Research Article

Asian Jour Hosp Phar, Vol: 5, Issue:4, 2025, 19-27

Asian Journal of Hospital Pharmacy

Content Available at www.ajhponline.com ISSN: 2583-0724

EVALUATING THE DECONTAMINATION EFFECTS OF HYPOCHLOROUS ACID WATER ON CYCLOPHOSPHAMIDE AND 5-FLUOROURACIL

Mayumi Nakaishi[1], Koki Takeda[2], Asako Nishimura[2], Akiko Kiriyama[3], Nobuhito Shibata[*4]

[1]Graduate Student, Department of Medical Pharmaceutics, Faculty of Pharmaceutical Sciences, Doshisha Women's College of Liberal Arts, Kyotanabe, Kyoto610-0395, Japan

[2]Assistant Professor, Department of Medical Pharmaceutics, Faculty of Pharmaceutical Sciences, Doshisha Women's College of Liberal Arts, Kyotanabe, Kyoto610-0395, Japan

[3]Professor, Department of Pharmacokinetics, Faculty of Pharmaceutical Sciences, Doshisha Women's College of Liberal Arts, Kyotanabe, Kyoto610-0395, Japan

[*4]Professor, Department of Medical Pharmaceutics, Faculty of Pharmaceutical Sciences, Doshisha Women's College of Liberal Arts, Kyotanabe, Kyoto610-0395, Japan

Received: 19 Aug 2025 Revised: 06 Sept 2025 Accepted: 10 Oct 2025

Abstract

Hypochlorous acid water (HAW) is widely used for disinfection in medical settings, yet its ability to decompose hazardous anticancer drug residues remains unclear. This study evaluated the decontamination efficacy of HAW on anticancer drugs by examining their decomposition kinetics, using sodium hypochlorite (NaClO) as a reference. Cyclophosphamide (CPA) and 5-fluorouracil (5-FU) were tested with 0.02% HAW, NaClO at 0.02%, 0.2%, and 2%, and ozone water. Decomposition kinetics were monitored, and cytotoxicity of drug–decontaminant mixtures was assessed using the MTT assay. HAW rapidly decomposed both drugs, with no detectable CPA after 5 minutes, while NaClO degraded CPA more slowly and showed concentration-dependent equilibrium for 5-FU. In the MTT assay, CPA mixed with either HAW or NaClO produced cytotoxic products, whereas 5-FU mixtures showed no cytotoxicity. These findings suggest that HAW is more effective than NaClO in decomposing CPA and 5-FU and could be a promising agent for removing anticancer drug residues, although the potential cytotoxicity of decomposition products should be considered when applying HAW for surface decontamination in clinical settings.

Keywords: HypochlorousAcid Water, Sodium Hypochlorite, Decontamination, Antineoplastic Drugs, Cytotoxicity, Pharmacy Practice.

***Corresponding Author**
Nobuhito Shibata

DOI: https://doi.org/10.38022/ajhp.v5i4.111

Introduction

In recent years, research on environmental and biological exposures to anticancer drugs has advanced significantly. Healthcare professionals and others handling anticancer drugs have become increasingly vigilant, raising awareness about the potential risks associated with their handling. Internationally, anticancer drugs are classified as hazardous drugs (HDs), making healthcare professionals particularly concerned about their potential carcinogenicity, teratogenicity, and mutagenicity.Guidelines for anti-exposure measures, whichtypically outline procedures for the preparation, transport, storage, administration, and disposal of anticancer drugs, have been established domestically, with each institution implementing facilities and systems accordingly [1].The first edition of the 2008 Guidelines for the Sterile Preparation of Injection Drugs and Anticancer Drugs strongly recommended neutralizing controlled areas contaminated with anticancer drugs byusing 1% sodium thiosulfate after inactivation with 2% NaClO, depending on the type of drug [2]. Meanwhile, the 2015 Joint Guidelines for Exposure Measures in Annual Cancer Drug Therapy has stated that using NaClO to inactivate HDs is ineffective. Only a high concentration of NaClO (5.25%) can be potent for inactivating certain anticancer drugs, and after its application, surfaces should be wiped with neutralizing sodium thiosulfate [3]. However, NaClO is not

recommended as a decontaminant because of its potential harmful effects on the human body and tendency to cause equipment corrosion. Additionally, NaClO can become mutagenic after reacting with certain anticancer drugs [1]. The latest 2019 Guideline for Occupational Exposure Measures in Cancer Drug Therapy indicates that the use of NaClO is a weak recommendation and its concentration is not specified for cases when HDs are spilled. The guideline also suggests combining the most effective and safe concentration of NaClO with other decontamination solutions. Additionally, the timing of neutralization with sodium thiosulfate should be determined after confirming the required degradation time for each HD, with careful consideration of the device material. The US guideline USP800 acknowledges NaClO as one of the agents used to inactivate HD but clarifies that it is not a decontamination solution capable of inactivating all substances. The ultimate goal is to remove HD from contaminated surfaces [4]. Thus, current evidence supports limited active use of NaClO as a decontamination solution for HD.

Previous studies have demonstrated that ozonated water, which is a strong oxidizing agent, can inactivate cyclophosphamide (CPA), 5-fluorouracil (5-FU), and gemcitabine (GEM), and it has thus been reported as a potential cleaning solution that can replace NaClO solution [5,6]; however,its practical application has not been implemented yet. Alternatively, hypochlorous acid water (HAW) has been shown to exhibit bactericidal, virucidal, and disinfecting properties at lower concentrations than NaClO and is often utilized in clinical practice as a bactericidal disinfectant with lower cytotoxicity. This is attributed to the higher proportion of highly oxidizing hypochlorous acid (HOCl) in HAW compared with NaClO; HOCl is approximately 80 times more bactericidal than hypochlorite (OCl^-) [7]. In the present study, we aimed to explore the potential ofHAWto degrade anticancer drugs. CPA and 5-FU, representative anticancer agents, were used as indicators of exposure to anticancer drugs. We compared the effects of serial dilutions of HAW and NaClO on the degradation of these drugs to elucidate the efficacy ofHAW as a cleansing solution.

Materials and Methods

Materials

CPA (pKa = 3.0, Log P = 0.478) and 5-FU (pKa = 8.01, Log P = −1.000) bulk powder (monohydrate, Lot: M1G0379 and anhydride, Lot: M2E8226) and MTT TS (Lot: LlT836 8) were purchased from NacalaiTesque, Kyoto, Japan. The chemical structures of CPA and 5-FUare shown in Fig. 1.

Ion-exchanged water was used as purified water. HAW was prepared using P-clear®(0.02% HAW), which was kindly provided by Shionogi Pharma Co., Ltd. Sodium hypochlorite (NaClO) solution (density 1.1 g/ml) was purchased from FujifilmWako Pharmaceutical Co., Ltd. (Lot: ESN0432) and diluted with purified water to concentrations of 0.02%, 0.2%, and 2%(v/v) for experimental purposes.Other drugsincludedover-the-counter special grade reagents.

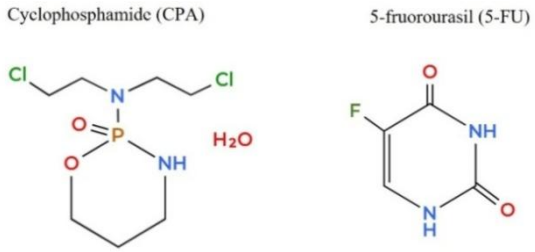

Fig 01: Chemical structures of antineoplastic drugs tested

ChemO3 COR-150 (manufactured by Nikkei Micron Co., Ltd.) was employed as the ozone water generator, and an ozone concentration of 5 ppm or more was used.

Decontamination Assessment

CPA or 5-FU bulk powder was dissolved in purified water to prepare serial dilutions of 50, 500, and 2,500 µg/ml. A 50 µl aliquot of the prepared CPAor 5-FU solution was dispensed in 1.5 ml polyethylene centrifuge tubes, followed by the addition of 200 µl of purified water and five decontamination solutions (ozone water, 0.02% v/v HAW, 0.02% v/v NaClO, 0.2% v/v NaClO, and 2% v/v NaClO) at room temperature. HAW and NaClO concentrations used in this study were based on their concentration ranges typically encountered in clinical settings for CPA and 5-FU. The final concentration of CPA or 5-FU immediately after agitation was 10, 100, or 500 µg/ml, and the initial concentration was 100% residual. After stirring, the mixture was allowed to react at standard temperature (25°C) for 30 min, and the residual CPA and 5-FU concentrations were measured using the HPLC method. Table 1 lists the HPLC measurement conditions for CPA and 5-FU.

Table 01: HPLC method for CPA and 5-FU

	CPA	5-FU
Column	Cosmoshil®C_{18} (5µm,4.6i.d. mm×mm)	Cosmoshil®C_{18} (5µm,4.6i.d. mm×mm)
Wave Length	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
Detection limits of CPA and 5-FU are 0.5 and 0.025µg/mL, respectively.		

Residual CPA and 5-FU

CPA or 5-FU bulk powder was dissolved in purified water to prepare serial dilutions of 500, 1,000, and 2,500 µg/ml. After adding 3 ml of 0.02% HAW, 0.02% NaClO, or 0.2% NaClO to CPA or 5-FU solution, 750 µl of the mixture was dispensed in 15 ml polypropylene tubes.Subsequently, 12 samples of 200 µl each were immediately dispensed in an HPLC sample cup. The final concentrations of CPA and 5-FU after agitation were 100, 200, and 500 µg/ml, and the residual rates were determined over time as 100% of the initial concentration immediately after agitation. The residual CPA or 5-FU concentrations were measured sequentially after the reaction using HPLC at every 10 min.

Cytotoxicity Assessment

The MTT assay was conducted to confirm the cytotoxicity of the reactant components after mixing the decontamination solution with or without the anticancer drug solution. Briefly, CPA or 5-FU bulk powder was dissolved in saline to prepare anticancer solutions at a final concentration of 45 µg/ml. Jurkat E6.1 cells, a cell line derived from human T-cell leukemia, was obtained from KAC Co. Ltd. (Kyoto, Japan) for the cytotoxicity assessment. Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% FBS and an antibiotic-mixed stock solution was used as the culture medium. The cells were cultured at 37°C in a 5% CO_2 incubator.Next, 100 µl of cell suspensions were seeded in 96-well microplates at a density of 2.5 × 10^5 cells/ml. After incubating in a 5% CO_2 incubator at 37°C for 24 h, 10 µl of a mixture containing anticancer solution and saline or each decontamination solution, prepared in microtubes in equal quantities of 100 µl each, was added to the cells. Subsequently, the cells were incubated for 1 or 24 h. Following this, 10 µl of MTT TS was added to each well, and the color reaction was performed for 3 h in an incubator. After the reaction, 100 µl of the solution was added to each well and mixed, and the precipitated formazan product was dissolved by pipetting. The absorbance was measured at 570 nm using a microplate reader (i-control 200 pro, TECAN).The cell viability (%) was calculated using the following equation:

Cell viability (%) = $(A_{sample}-A_{blank})/(A_{control}-A_{blank}) \times 100$

WhereA_{blank} is the absorbance of the background measurement well, $A_{control}$ is the absorbance of the negative control added only to the cells, and A_{sample} is the absorbance of the drug-treated group.

Data Analysis

Numerical data are expressed as the mean ± standard deviation (SD), unless otherwise indicated.The reproducibility of the HPLC analysis was confirmed by the coefficient of variation (%) of the measured values in repeated measurements.The rate constants for drug degradation were estimated using Phoenix 64 WmNonlin (Ver. 8.3.3.3.33), assuming a first-order degradation process. Repeated one-way ANOVA was performed to analyze the wash-off effects of CPA and 5-FU, as well as for multigroup comparisons in the MTT assay. A risk ratio of 0.05% or less was considered significant.

Results

Effect of various decontamination solutions on CPA and 5-FU degradation

Table 2 displays the residual rates of anticancer drugs after addition of purified water versus the five decontamination solutions to CPA and 5-FU solutions prepared at high (500 µg/ml), moderate (100 µg/ml), and low (10 µg/ml) concentrations for 30 min.

Table 02: Effects of decontamination liquids on the residual rates (%) of CPA and 5-FU

Decontam-inationsolu tuion	CPA initial concn. (µg/mL)			5-FU initial concn. (µg/mL)		
	500	100	10	500	100	10
Purified Water	100	100	100	100	100	100
Ozone Water	94.25 ± 2.83	92.87 ± 1.13	86.08 ± 6.32	96.0 ± 1.10	81.76 ± 1.62	ND
0.02v/v% HAW	0.01 ± 0.02	ND	ND	75.42 ± 1.22	33.38 ± 0.76	ND
0.02v/v% NaClO	89.25 ± 2.59	86.42 ± 0.86	87.38 ± 5.10	78.56 ± 1.16	27.70 ± 1.11	ND
0.2v/v% NaClO	44.78 ± 4.82	32.78 ± 1.46	33.41 ± 2.90	0.44 ± 0.15	0.04 ± 0.05	ND
2.0v/v% NaClO	0.21 ± 0.04	ND	ND	ND	ND	ND
ND indicates that the measurement is below the detection limit of HPLC						

At high concentrations of CPA (500 µg/mL), the residual rates were in the following order: 0.02% HAW < 2% NaClO< 0.2% NaClO< 0.02% NaClO< ozone water < purified water. At moderate concentration (100 µg/ml), the residual rates were in the following order: 0.02% HAW ≒ 2% NaClO< 0.2% NaClO< 0.02% NaClO< ozone water < purified water, and the residual rates of 0.02% HAW and 2% NaClO were within the detection limit. A similar trend was observed in the residual rates at low concentration of CPA (10 µg/ml), and the residual rates of 0.02% HAW and 2% NaClO were within the detection limit.At high concentrations (500 µg/ml) of 5-FU, the residual rates exhibited the following order: 2% NaClO< 0.2% NaClO< 0.02% HAW < 0.02% NaClO< ozone water < purified water. For moderate concentrations (100 µg/ml), the order was 2% NaClO< 0.2% NaClO< 0.02% NaClO< 0.02% HAW < ozone water < purified water. At both high and medium concentrations of 5-FU, the residual rates with 2% NaClO were not detectable. In the case of low concentration (10 µg/ml), residual rate was only detected for purified water. Further, 0.02% HAW showed the strongest decomposition effect ($p < 0.01$) at all CPA concentrations—almost 100% decomposition within 30 min of addition. On the other

hand, for 5-FU, high decontamination efficacy was observed at onlythelow concentration (10 μg/ml).

Changes in residual CPA and 5-FU after addition of various decontamination solutions

Figure 02 shows the time course of the residual rates of CPA and 5-FU every 10 min following the addition of different decontamination solutions.

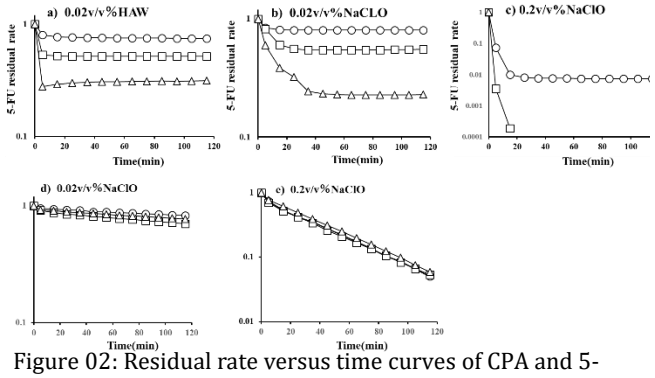

Figure 02: Residual rate versus time curves of CPA and 5-FU with detergents

Three initial concentrations of CPA or 5-FU were tested. Key: △; 100 μg/mL, □; 200μg/mL, ○; 500μg/mL

The residual rates are represented relative to the initial concentration of each anticancer agent. Additionally, for all time-courses, assuming first-order decomposition, and the decomposition rate constants of the anticancer drugs were determined using the nonlinear least squares method and is presented in Table 03.

Table 03: Decomposition rate constants (min^{-1}) of CPA and 5-FU in the presence of decontamination solutions

	CPA initial concn. (μg/mL)			5-FU initial concn. (μg/mL)		
	500	200	100	500	200	100
0.02v/v% HAW	–*	–*	–*	4.34 ×10^{-2}	12.56 ×10^{-2}	25.64 ×10^{-2}
0.02v/v% NaClO	1.99 ×10^{-3}	2.58 ×10^{-3}	1.86 ×10^{-3}	3.39 ×10^{-2}	5.54 ×10^{-2}	10.30 ×10^{-2}
0.2v/v% NaClO	2.54 ×10^{-2}	2.40 ×10^{-2}	2.37 ×10^{-2}	5.08 ×10^{-1}	11.31 ×10^{-1}	–*
2.0v/v% NaClO	–*	–*	–*	–*	–*	–*

Asterisk indicates that the residual rete-time curve was not available because of ND in measurements. These values were obtained corresponding to the residual rate-time curve in Figure 02.

Cases, in which the residual rate fell below the detection limit within 30 min, as is evident from the results in Table 2, were excluded. Ozone water was also excluded because its effectiveness was deactivated in less than 30 min [8]. Furthermore, for CPA, owing to its rapid decomposition with 0.02% HAW at all initial concentrations, only decomposition with NaClO was investigated. The decomposition reactions of 5-FU with various detergents were promptly observed, and the decomposition rate constants were calculated using the measured values from 2–3 points up to 20 min after the reaction.

When NaClO was added to the CPA solution, the logarithmic residual rate of CPA decreased linearly over time, suggesting the involvement of a first-order degradation reaction mechanism in CPA decomposition. The first-order degradation rate constants for CPA in 0.02% NaClO solution were 1.99×10^{-3}, 2.58×10^{-3}, and 1.86×10^{-3} min^{-1} for initial CPA concentrations of 500, 200, and 100 μg/ml, respectively. The respective values for CPA in 0.2% NaClO solution were 2.45×10^{-2}, 2.40×10^{-2}, and 2.37×10^{-2} min^{-1}.However, when 0.02% HAW or 0.02% or 0.2% NaClO solution was added to the 5-FU solution, an equilibrium was observed over the time courses of the 5-FU residual rates. The 5-FU residual rateat equilibrium decreased at lower initial 5-FU concentrations (Fig. 2a-c). However, when 0.02% HAW was used, the residual rate reached equilibrium 5 min after the addition for all three initial concentrations. In contrast, for 0.02% NaClO solution, equilibrium was reached after 20–30 min of addition, and the residual rate at equilibrium was similar to that when 0.02% HAW solution was added for allinitial concentrations of 5-FU. Furthermore, compared with the other two decontamination solutions, 0.2% NaClO solution resulted in strong degradation of 5-FU. Even under high initial concentration of 5-FU at 500 μg/ml, equilibrium in the residual rate was observed.The residual rate reached equilibrium in approximately 20 min, similar to the conditions observed for lower initial levels of 5-FU (Fig. 2c).Because a linear relationship was not observed in the residual rate-time curve for the degradation of 5-FU, the degradation rate constant was calculated using the initial measured values of 2–3 points. In the case of 0.02% HAW, the decomposition rate constants for 5-FU at initial levels of 500, 200, and 100 μg/ml were $4.34\times10^{2-}$, 12.56×10^{-2}, and 25.64×10^{-2} min^{-1}, respectively.In the case of 0.02% NaClO solution, the values were 3.39×10^{-2}, 5.54×10^{-2}, and $10.30\times10^{-2}min^{-1}$, respectively. In case of 0.2% NaClO solution, the values were 5.08×10^{-1} and 11.31×10^{-1} min^{-1} for the two initial levels of 5-FU, 500 and 200 μg/ml, respectively.

Relationship between the residual rate and decomposition rate in 5-FU degradation after addition of each decontamination solution

Based on the residual rate-time curve of 5-FU shown in Fig. 2, the degradation rate was calculated for each initial concentration of 5-FU and the residual concentration of 5-FU at equilibrium.The relationship between the decontamination effect of 0.02% HAW and NaClO solutions was investigated.Figure 3 illustrates the relationship between the degradation rate and residual rate at each initial concentration of 5-FU when each decontamination solution was added.

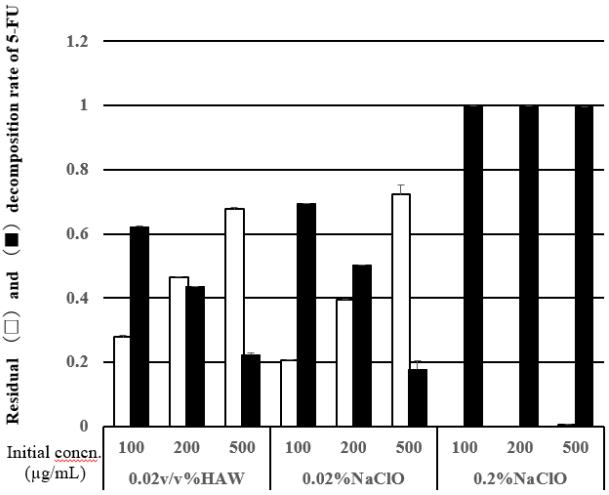

Fig 03: The relationship between residual rate and decomposition rate of 5-FU after the addition of various detergents

Three initial concentrations of 5-FU were tested. The decomposition rate is defined as one minus the residual rate relative to the initial concentration. The average values of the data for 5-FU charts (a to c) as shown in Fig. 2, starting from 25 minutes onwards when equilibrium was reached, were calculated, and then determined the residual rate.

The residual rate at equilibrium was calculated from the values in the last 10 points after the reaction in Fig. 02.

When 0.02% HAW added, the decomposition rate decreased with an increase in the initial 5-FU concentration. The decomposition rates were 69%, 48%, and 25% of for the initial 5-FU concentrations of 100, 200, and 500 μg/ml, respectively. When 0.02% NaClO solution was added, the decomposition rates of 5-FU were 70%, 56%, and 20%, respectively. However, when 0.2% NaClO solution was added, 100% decomposition was observed for initial 5-FU concentrations of 100 and 200 μg/ml, while 99% decomposition was achieved for the 500 μg/ml concentration.

Cytotoxicity of various anticancer agent and decontamination solution mixtures

Cell viability (%) was assessed using the MTT assay after exposure to anticancer drug (CPA or 5-FU) solution alone, each decontamination solution alone, and a mixture of anticancer drugs with various decontamination solutions. Figures 4 and 5 show the results of CPA and 5-FU incubated with various decontamination solutions for 1 or 24 h. Normal saline was added to the control and the cells were incubated.

After 1 h of CPA addition, cell viability was 90.8% ± 9.8%, decreasing to 82.7% ± 8.1% at 24 h. For 5-FU, cell viability was 93.6% ± 5.3% at 1 h and significantly dropped to 56.2% ± 3.9% at 24 h. Both CPA and 5-FU exhibited increased cytotoxicity at 24 h compared with 1 h, with 5-FUshowing agreater impact on cell viability over time. When treated.

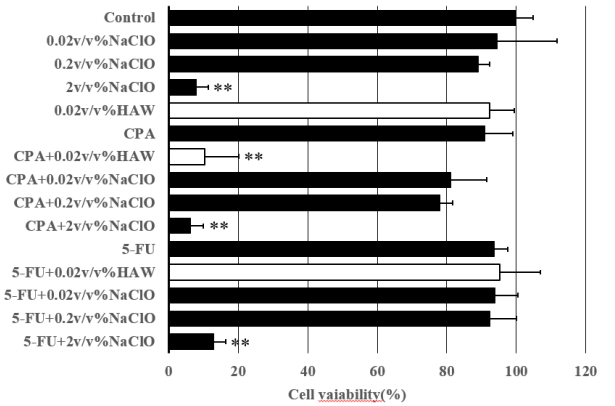

Fig 04: Results of MTT assay for various detergents with and without antineoplastic drugs after 1hr incubation Open columns represent 0.02v/v%HMA with or without antineoplastic drugs. Each bar represents the mean±SD of 5 determinations. **) Significantly difference from control or detergent only ($p<0.01$).

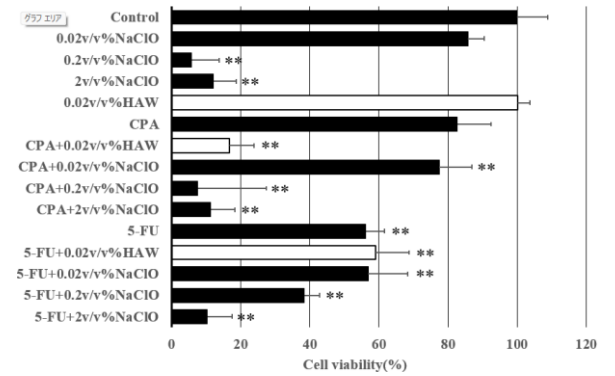

Fig 05: Results of MTT assay for various detergents with and without antineoplastic drugs after 24hrs incubation Open columns represent 0.02v/v%HMA with or without antineoplastic drugs. Each bar represents the mean±SD of 5 determinations. **) Significantly difference from control or detergent only ($p<0.01$).

with 2% NaClO alone, cell viability was 7.9% ± 6.5% at 1 h and 12.2% ± 3.4% at 24 h. Mixing with CPA caused the cellviability to be 6.2% ± 6.9% at 1 h and 11.4% ± 3.7% at 24 h, which were comparable to the effects of 2% NaClO alone. Similarly, when 5-FU and 2% NaClO were mixed, cell viability was 13.0% ± 7.3% at 1 h and 10.3% ± 3.4% at 24 h, indicating high cytotoxicity similar to CPA.Similartothe results observed when CPA was used alone, there was little difference in the results when 2% NaClO solution was applied alone. This is because 2% NaClO is considered strongly cytotoxic. When 0.2% NaClO was used alone, cell viability was 89.2% ± 8.0% at 1 h and significantly decreased to 5.8% ± 3.2% at 24 h, demonstrating increased cytotoxicity with prolonged treatment. When mixed with CPA, cell viability was 77.9% ± 19.8% at 1 h and 7.6% ± 3.8% at 24 h, which was comparable to using the decontamination solution alone. However, mixing with 5-

FU resulted in a cell viability of 92.4% ± 4.4% at 1 h and 38.4% ± 7.6% at 24 h, indicating a 30% reduction in cytotoxicity compared with decontamination alone. In contrast, 0.02% NaClO alone showed slight cytotoxicity after 24 h, with cell viability at 94.4% ± 4.7% at 1 h and 85.8% ± 17.2% at 24 h. CPA increased cytotoxicity by approximately 10% and 5% at 1 and 24 h, respectively. Mixing with 5-FU resulted in an approximately 25% increase in cytotoxicity after 24 h compared with decontamination alone. On the other hand, the cell viability with 0.02% HAW alone was 92.4% ± 3.5% at 1 h and 100.2% ± 7.1% at 24 h, and no cytotoxicity was observed after 24 h. When mixed with CPA, the cell viability was 10.4% ± 7.1% at 1 h and 16.7% ± 9.7% at 24 h, showing approximately 80% cytotoxicity compared with 0.02% HAW alone. Additionally, when mixed with 5-FU, the cell viability was 95.1% ± 9.5% at 1 h and 59.1% ± 11.8% at 24 h, indicating approximately a 40% increase in cytotoxicity after 24 h compared with using 0.02% HAW alone.

Discussion

This study demonstrated the practical application of anticancer drugs in routine preparations and contribute to the development of efficient yet simple decontamination methods for safety cabinets and similar environments. The experimentally determined concentrations of anticancer drugs represent the maximum levels typically administered in routine clinical practice. Although various types of anticancer agents exist, this study focused on CPA and 5-FU because of their versatility.CPA is widely used in the treatment of solid tumors (e.g., breast, ovarian, and lymphoma) and blood cancers (e.g., malignant lymphoma), and has demonstrated effectiveness across a broad spectrum of cancer types [9]. Meanwhile, 5-FU is used in the treatment of gastrointestinal cancer, breast cancer, and certain skin disorders [10]. While the optimal treatment plan varies for every patient based on individual conditions, cancer type, and cancer stage, CPA or 5-FU is part of representative regimens such as FOLFOX (5-FU, oxaliplatin, and leucovorin), AC (adriamycin and CPA), CHOP (CPA, doxorubicin, vincristine, and prednisone), R-CHOP (rituximab, CPA, doxorubicin, vincristine, and prednisone), CMF (CPA, methotrexate, and 5-FU), CAF (CPA, doxorubicin, and 5-FU), and FEC (5-FU, epirubicin, and CPA) [11-16].The potent NaClO solution, typically recommended as a cleansing agent for anticancer agents in safety cabinets, reacts with water molecules via hypochlorite ions. This reaction involves the removal of electrons from other organic molecules, resulting in the formation of chloride and hydroxide ions, which exhibit an oxidizing effect. As indicated in Table 2, the residual amount of both CPA and 5-FU decreased as the concentration of the NaClO solution increased during decontamination. Additionally, we confirmed that CPA and 5-FU remained stable when cleaned with purified water, invert soap water, and ethanol in safety cabinets, and their residual rates remained unchanged after mixing(data not shown). Therefore, these decontaminationmethods can only physically remove anticancer drugs without their inactivation, which can have severe implications on the environment upon disposal. Moreover, for treating anticancer drugs residues in waste generated from bed baths, it is recommended to use an appropriately diluted NaClO solution as a decontaminant, considering the environmental impact. However, it is important to note that NaClO is a strong oxidizing agent with potent alkaline properties, which can corrode any metal instruments in the environment and damage the skin and mucous membranes when used as a decontamination solution[1]. Considering reports indicating the potential of the solution to react with certain anticancer drugs, resulting in mutagenicity and concentration-dependent cytotoxicity [1], it is crucial to explore methods for minimizing the use of NaClO solution.This Includes careful consideration of its concentration and reaction time and developing strategies minimizing its use to ensure its effective and safe utilization.

On the other hand, HAW, which has recently gained attention as a sterilizing and antiviral agent, is a slightly acidic electrolytic water solution with a pH of 6.0 ± 0.5. This is achieved by adding dilute hydrochloric acid to a sodium hypochlorite solution, resulting in an increased ratio of hypochlorite molecules in the aqueous solution [17]. Although the oxidizing power of hypochlorite molecules is primarily dominated by chlorine ions, the electrons of these ions are separated from the oxygen atom, rendering them in a more unstable state. Consequently, unstable chlorine atoms are known to exhibit potent bactericidal and oxidative effects at lower concentrations than NaClO. This is achieved by extracting electrons from the surrounding organic matter to form stable chloride ions.Notably,HAW is approved as a food additive by the Ministry of Health, Labour, and Welfare. However, its usage as a detergent for removing anticancer drugs has not yet been outlined in the guidelines. HAW utilized in this study had an effective chlorine concentration of 200 ppm (0.02% v/v).

Figure 2 and Table 2 clearly show that the NaClO solution, designated as the guideline wash solution, effectively degraded CPA in a concentration-dependent manner. CPA degradation followed a first-order degradation mode and was eliminated regardless of the initial concentration. In contrast, 0.02% HAW exhibited a remarkable decontamination effect, degrading nearly 100% of CPA in a significantly shorter time than the equivalent effective chlorine concentration of 0.02% NaClO solution.For 5-FU, the residual rate showed a concentration-dependent pattern that was almost equivalent to 0.02% NaClO solution. However, the residual rate increased with increasing initial 5-FU concentrations, and unlike CPA, 5-FU did not exhibit first-order degradation. The residual rate reached equilibrium 30 min after the reaction. A comparison of the residual rate-time curves for 5-FU in 0.02% HAW and 0.02% NaClO revealed that equilibrium was achieved in both cases. The magnitude of the residual

rate at equilibrium was dependent on the initial 5-FU concentration (Fig. 2).In contrast, CPA rapidly degraded in 0.02% HAW at any initial concentration, making it impossible to obtain a residual rate-time curve. Despite the absence of a curve for 0.02% HAW, considering the first-order degradation of CPA in 0.02% or 0.2% NaClO solution, it was inferred that the degradation mechanism involving hypochlorite ions differed between CPA and 5-FU. Moreover, the degradation of 5-FU by hypochlorite ions within the concentration range of these decontamination solutions and anticancer agents terminated quickly based on the concentration of hypochlorite ions, halting the degradation reaction upon ion consumption. Conversely, the degradation and elimination of CPA, exhibiting primary degradation, increased the degradation rate constantly with increasing concentrations of NaClO solution, accelerating the degradation process. Despite the constant degradation, irrespective of the initial CPA concentration, it was postulated that the radicals generated in the degradation process underwent sequential reactions.These findings underscore that 0.02% HAW surpassed the efficacy of 0.02% NaClO solution in CPAdecontamination, and that the mode of degradation varied with the type of anticancer agent. Additionally, it was observed that 0.02% HAW outperformed ozone water in removing both CPA and 5-FU.

The major metabolites of CPA *in vivo* are known to be 4-hydroxycyclophosphamide, oxophosphamide, and chloroethylphosphoramide, all of which are involved in the alkylation of cancer DNA and act to inhibit cancer cell growth [18]. On the other hand, the major metabolites of 5-FU *in vivo* are 5-fluorouridine and 5-fluorodeoxyuridine triphosphate, which inhibit protein synthesis and DNA synthesis, respectively, in cancer cells [19]. These are the active metabolites formed during the metabolism of CPA and 5-FU *in vivo*. A previous studyhas reported the formation of the active metabolite 4-hydroxy-cyclophosphamide when CPA and NaClO are mixed [20]; thus,the formation of various active molecular species when CPA or 5-FUwas decontaminated with hypochlorite water or NaClO solutions of various concentrations cannot be ruled out. In this study, it was not possible to determine the molecular species that formed due to the combination of detergents and anticancer agents. However, we investigated whether the degradation-generated molecular species of 0.02% HAW, which had excellent degradation effects on CPA and 5-FU, were active in the reaction between detergent and anticancer agents.The effect of a mixture of decontamination solutions and anticancer agents on biological membranes was verified using the MTT assay. When the cytotoxicity of CPA, 5-FU, each decontamination solution, and a mixture of anticancer agents and decontamination solutions was investigated, as shown in Figs. 4 and 5, 2% NaClO alone was already the most cytotoxic from the first hour of culture, and the effects of reactants with CPA or a mixture of 5-FU could not be ascertained (Fig. 4). In addition, 0.2% NaClOalone had similar cytotoxicity to 2% NaClO 24 h after culture (Fig. 5). Therefore, NaClO described in the guidelines was the most cytotoxic in the range of concentrations used.In contrast, 0.02% HAW alone was not cytotoxic at 1 or 24 h after incubation, suggesting little damage to the biological membranes (Figs. 4 and 5). 5-FU was more cytotoxic than CPA after 24 h of incubation (Fig. 5). However, when 0.02% HAW and anticancer drugs were mixed, cytotoxicity was three times greater in the culture mixed with CPA than in that mixed with 5-FU at 24 h (Fig. 5). The degradation products of 5-FU by 0.02% HAW were not considered to be cytotoxic because the cytotoxicity of 5-FU alone was similar to that of 0.02% HAW in the 24-h culture. Conversely, when CPA was mixed with 0.02% HAW, cytotoxicity significantly increased compared with CPA alone, indicating that 0.02% HAW degraded CPA and produced cytotoxic active degradation products.

As indicated earlier, HAW is an extremely unstable decontamination solution; therefore, in this study, its concentration was limited to 0.02%. However, CPA decontamination demonstrated a decomposition effect equivalent to 2% NaClO solution mentioned in the guidelines (Table 1). On the other hand, the efficacy of 0.02% HAW against 5-FU did not surpass that of 2% NaClO solution; however, complete decomposition was observed when the initial concentration of 5-FU was relatively low. Additionally, it was evident that the decontamination effects of HAW and NaClO on 5-FU did not progress further once they were consumed in the reaction process (Fig. 2). Considering these findings, it is believed that by substituting the NaClO solution specified in the guidelines with 0.02% HAW for decontamination, complete decomposition and decontamination of 5-FU could be achieved by incorporating several steps with HAW.

Based on the above results, it was revealed that HAW has decontamination effects on anticancer drugs equivalent to or even greater than those of NaClO at low concentrations. Therefore, considering that low concentrations of HAW pose minimal harm to the human body, HAW can be used as a decontamination solution suitable for the prevention of exposure to anticancer drugs by replacing NaClO. However,HAW, like NaClO, has the potential to produce hazardous decomposition products when mixed with CPA; thus, ensuring the safety of the decomposition products during decontamination is crucial. By positioning HAW as a decontamination solution for the prevention of anticancer drug exposure and combining it with other decontamination solutions that allow for physical removal, a safe and convenient decontamination protocol for anticancer drug exposure can be established.

Conclusion

Based on a kinetic examination of the decontamination effects of HAW on representative anticancer drugs, CPA and 5-FU, as indicators of anticancer drug exposure, HAW was found to exhibit a more potent decomposition effect on both CPA and 5-FU than diluted NaClO solutions and ozone

water, establishing its efficacy as a candidate decontamination solution. Additionally, based on the results of the MTT assay, there is a concern about the potential cytotoxicity resulting from the decomposition products generated by the reaction with anticancer drugs. Nevertheless,by appropriately combining HAW with other decontaminants, a safe and effective decontamination protocol can be established.

Conflict of Interest

All authors declare that they have no conflicts of interest related to this study.

References

1. JSCN, JSMO, & JASPO. (2019). Joint guidelines for safe handling of cancer chemotherapy drugs. Kanehara Publishing Company.
2. Japan Hospital Pharmacist's Association. (2008). Guideline for aseptic preparation of injections and anticancer drugs. Yakujinippou.
3. Kanehara Publishing Company. (2015). Guidelines for preventing occupational exposure in cancer chemotherapy drugs. Kanehara Publishing Company.
4. United States Pharmacopeia. (2020). USP general chapter <800> hazardous drugs—Handling in healthcare settings. ttps://www.usp.org/compounding/general-chapter-hazardous-drugs-handling-healthcare
5. Iwasaki, R., Tanaka, Y., Kato, M., & Yamada, S. (2025). Removal efficiency of cyclophosphamide by hypochlorous acid solution generated from NaCl electrolysis. Journal of Environmental Chemical Engineering, 13(2), 112345. https://pubmed.ncbi.nlm.nih.gov/39656713/
6. Asrari, M., Rezaei, M., &Karimi, H. (2024). Study of cyclophosphamide removal using sodium hypochlorite in healthcare wastewater. Applied Research in Water and Wastewater, 11(1), 45–52. https://arww.razi.ac.ir/article_3144.html
7. Nasiłowska, E., Kocot, A., &Dobrzyńska, A. (2024). Decontamination effect of hypochlorous acid dry mist on healthcare surfaces contaminated with biological agents. International Journal of Environmental Research and Public Health, 21(3), 1156. https://pmc.ncbi.nlm.nih.gov/articles/PMC11241407/
8. Yoshichika, H., & Katsuhiko, N. (2019). Effect of citric acid on prolonging the half-life of dissolved ozone in water. Food Safety (Tokyo), 7, 90–94. https://doi.org/10.14252/foodsafetyfscj.D-19-00005
9. Naoki, I. (2017). Antiemetic therapy for non-anthracycline and cyclophosphamide moderately emetogenic chemotherapy. Medical Oncology, 48, 77–83. https://doi.org/10.1007/s12032-017-0937-y
10. Anai, V. A., Dilawar, H., Mehrab, P., Amin, S., Razieh, B., Abbas, R., Dora, I., & Ana, M. D. (2023). 5-Fluorouracil nano-delivery systems as a cutting-edge for cancer therapy. European Journal of Medicinal Chemistry, 246, 114995.https://doi.org/10.1016/j.ejmech.2022.114995
11. Lai, W. W.-P., Tien, C.-H., & Tang, Z.-S. (2023). Removal of the chemotherapeutic drugs 5-fluorouracil and cyclophosphamide by the UV/peracetic acid process: Reaction kinetics and mechanism. Journal of Water Process Engineering, 56, 104186.
12. Hayes-Porter, A., et al. (2025). Evaluation of a hazardous drug surface contamination monitoring program in a community hospital. American Journal of Health-System Pharmacy, 82(18), 1006–1014.
13. Sessink, P. J. M., et al. (2024). Longitudinal evaluation of environmental contamination with hazardous drugs in a hospital pharmacy and wards using CSTD. Journal of Oncology Pharmacy Practice, 30(X), ePub ahead of print.
14. Walton, A. L., Leach, S., & Ellis, J. (2025). Assessing etoposide and cyclophosphamide contamination and current cleaning practices in patient bathrooms. Clinical Journal of Oncology Nursing, 29(2), 145–152. https://onf.ons.org/publications-research/cjon/29/2/assessing-etoposide-and-cyclophosphamide-contamination-and-current
15. Vermette, M. L., Reith, M. E., &Rinfret, A. (2024). Wipe sampling of antineoplastic drugs from workplace surfaces: An exposure assessment study. Journal of Hazardous Materials Advances, 12, 100430. https://www.sciencedirect.com/science/article/pii/S2773049224000023
16. Portilha-Cunha, M. F., et al. (2025). Tackling antineoplastic drugs' contamination in healthcare: Evaluation of cleaning protocols for hazardous drug residues. Journal of Occupational and Environmental Hygiene, 22(4), 200–212. https://www.tandfonline.com/doi/full/10.1080/15459624.2025.2449945
17. AIHA (American Industrial Hygiene Association). (2023). Hazardous drug surface contamination—Guidance document.
18. NIOSH. (2025). NIOSH List of Hazardous Drugs in Healthcare Settings. DHHS (NIOSH) Publication No. 2025-103.
19. Huehls, A. M., Huntoon, C. J., Joshi, P. M., Baehr, C. A., Wagner, J. M., Wang, X., Lee, M. Y. M., &Karnitz, L. M. (2016). Genomically incorporated 5-fluorouracil that escapes UNG-initiated base excision repair blocks DNA replication and activates homologous recombination. Molecular Pharmacology, 89, 53–62. https://doi.org/10.1124/mol.115.100164
20. ŠtenglováNetíková, I. R., Petruželka, L., Šťastný, M., &Štengl, V. (2018). Safe decontamination of cytostatics from the nitrogen mustards family. Part one: Cyclophosphamide and ifosfamide. International

Journal of Nanomedicine, 26, 7971–7985. https://doi.org/10.2147/IJN.S159328