AC, Shimadzu Corporation) were employed for the analysis. Quantitative analyses were conducted using the absolute calibration curve method. The calibration curve for CPA exhibited good linearity through the origin ($R^2 > 0.999$), with a minimum detection limit of 0.1 ng/mL. Similarly, the calibration curve for 5-FU displayed good linearity through the origin ($R^2 > 0.992$), with a minimum detection limit of 0.05 ng/mL. Additional LC-MS/MS measurement conditions are presented in Table 1.

2.7 Measuring the residual CPA and 5-FU quantities after a single cleaning with decontamination solution and BSC-attached UV lamp irradiation:

To measure the residual quantity after a single cleaning with decontamination solution and UV lamp irradiation within the BSC, the analysis was outsourced to Shionogi Pharma Co., Ltd. (Osaka, Japan), and performed using the quadrupole tandem LC-MS/MS method. The detection limits for CPA and 5-FU were 0.02 ng/mL and 0.1 ng/mL, respectively.

2.8 Data analysis:

Numerical data are presented as means ± SD unless otherwise noted. The HPLC analysis reproducibility was confirmed by the coefficient of variation (CV, %) of the measured values in the repeated measurements. The rate constants for drug degradation were estimated using Phoenix 64 WinNonlin (Ver. 8.3.3.3.33), assuming a first-order degradation process. Repeated one-way analysis of variance (ANOVA) and two-way ANOVA were performed to investigate the effects of decontamination agents and UV irradiation on each anticancer drug. A risk ratio of 0.05% or less was considered significant.

3. RESULTS

3.1 Decomposition effect of BSC-attached UV lamps:

The anticancer drug recovery rate from stainless steel sheets was 93% (n = 10, CV = 0.7%) for CPA and 95% (n = 10, CV = 2.7%) for 5-FU when anticancer drug solutions were dropped onto 2 cm × 2 cm stainless steel sheets (thickness 0.1 mm) and dried. This indicates that the anticancer drug recovery procedure on stainless steel sheets was performed

accurately and quantitatively. Fig. 2 and Table 2 show, respectively, the change over time in the residual quantity of CPA or 5-FU and their kinetic degradation parameters obtained from the residual amount-time data for CPA or 5-FU after dropwise drying on a 2 cm × 2 cm stainless steel sheet followed by UV lamp irradiation in the BSC for 16 h.

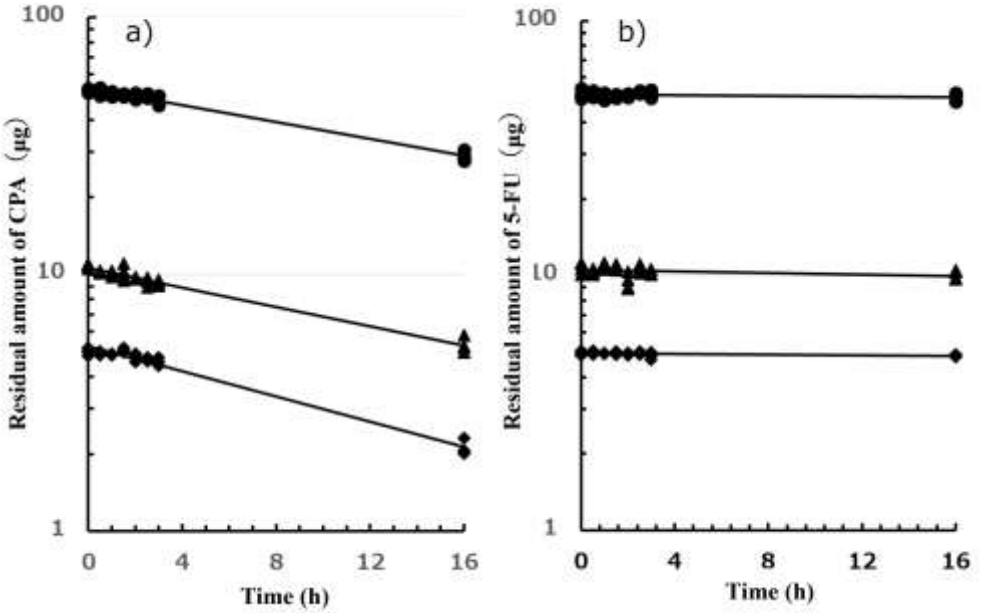

Figure 2. Degradation of (a) CPA and (b) 5-FU plotted as a function of logarithmic residual quantities during UV irradiation
Each regression line was fitted via the nonlinear least squares method, Phoenix 64 WimNonlin (Ver. 8.3.3.33). Initial quantities of CPA (a) and 5-FU (b) loaded on 2 cm × 2 cm stainless sheets were 5 μg (◆),10 μg (▲) and 50 μg (■). Residual quantities were calculated via dividing the quantity of antineoplastic drugs at time t after UV irradiation by their initial quantities.

Table 2: Kinetic analysis for the degradation of CPA and 5-FU under UV irradiation for 16 hours

	Initial loaded CPA (mg)			Initial loaded 5-FU (mg)		
	5	10	50	5	10	50
Slope of regression line	− 0.025*	− 0.019*	− 0.016*	− 0.001*	− 0.0015*	− 0.001*
Coefficients of determination	0.978*	0.974*	0.976*	0.217	0.078	0,087
X_0 (mg)	5.3	10.3	53.3	5.0	10.5	51.5
K (h^{-1})	0.057	0.043	0.038	0.002	0.003	0.002
$T_{1/2}$ (h)	12.2	16	18.3	441.6	221.6	427.0

The values are calculated by fitting a logarithmic regression line between residual amount and time. Significances for the slope of regression line and coefficient of determination were determined by distribution F-test. *): $p<0.05$. Kinetic Parameters were estimated by WinNonlin (Ver.8.3.3.33). X_0: estimated initial loading dose (mg); k: degradation rate constant (h^{-1}); $T_{1/2}$: half-life (h).

For CPA, the residual quantities decreased over time at all initial loads. The mean residual quantities (residual rates) at 16 h after UV irradiation were 2.1 ± 0.1 μg (42%), 5.3 ± 0.4 μg (49%), and 28.2 ± 0.5 μg (55%), respectively, compared to the initial loading doses of 5 μg, 10 μg, and 50 μg (Fig. 2a). The residual CPA quantity in the CPA residual rate-time curve was assumed to follow a first-order degradation reaction, and the UV degradation rate constants for each initial load were calculated to be 0.057 h^{-1}, 0.043 h^{-1}, and 0.038 h^{-1}, respectively ($p < 0.01$). Conversely, for 5-FU, the mean residual quantities (residual rates) for the same initial loads were 4.8 ± 0.0 μg (96%), 10.2 ± 0.4 μg (102%), and 50.1 ± 2.0 μg (100%), respectively (Fig. 2b). The residual quantities did not decrease on the stainless-sheets over time at any loading dose. Assuming that 5-FU degradation was due to a first-order reaction, the degradation rate constants were calculated to be 0.002 h^{-1}, 0.003 h^{-1}, and 0.002 h^{-1}; those parameter values did not show predominance at any initial concentration (Table 2).

3.2 Single wiping effect on CPA and 5-FU with various decontamination solutions:

Table 3 shows the effect of single wiping with various decontamination solutions on CPA and 5-FU.

Table 3. Effects of one-time wiping with representative decontamination solutions on the residual amount of CPA loaded on 10 cm × 10 cm stainless sheet

CPA		
Decontamination solution	Residual amount (ng)	Mean residual amount per uniti areas (ng/cm^2)
0.02% (v/v) NaClO	95.1±91.0	1.0±0.9
0.2% (v/v) NaClO	30.4±32.6	0.3±0.3
2% (v/v) NaClO	48.2±52.5	0.5±0.5
Distilled water	29.4±22.6	0.3±0.2
0.1% (v/v) benzalkonium chloride	223.1±407.3	2.2±4.1
Disinfectant ethanol	1460.5±730.7	14.6±7.3
5-FU		
Decontamination solution	Residual amount (ng)	Mean residual amount per uniti areas (ng/cm^2)
0.02% (v/v) NaClO	1346.89±1979.8	13.5±19.8
0.2% (v/v) NaClO	318.2±454.8	3.2±4.5
2% (v/v) NaClO	329.0±250.4	3.3±2.5
Distilled water	1320.7±1478.8	13.2±14.8
0.1% (v/v) benzalkonium chloride	144.6±1019.0	14.4±10.2
Disinfectant ethanol	30.4±32.6	0.3±0.3

For CPA, when comparing the average residual CPA quantities with various decontamination solutions after one-time cleaning, the decontamination effect magnitude was ranked as follows: purified water ≧ 0.2 v/v% NaClO > 2% NaClO > 0.02% NaClO > 0.1% BKC solution > ethanol for disinfection. For CPA, a single wipe with purified water showed the lowest residual quantity and was the most effective. However, 0.1% BKC solution and ethanol for disinfection showed significantly higher residual quantities after a single wiping, with quantities 8.3 and 18.3 times higher than those for purified water, respectively, indicating that wiping with ethanol was the least effective. Decontamination with purified water or 0.1% BKC showed no significant difference in the 5-FU residual quantity, but purified water tended to be more effective. For 5-FU, the decontamination effect magnitude was: 0.2 v/v% NaClO ≒ 2% NaClO > purified water ≒ 0.02% NaClO > 0.1% BKC solution > ethanol for disinfection. For 5-FU, a single wiping with 0.2 v/v% NaClO resulted in the lowest residual volume. However, cleaning with purified water was almost equivalent to 0.02% NaClO, and the residual quantity after a single wiping was lower than that with 0.1% BKC solution or disinfectant ethanol. The residual quantity with 0.1% BKC solution or disinfectant ethanol was 1.1 and 24.1 times higher than that with purified water, indicating that cleaning with disinfectant ethanol was the least effective for decontamination, as observed with CPA.

3.3 Effect on residual CPA and 5-FU levels after wiping once with decontamination solutions and BSC UV lamp exposure:

Purified water and 0.1% BKC solution were used for the decontamination solution. Table 4 shows the two-way ANOVA results for CPA and 5-FU residual rates (%), considering two levels (cleaning with decontamination solution and UV irradiation) and two factors (decontamination solution type used for wiping and UV irradiation use). Here, we compared the residual rates of anticancer drugs dropped on a 10 cm × 10 cm stainless steel sheet after wiping with purified water or a 0.1% BKC solution, followed by 16 h of UV lamp irradiation. For CPA, the mean residual rate after cleaning with purified water as a decontamination solution was $0.80 \pm 0.06 \times 10^{-3}$ (%) and $2.01 \pm 1.65 \times 10^{-3}$ (%) in the presence and absence of UV irradiation, respectively. The mean residual rate after wiping with 0.1% BKC solution as a decontamination solution was $5.65 \pm 2.21 \times 10^{-3}$ (%) and $46.10 \pm 32.30 \times 10^{-3}$ (%) in the presence and absence of UV irradiation, respectively. Although the residual rate decreased by approximately 50% after UV irradiation, the mean residual rate decreased by an order of magnitude when purified water was used as a decontamination solution. For 5-FU, the average residual rates after cleaning with purified water as a decontamination solution were $3.19 \pm 0.44 \times 10^{-3}$ (%) and $4.34 \pm 2.19 \times 10^{-3}$ (%) in the presence and absence of UV irradiation, respectively. The average residual rates after wiping with 0.1% BKC solution as a decontamination solution were $48.20 \pm 34.29 \times 10^{-3}$ (%) and $39.80 \pm 47.90 \times 10^{-3}$ (%), respectively. The UV irradiation effect was negligible, using purified water as a decontamination solution loweredthe average residual rate by one order of magnitude. The two-way ANOVA ($p < 0.05$) result indicates that using purified water as a decontamination solution contributed to a lower residual CPA or 5-FU rate than with UV irradiation. Cleaning with purified water was more effective than that with 0.1% BKC solution. Conversely, although the decontamination effect of combining cleaning with decontamination solution and UV irradiation was not significant for either anticancer drug, comparing the p-values for each anticancer drug interaction showed that the CPA p-value was

small at 0.07 (vs. 0.51), indicating that using purified water and UV irradiation for CPA decontamination was more effective than using 0.1% BKC solution.

Table 4. Effects of one-time wiping with decontamination solution plus UV lamp irradiation on residual rate (%) of CPA and 5-FU loaded on 10 cm × 10 cm stainless sheet

CPA	Decontamination solution	UV irradiation	Residual rate $\times 10^{-3}$(%)
	Distilled water	–	2.10±1.65
	0.1% (v/v) benzalkonium chloride	–	46.10±32.30
	Distilled water	+	0.80±0.06
	0.1% (v/v) benzalkonium chloride	+	5.65±2.11

ANOVA	factor	Degree of freedom	Deviation square sum	Unbiased dispersion	Dispersio ratio	p-value	Verdict
	All	9	4173.1				
	UV irradiation	1	822.6	822.6	2.52	0.11	n.s.
	Decontamination solution	1	494.3	494.3	1.51	0.03	P<0.05
	Interaction	1	570.6	570.6	4.63	0.07	P=0.07.
	Error	6	2285.6	326.5			

5-FU	Decontamination solution	UV irradiation	Residual rate $\times 10^{-3}$(%)
	Distilled water	–	4.34±2.19
	0.1% (v/v) benzalkonium chloride	–	39.80±47.90
	Distilled water	+	3.10±0.44
	0.1% (v/v) benzalkonium chloride	+	48.20±34.29

ANOVA	factor	Degree of freedom	Deviation square sum	Unbiased dispersion	Dispersio ratio	p-value	Verdict
	All	9	9346.38				
	UV irradiation	1	150.08	150.08	0.24	0.64	n.s.
	Decontamination solution	1	5210.07	5210.07	8.47	0.03	P<0.05
	Interaction	1	296.46	296.46	0.48	0.51	n.s.
	Error	6	3689.77	614.96			

The residual rate (%) means residual amount of CPA or 5-FU against the individual amounts loaded on the 10 cm × 10 cm stainless sheet. Each value represents the mean±SD of six experiments.

4. DISCUSSION

In recent years, cleaning of work environments has been emphasized as an important measure for contamination control; however, standardized cleaning methods have not yet been established. In the 1990s, potassium permanganate and sodium hypochlorite (NaClO) were reported to effectively degrade anticancer drugs. The American Society of Health-System Pharmacists recommends cleaning with NaClO, the National Institute for Occupational Safety and Health advises the use of appropriate cleaning agents, and the Society of Hospital Pharmacists of Australia recommends the use of strong alkaline cleaners[19]. In wipe tests for anticancer drugs, NaClO demonstrated the highest effectiveness, while household detergents also achieved removal rates exceeding 90%. Nevertheless, no single cleaning agent is currently effective against all anticancer drugs, making it necessary to select different agents according to the physical properties of each drug[3,4]. Furthermore, the Australian Society of Hospital Pharmacists' Code of Practice for the Safe Handling of Cytotoxic Drugs recommends wiping the interior surfaces of biological safety cabinets (BSCs) with alcohol at the beginning and end of injectable anticancer drug preparation and disinfecting the cabinet floor with ethanol at the end of preparation[20]. At least once a week, it is recommended that the interior surfaces of the BSC be wiped with an appropriately diluted strong alkaline or neutral surfactant, purified water, and disinfectant ethanol. Similarly, European guidelines acknowledge the effectiveness of NaClO, but place strong emphasis on safety issues such as corrosion, chlorine gas generation, and material compatibility[21]. Overall, NaClO has been widely accepted as an effective decontamination method, but the precautions and practical emphases regarding its use vary among regions. However, in the United States, Australia, Europe, and Japan, none of the guidelines refer to the decontamination effect of UV lamp irradiation during the period between the end of BSC use and the next use. Therefore, in this study, we investigated the decontamination effects of anticancer drugs in BSCs by UV irradiation in comparison with cleaning using decontamination solutions.

The study endpoint was set at 16 hours, considering an anticancer drug preparation cycle in daily practice. The residual CPA quantity decreased with the UV irradiation duration; the degradation rate constant was independent of the initial CPA dose, indicating that CPA underwent a first-order degradation reaction under UV irradiation (Table 2). However, the 5-FU decrease in UV persistence over time after 16 h was less and the UV lamps were unable to decompose the remaining 5-FU during daily operations. The maximum cumulative light in the BSC used in this study was 5875.2 mJ/cm^2, which was calculated considering the UV lamp illumination. Associated UV lamps are designed to emit 254 nm germicidal rays most efficiently, accounting for approximately 90% of the UV and visible radiation, with little UV radiation emitted at 185 nm. UV light is thought to decompose organic compounds via hydroxyl radical and ozone formation, with directly breaking organic chemical bonds[22]. Environmental water and oxygen are involved in the decomposition process. A 185 nm UV wavelength reacts with water to generate hydroxyl radicals and with oxygen to generate ozone. A 254 nm UV wavelength decomposes ozone to form reactive oxygen or causes ozone to react with reactive oxygen and water to generate hydroxyl radicals[23]. Therefore, CPA degradation via UV irradiation is thought to involve direct action and radical formation reactions. Kim et al.[24] studied the scavenging properties of pharmaceutical components via UV treatment and indicated that drugs that were less likely to be degraded by UV alone were more likely to be degraded by both 185 and 245 nm wavelengths when 30 drugs were dissolved in water and irradiated with UV alone or a 245 nm wavelength, because 185 nm UV generates hydroxyl radicals from water, thereby promoting a direct degradation effect by 254 nm UV. The UV lamp used in this study was ozone-less; hence, environmental ozone was considered to not have contributed to CPA degradation, and no direct chemical bond cleavage by 254 nm UV was assumed possible for 5-FU, although contributions of ozone-related radical reactions were insignificant. In our experiment, CPA or 5-FU was loaded onto a stainless-steel sheet and dried. Water is necessary for UV degradation; therefore, the residual 5-FU was measured using UV irradiation without drying in a preliminary experiment. 5-FU was found to be less sensitive to 254 nm UV than CPA (data not shown). Therefore, 5-FU was not degraded at the same UV dose, probably because of bonding state of the atoms that make up the molecule; however, the details are unclear.

Ozone is reactive and utilized for deodorization, decolorization, and disinfection. However, its exposure to the human body can cause throat irritation, upper respiratory tract irritation, cough, and dyspnea. The Japan Society for Occupational Health working environment standards set the permissible ozone concentration at 0.1 ppm[25], and safety management standards for ozone-generating equipment does not allow this concentration to exceed. The ozone-less UV lamp used in this study did not affect the environment or human body. A bactericidal UV lamp can be easily activated only after completing anticancer drug preparation. This is a safe and cost-effective solution that does not generate waste. As shown in Table 3, for typical decontamination solutions described in the Occupational Exposure Guidelines (Fiscal 2019 Edition)[3], the CPA or 5-FU removal rate after wiping once was the highest when purified water was used, and least for 0.1% BKC solution and ethanol. Although the physically removing anticancer drugs contributed significantly to cleaning, CPA, which has a higher lipid solubility, dissolved more easily in ethanol than in water because of its 0.478 log P. However, the disinfection effectiveness of purified water > 0.1% BKC solution > ethanol shows that the contribution of physical removal is superior. Conversely, as the log P of 5-FU is -1.000, 5-FU has high solubility in water and low solubility in ethanol. Therefore, its solubility in purified water is superior to that in ethanol. The decontamination effect of 0.1% BKC solution was higher than that of ethanol for both CPA and 5-FU, but inferior to that of purified water. Decontamination solutions containing alcohol and surfactants have a weaker physical removal effect.

The Occupational Exposure Control Guidelines (2019 edition)[4] state that NaClO is among the typical decontamination solutions described for anticancer drug decontamination. However, NaClO solution is weakly recommended for daily BSC cleaning. Clinical NaClO concentrations are 0.01 to 0.05 v/v for hand/skin antisepsis, 0.005 to 0.01 v/v for surgical site (surgical field) skin and mucous membrane antisepsis, 0.02 to 0.05 v/v for disinfecting medical equipment, operating rooms, hospital rooms, furniture, instruments, etc., and 0.1 to 1 v/v% for waste and hepatitis B virus antisepsis. According to a report by Okawa and Shiraishi et al.[26,27] investigating the effects of NaClO on stainless steel, after immersing it in a 0.05 v/v% NaClO solution for 72 h showed no significant effects. However, when it was immersed in a 0.1 v/v% NaClO solution, some rusting occurred. Therefore, using 0.1 v/v% or higher concentrations for cleaning BSC, which mostly comprise a stainless-steel work surface, could irreversibly damage it. The USP 800 [4] also mentions using oxidizing agents such as NaClO and peroxides to inactivate HD. However, the product hazard, respiratory disorders, and equipment damage have been indicated to be related to NaClO usage, which requires neutralization with sodium thiosulfate and wiping with disinfecting alcohol, purified water, and detergent for removal. Physically removing HD is important, and periodically cleaning BSCs (Containment Primary Engineering Control) is recommended. Cleaning and disinfection operations, including HD removal using purified water, detergents, surfactant solutions, etc., are also required.

Many medical institutions in the country utilize purified water, surfactant solutions, and disinfectant ethanol for the daily BSC cleaning. Surfactants and ethanol are commonly employed as antiseptic solutions for infection control in medical settings, and in BSCs, they are bactericidal and a significant disinfectant. Therefore, they are used for wiping and

cleaning for infection control. However, this study concluded that cleaning with a 0.1% BKC solution and disinfectant ethanol resulted in a lower and significantly lower removal rate than that with cleaning with purified water, respectively. Therefore, cleaning once with a detergent solution and ethanol was considered insufficient for anticancer drug decontamination.

Cleaning with the decontamination solution contributed more than exposure to UV irradiation, as indicated in Table 4. This observation supports the effectiveness of physically removing anticancer drugs. When purified water and a 0.1% BKC solution were used as decontamination solutions, the decontamination effect was higher than when wiped with purified water. CPA could be decontaminated more effectively via wiping once with purified with a 16 h UV irradiation; this aligns with the UV irradiation experiment in Fig. 2. Conversely, when only a 0.1% BKC solution was used, the decontamination effect was lower for both CPA and 5-FU compared to purified water usage. The anticancer exposure index for CPA proposed by Sessink[28] has already been published, according to which, the residual quantity on stainless steel plates shown in Table 3 was classified as risky. Even wiping once with purified water was able to reduce the environmental contamination to the targeted level (< 0.1 ng/cm^2) with or without UV irradiation. However, stainless-steel sheets wiped with a 0.1% BKC solution showed a caution level (1−10 ng/cm^2) with or without UV irradiation. No specific exposure index values have been set for 5-FU; however, the residual quantity after disinfection with 0.1% BKC solution was high, regardless of UV irradiation use. This suggests that cleaning with a 0.1% BKC solution alone was insufficient for decontamination.

5. CONCLUSION

We re-examined the decomposition effect of anticancer drugs using a UV lamp attached to the BSC, and pure water, 0.1% BKC solution, and disinfection ethanol for BSC interiors. Wiping with purified water was most effective in physical decontamination. Approximately 50% CPA was degraded in a dose-independent manner after UV irradiation for 16 h, whereas 5-FU did not show similar results. We conclude that physical decontamination with purified water is effective for decontaminating HDs in BSC, and that some anticancer agents are effectively degraded by the attached UV lamp. Guideline adherence, determining decontamination solution combinations, and UV irradiation are considered important for developing convenient and safe routine decontamination procedures, despite requiring further studies for other anticancer agents.

6. ACKNOWLEDGEMENT

The authors thank Shionogi Pharma Co. Ltd. for their cooperation for the paid rapid determination of CPA and 5-FU concentrations via LC/MS/MS for some samples.

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