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Evaluation of Environmental Contaminations and Occupational Exposures Involved in Preparation of Chemotherapeutic Drugs

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Many healthcare workers are concerned about the risk of occupational exposures to hazardous drugs. The Japanese Society of Hospital Pharmacists (JSHP) revised the ''Guidelines for the Handling of Antineoplastic Drugs in Hospitals'', however, the precautions and awareness of handling drugs varied in institutions. We assessed the levels of environmental contaminations in our hospital and urinary excretion of cyclophosphamide (CP) and ifosfamide (IF) in pharmacists and nurses. In environmental studies, we obtained samples by wiping the surfaces around two biological safety cabinets (BSCs) on eight days for four months. One BSC was equipped in hospital pharmacy and the other was equipped in an oncology ward, and used for preparing chemotherapeutic drugs for outpatients and for inpatients, respectively. We obtained the urine samples from 6 pharmacists and 2 nurses. We used solid phase extraction (SPE) as a convenient extraction procedure and liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) for the analysis of the samples. CP was detected on the working surfaces inside both BSCs, and detected at low levels on the back surfaces of the BSCs and at the working tables around the BSCs. IF over the LLOQ was not detected in both BSCs. CP and IF were not detected in all urine samples of pharmacists and nurses. Detection frequencies and amounts of these drugs were low levels, compared with previous reports in Japan, and our results showed that improving awareness about handling hazardous drugs could reduce the risk of the occupational exposures.

Key words——cyclophosphamide; occupational exposure; environmental contamination; solid phase extraction; liquid chromatography/mass spectrometry/mass spectrometry

INTRODUCTION

Currently, many healthcare workers are concerned about the risk of occupational exposure to hazardous drugs. In 1979, Falck[1] reported that mutagens were detected in the urine samples of nurses involved in chemotherapy. Since then, many reports have been published about the presence of urinary mutagen,[2–7] and the detection of unchanged hazardous drugs in the urine samples of hospital workers.[6,8–14] Recently, hospital pharmacists in Japan have been required to prepare hazardous drugs. In 2005, the Japanese Society of Hospital Pharmacists (JSHP) revised the ''Guidelines for the Handling of Antineoplastic Drugs in Hospitals'', and recommended standard precautionary measures for using the laminar flow cabinet, masks, gloves, caps, disposable nonwoven clothes, and luer-lok syringes. In addition, the JSHP recommended the use of closed-system devices to limit the aerosols of hazardous drugs. However, these devices are not commonly used in Japan mainly because of their cost. Validations of the various methods to avoid occupational exposures are required.

Previously, we focused on occupational exposures to epirubicin and reported that the surface of biological safety cabinet (BSC) and ambient environments were contaminated by epirubicin during preparation by pharmacists. However, epirubicin was not detected in the urine samples of healthcare workers involved in chemotherapy.[15]

In this study, we focused on occupational exposures to cyclophosphamide (CP).[16] CP is an alkylating agent, which is converted to its active metabolite phosphpramide mustard. Although the International Agency for Research on Cancer (IARC) has classified CP as a human carcinogen,[17] it is widely used for the treatment of various solid tumors and pretreatment for hematopoietic stem cell transplantation. CP is widely used as a marker of occupational exposure because it can be detected easily. We assessed the levels of environmental contaminations in hospital pharmacy and oncology ward, and urinary excretion of

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CP and ifosfamide (IF), which could be simultaneously quantified, using a convenient solid phase extraction (SPE) pretreatment, to evaluate exposure level and effectiveness of the precautionary measures taken in our hospital.

MATERIALS AND METHODS

CP, IF, prednisolone (PSL), and formic acid were purchased from Wako Pure Chemical (Biochemical reagents; Osaka, Japan). Distilled water, methanol, and acetonitrile were also purchased from Wako Pure Chemical (HPLC grade reagents).

Sampling procedures of ambient environments were as follows. In our hospital, chemotherapeutic drugs for outpatients were prepared on the BSC in hospital pharmacy, and were delivered to chemotherapeutic room for outpatients, whereas, we equipped the BSC at the corner of the nurse station in an oncology ward, and prepared drugs for inpatient chemotherapy. We collected samples from the surfaces of and around both the BSC in the hospital pharmacy and the BSC in an oncology ward on eight days for four months (Fig. 1). The procedures to obtain samples were modified by previous reports.[9,13,18,19] Briefly, we first applied 500 ng PSL/50 μl methanol to the sampling spots as internal standards. After the spots were air-dried, we wiped 800 cm^2 area (20 cm$\times$40 cm) with a sheet of Kimwipe® S-200 (120 mm$\times$215 mm, Nippon Paper Crecia, Tokyo, Japan) wetted with 1.5 ml of 20% methanol. Subsequently, we wiped off the surface with another sheet of dry Kimwipe®. We placed both sheets in 50 ml polypropylene conical tube, added 20% methanol 10.5 ml, and shook the tube for 30 min, 2000 rotations per min. We loaded 4 ml of the extracts obtained in a 96-well SPE plate (InertSep® 96 WP Pharma; polymer sorbent, 30 mg; GL Sciences, Tokyo, Japan), which was previously conditioned with 2 ml methanol and equilibrated with 1 ml water. We washed the SPE plate with 1 ml water and dried the plate under reduced pressure (0.02 MPa) for 10 min using an aspiration manifold (3M; Tokyo, Japan). Analyte was eluted with 400 μl methanol and the plate was similarly dried out.

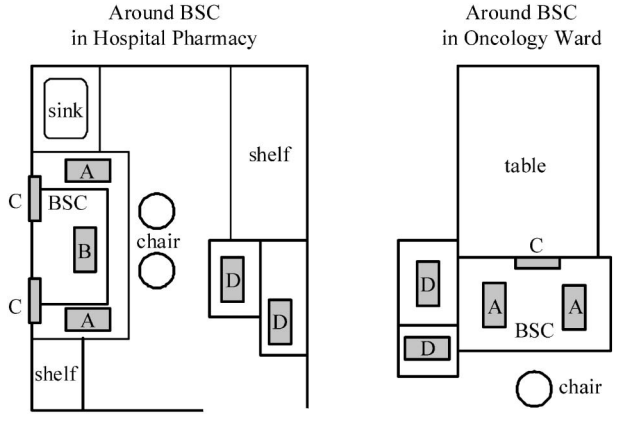

Fig. 1. Floor Layout around BSC and Sampling Spots
A) Working surfaces inside BSC, B) Under superabsorbent sheet in BSC, C) Back surfaces in BSC, D) Working tables around BSC.

Urine samples were collected in the following 3 ways. (i) 24-h sampling (1 pharmacist, 6 urine samples); the volume of urine voided each time was measured over a 24-h period after preparing CP, and 50 ml of each urine sample was collected into a polypropylene conical tube. (ii) Spot sampling (6 pharmacists, 22 urine samples); urine volumes were measured once or twice on voiding (6–10 h or 20–24 h after preparing CP) and the urine samples were collected in the same manner as the 24-h samples. (iii) Spot sampling (2 nurses, 7 samples); urine volumes were measured after each voiding on-duty after treating patients with CP, and the urine samples were collected in the above-mentioned manner. The doses of CP prepared and treated, amounts of CP handled, urine volume, and voiding time were shown in Table 1. IF was not prepared and treated during this examination. All urine samples were stored at -20°C until analysis. Urine samples from a healthy volunteer were also stored and analyzed for CP and IF levels. Urine samples containing 0 to 5000 ng of CP and IF were used to plot a calibration curve.

Urine extraction procedures were as follows. We added 500 ng PSL as an internal standard and 3 ml phosphate buffered saline (PBS, 100 mM, pH 7.0) to a 5 ml aliquot of urine samples. After mixing, these samples were loaded on SPE cartridges (Bond Elut® LRC C18; sorbent mass, 500 mg; column reservoir, 10 ml; Varian, California, USA), which were previously conditioned with 3 ml methanol and equilibrated with 3 ml water. SPE cartridges were used with an aspiration manifold (GL Sciences, Tokyo, Japan) under reduced pressure. Cartridges were washed with 3 ml PBS and 5 ml 30% methanol and dried under reduced pressure for 3 min. Analyte was eluted with 1 ml methanol and dried out by previous procedure.

Liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) analysis was performed

Table 1. The number of CP Prepared and Treated, Amounts of CP Handled, Urine Volume, and Voiding Time

Personnel	Pharmacists (P) or nurses (N)/ gender	Operation	sample no.	CP preparation numbers/amounts	Urine volume/ time after preparation (or time after administration)
1	P/male	oncology ward	1	1/2100 mg	270 ml/0–5 h
			2		260 ml/5–9 h
			3		290 ml/9–10.5 h
			4		170 ml/10.5–14 h
			5		340 ml/14–20 h
			6		150 ml/20–24 h
			7	1/1400 mg	160 ml/7 h
2	P/male	oncology ward	8	1/800 mg	100 ml/8.2 h
			9		150 ml/20.5 h
			10	1/1400 mg	80 ml/9.5 h
			11		250 ml/21.0 h
			12	1/1700 mg	150 ml/9.3 h
			13		250 ml/21.3 h
			14	2/2200 mg	35 ml/8.3 h
			15	3/3200 mg	150 ml/32.3 h
			16	1/1400 mg	100 ml/8.0 h
			17		250 ml/20.8 h
			18	1/1150 mg	200 ml/9.8 h
			19		250 ml/20.6 h
			20	2/1650 mg	unclear/9.0 h
			21		unclear/23.0 h
3	P/male	oncology ward	22	1/1100 mg	130 ml/8.3 h
4	P/female	outpatient chemotherapy	23	1/715 mg	55 ml/7.0 h
			24		80 ml/22.0 h
5	P/female	outpatient chemotherapy	25	1/1600 mg	110 ml/6.0 h
			26		160 ml/21.0 h
6	P/male	outpatient chemotherapy	27	3/2290 mg	220 ml/42.8 h
			28	2/1400 mg	225 ml/7.3 h
7	N/female	outpatient chemotherapy	29	5/3865 mg	250 ml/0–0.5 h
			30		300 ml/0.5–4.5 h
			31		210 ml/4.5–5.5 h
			32		230 ml/5.5–8.5 h
8	N/female	outpatient chemotherapy	33	3/2337 mg	300 ml/0–1 h
			34		300 ml/1–5 h
			35		200 ml/5–6 h

on a VLC-MS/MS LCQ system (Thermo Quest, current Thermo Fisher Scientific, Kanagawa, Japan). An octadecyl silyl column (Inertsil® ODS-3; 150 mm×2.1 mm; particle size, 3 μm; GL Sciences, Tokyo, Japan) with a guard column (cartridge guard-column E®; 20 mm×2.0 mm; particle size, 3 μm, GL Sciences) was used for the separation. A mixture of acetonitrile, methanol, and 0.1% formic acid in distilled water (25 : 5 : 70 (v/v)) was used as a mobile phase A. A mixture of acetonitrile and 0.1% formic acid in distilled water (70 : 30 (v/v)) was used as a mobile phase B. The flow rate was 0.2 ml/min. Gradient elution was performed in the following manner: 0% B to 70% over 14 min; 70% B to 100% over 2 min. Finally, isocratic elution was performed using 100% B over 5 min. Subsequently, the concentration of B was linearly decreased to 0% in 2 min and equilibrated for 4 min at A 100%. Total run time was 27 min. Injection volume was 20 μl for each sample and retention times for CP, IF, and PSL were 11.1, 10.6, and 12.7 min, respectively. To prevent contamination of mass detector, we rejected the urine eluted in the initial 6 min and after 14 min.

The ion-trap mass spectrometer was equipped to LCQ system. An electron spray ionization (ESI) source with an ion spray voltage of 30 V. Precursor ions for MS/MS fragmentation were each $[MH^+]$ ions; the peaks obtained at m/z 261, 261, and 361 corresponded to CP, IF, and PSL, respectively. Collision energies of CP, IF, and PSL were 37%, 37%, and 24%, respectively. Fragment peaks were obtained at m/z 140, 182, and 343 for CP, IF, and PSL, respectively.

Lower limit of quantitation (LLOQ) was determined using a signal-to-noise ratio of 10, and lower limit of detection (LLOD) was determined by corresponding concentration of 3σ of the lowest concentration of calibration curves. Urine samples in which presence of trace amounts of CP and IF could not be ruled out, as confirmed from precursor ion peaks at m/z 261 and fragment ion peaks at m/z 233, were reanalyzed after the following evaporating procedure: 700 μl of SPE eluate was evaporated at 35℃ for 3.5 h and then the residue was dissolved with 70 μl of methanol, vigorously vortexed, and analyzed.

RESULTS

In an examination of environmental contaminations, calibration curves were fitted by linear regression 5–5000 ng/wipe (=800 cm^2) for CP and IF, and LLODs were 2 ng/wipe for CP and IF.

Table 2 showed the positive ratio of detection and amounts of CP and IF. CP was detected occasionally in both BSCs. Amounts of CP detected varied widely (8～1869 ng/wipe); the working surfaces inside BSCs were unexpectedly contaminated, meanwhile, contaminations of the back surfaces in BSCs and working tables around BSCs were at low levels. IF over the LLOQ was not detected in both BSCs.

In urinary excretion monitoring, calibration curves were fitted by linear regression 2–5000 ng/ml urine for CP and 1–5000 ng/ml urine for IF without an evaporation step. LLOQs of CP and IF were improved to 0.4 ng/ml urine after an evaporation step. The values of LLODs with and without an evaporation step, however, were equal; 0.4 ng/ml urine for CP and IF.

CP and IF were not detected in all urine samples.

DISCUSSION

Many reports,[2–14] in addition to the report published by Falck[1] in 1979, have led to increased concerns about the occupational exposures of hospital

Table 2. Frequencies and Amounts of CP and IF Detected in Wipe Samples (ng/wipe)

Place	Sampling points	CP		IF	
		Positive ratio	Amounts	Positive ratio	Amounts
hospital pharmacy	A) Working surfaces inside BSC	4(1)/16	26～938	0/16	—
	B) Under superabsorbent sheet in BSC	1/8	18	0/8	—
	C) Back surfaces in BSC	3(2)/14	54	0/14	—
	D) Working tables around BSC	2/21	8～31	0/21	—
oncology ward	A) Working surefaces inside BSC	2/16	451～1869	1(1)/16	—
	C) Back surfaces in BSC	1/8	23	1(1)/8	—
	D) Working tables around BSC	0/13	—	0/13	—

Numerals in parentheses mean numbers of samples under LLOQ (5 ng/wipe).

workers to hazardous drugs; however, contradictory results regarding the same have been reported. Tomioka[20] monitored occupational exposures to hazardous drugs by routes of exposures and the levels at which the drugs exert their effects; *i.e.*, (i) external exposures, exposure to airborne drugs and drugs deposited on the working table; (ii) internal exposures, presence of drugs or their metabolites in blood and urine; (iii) cellular level effects, presence of mutagens in urine and frequency of sister chromatid exchanges (SCEs); and (iv) effects on individual level, susceptibility to cancer and effects on reproduction.

In the studies about the effects of occupational exposures at the cellular level, several groups reported that significant differences were observed between the frequencies of SCEs,[3] chromosome aberration (CA),[21] micronuclei (MN),[4] and commet assay,[7] while other groups reported no significant differences between the frequency of SCEs,[2,4,5] CA,[2] and MN.[2] Further, only Roth[2] and Kopjar[7] reported obvious correlation between cellular level effects of hazardous drugs and working environments. Roth[2] reported that pharmacists who took all possible precautions showed no significant differences and Kopjar[7] reported that statistically significant differences were observed between occupational exposures occurring with and without using laminar flow cabinet.

In internal exposure studies[6,8–12,14] and in external exposure studies,[9,13,22,23] CP was frequently used as a marker of occupational exposures because of its slight volatility,[16] human genotoxicity,[16,24] reproductive toxicity,[25] carcinogenity,[17] and ease of detection.[26,27] Among several reports on occupational CP exposures in healthcare workers, some showed the presence of CP in the urine samples of nurses and pharmacists,[6,8–14] while others did not.[23,28] Although CP was more frequently detected in nurses than in pharmacists,[29] amounts of CP excreted varied considerably in individuals, and these amounts did not correlate with the frequencies of handling drugs and the years of working.

In Japan, the proposal of the JSHP led to an increased concern among hospital workers about the exposures to hazardous drugs. The JSHP[30] reported that occupational exposures to CP *via* internal exposures and external exposures greatly varies in hospitals and in individuals even in other countries.

Thus, we investigated whether the standard precautionary measures that we took in our hospital (Table 3) were adequate to prevent occupational exposures. We started preparing chemotherapeutic drugs for inpatients since February 2003, and for outpatients since November 2003. We adopted the standard precautionary measures to avoid occupational exposure from the beginning,[15] and we improved measures accordingly. For example, we switched the material of gloves from latex to nitrile rubbers because latex gloves were suspected to facilitate the permeation of hazardous drugs.[12,13]

Table 3. Standard Precautionary Measures to Prevent Occupational Exposures Taken in Our Hospital

No	Standard precautionary measures
1	Prepare hazardous drugs in a centralized area restricted to authorized personnel with expertise in the preparatory techniques and the characteristics of these drugs.
2	Prepare these drugs in a biological safety cabinet Class II Type B.
3	Place a superabsorbent sheet on the work counter inside the preparation cabinet. (Only at the BSC in hospital pharmacy, not use at the BSC in an oncology ward.)
4	Use syringes with *Luer-Lok-type* fittings for preparing these drugs.
5	Use gowns made of a lint-free, low-permeability fabric. The gown should have a closed front, long sleeves, and elastic or knit-closed cuffs.
6	Use disposable nitrile rubbers gloves doubly, and change gloves every hour or on accidental exposures.
7	Wear a disposable mask and cap.
8	Remove protective clothing carefully to avoid spreading contamination.
9	Maintain a negative pressure in the drug vials.
10	Always close the cover of trash boxes used to dispose of these drugs.
11	Using a rotating schedule, as limitation the successive preparation time to 1 hour.

These measures are based on the guidelines recommended by the National Institute for Occupational Safety and Health.

While the JSHP[31] and Mochizuki[32] recommended use of sodium hydroxide (NaOH) solution for wiping solvent, this solution decomposed paclitaxel and docetaxel.[32] We selected 20% methanol as wiping solvent, because we will extend this measurement system to simultaneous determination of multidrugs,[13,18,33] and confirmed this solvent was adequate to SPE extraction with high recovery.

We adopted convenient procedures because routine monitoring was important to survey occupational ex-

posure risk. Although daily uptake was estimated from 24-h urine sampling, we were able to examine only 1 person using this approach, as continuous sampling was a burden for the subjects. Others were examined for 2-points samplings. Sampling times were referred to urine excretion peaks, about 6–10 h after CP administration.[14,34]

We selected SPE[27,35] as a pretreatment and the LC/MS/MS[33,35,36] system for analyzing the samples. SPE pretreatment reduces matrix effects, which affect mass analysis, more effectively than protein precipitation or liquid-liquid extraction,[37] and our intensive washings with 30% methanol significantly reduced trash backgrounds from urine. LC/MS/MS avoided laborious derivatization procedures such as heating up and evaporation, and therefore, reduced the chances of the error. Although MS/MS analysis did not improve LLODs, it specifically identified CP and IF from impurities better than single MS analysis. Saturated vapors of CP solution at 37°C show increased mutagenic potential[16] and routine analyses without heating the samples are very useful. Because we directly analyzed eluate from SPE without evaporation, LLOQs were higher than that previously reported with an evaporation step,[26,27] but LLODs were equivalent to that previously reported. Thus, we analyzed the urine samples using 2-tiered approaches, without or with evaporation.

In an environmental contaminations study, frequencies and amounts of CP detected were low levels in our hospital, compared with previous reports in Japan,[30,38] though the working surfaces inside BSCs were unexpectedly contaminated.

Because carcinogenicity of an alkylating agent is related to continuous exposures and cumulative amounts,[39] an estimation of absorbed CP from urinary excretion is important. An absorption of CP was similar in oral or intravenous administration, and mainly 10–20%[11,40–44] of unchanged CP was excreted into urine. Further, 1% of unchanged CP is excreted when CP is administered by dermal route.[10]

Inhalation and dermal penetration were presumed to be the main routes of occupational exposures. Sessink,[12] Minoia,[13] and McDevitt[45] compared the urinary CP excretion levels and concentration of CP in air during preparation of drugs. They concluded that the amounts of CP inhaled were much lower than amounts CP excreted in urine, and that inhalation was not the main route of exposures resulting in high levels of CP. In Japan, Yabunaka[46] reported that volatilization of CP was not significant in usual preparation. Fransman[29,47] reported that dermal exposure predominantly occurred on the hands and sporadically on the forehead and forearms. In addition, he mentioned that greater than 90% exposures were prevented by using latex gloves at the time of preparation.

Ensslin[11] estimated that 0.0025% and 0.00045% of handled CP and IF were taken up, and possible precautions such as wearing proper protective clothing, safely handling drugs, and vertical laminar flow cabinet should be adopted.

We limited the successive preparation time to 1-h; therefore, amounts of preparation per capita in our hospital were much lower than other hospitals. Additionally, adept pharmacists educated newcomers according to our manual for handling hazardous drugs, which emphasized contact precaution. CP and IF were not detected in urine, due to these precautionary measures.

Sessink[8] and Sorsa[48] estimated the carcinogenic risk by CP absorption. Sessink[8] presumed that urinary excretion of unchanged CP was 1–5% and 180 ng of CP was continuously excreted in urine for 200 days $\times$40 years, and estimated that the occupational risk of developing cancer was 120–600 per million by extrapolation of incidences of tumors in rats, and proposed regular monitoring of urinary CP excretion.

Recently, the closed-system devices for preparation have been introduced in several hospitals in Japan. Yoshida[38] reported that use of closed-system devices could reduce occupational contaminations. Although these devices are useful for aerosol trappings, they are not versatile. We have adopted various precautionary measures in our hospital, but we have not employed these devices. Our result showed that improved awareness regarding handling of hazardous drugs could reduce the risk of occupational exposures.

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