



Cyclophosphamide Levels in Sites of Preparation and Administration of Antineoplastic Drugs

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SUMMARY. The extensive use of antineoplastic agents in chemotherapy may be at risk to health care workers involved in the preparation and administration of these drugs. In this study cyclophosphamide, a drug classified as a human carcinogen, was quantified by adapting a previous analytical method using gas chromatography coupled to mass spectrometry (GC-MS) after solid phase extraction with diatomaceous earth. The drug was measured by analysis in surfaces (wipe samples) and gloves, collected from four different hospitals, before and after the practice of cleaning procedures, and the use of a closed-system device for the preparation and administration. Validation results were satisfactory and cyclophosphamide levels ranging from below the quantification limit to 141000 ng. Our findings demonstrated that surfaces and materials contamination was found in all hospitals during the traditional open technique for preparation and administration of cyclophosphamide and a significant reduction in contamination when a closed-system device was used. However, some values were considered unexpected, especially those obtained from samples collected after the cleaning surfaces.

RESUMEN. “Niveles de Ciclofosfamida en Puestos de Preparación y Administración de Drogas Antineoplásicas”. El uso extenso de agentes antineoplásicos en quimioterapia se considera un riesgo relevante para el personal implicado en la preparación y la administración de estos medicamentos. En este estudio la ciclofosfamida, un fármaco clasificado como carcinógeno humano, fue cuantificada adaptando una metodología anterior usando la cromatografía de gas/ espectrometría de masa (GC-MS) después de la extracción de las muestras con fase sólida de tierra de diatomeas. La medición del fármaco fue realizada por análisis en las superficies (wipe test) y los guantes, recogidos a partir de cuatro diversos hospitales, antes y después de la práctica de limpieza, y el uso de un dispositivo de sistema cerrado para la preparación y la administración. Los resultados de la validación fueron satisfactorios y los niveles del ciclofosfamida fueron inferiores al límite de cuantificación a 141000 ng. Los resultados demostraron que la contaminación de las superficies y de los materiales fue encontrada en todos los hospitales durante la técnica abierta tradicional para la preparación y administración del ciclofosfamida y que hubo una reducción significativa en la contaminación cuando se utilizó el sistema cerrado. Algunos valores fueron inesperados, especialmente los obtenidos a partir de muestras recogidas después de la limpieza.

INTRODUCTION

A hospital, like any other work environment, presents numerous health risks to workers that include chemical, physical and biological agents. Among chemical agents, exposure to antineoplastic drugs (ANDs) is particularly important. The use of this category of drugs has progressively increased over the last decades as a result of the growing number of tumor cases diagnosed and the need for new formulations that can offer a better life quality to patients ^{1,2}.

Groups exposed to antineoplastic drugs include patients, individuals working in the pharmaceutical industry, workers who prepare and administer the drugs, cleaning personnel, and family members of patients and researchers ³.

Hospital personnel may be exposed by inhalation of aerosols, drug droplets and dust, or by direct skin contact, which is considered to be main exposure route. Inadvertent ingestion is also possible, as are accidental events, such injuries from sharps. Handling storage, transport,

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and disposal of these drugs are task which may lead to exposure ⁴.

Comparison of results of periodic monitoring studies of exposed and non-exposed sanitary groups has demonstrated a higher frequency of symptoms, with a cancer risk, in health care workers occupationally exposed to antineoplastic agents ^{5,6}.

Regardless of the program adopted to contain the occupational risks of exposure to hazardous substances, in the case of antineoplastic drugs this program should be constantly revised and the entire personnel involved in drug handling should be informed and trained to ensure that the safety recommendations are being followed ⁷.

Environmental and biological monitoring procedures are important in this planning. In order to assure an accurate risk assessment, the monitoring strategy should meet the following: information about the quantity of substance manipulated and mode of diffusion of the drugs in the environment, characterization of the area of highest contamination, knowledge about the main routes of absorption of the agent and effectiveness of the personal protective equipment and biological safety cabinets (BSCs) ^{2,8,9}.

Since workers are exposed to a wide variety of antineoplastic agents, it is necessary to identify certain substances that can be used as indicators. These can include cyclophosphamide, 5-fluorouracil and platinum compounds. These drugs can be detected on surfaces, environmental and biological matrices, according to the following priorities: a) measurements on surfaces samples; b) measurements on biological samples and c) measurements on environmental samples ^{6,9,10}. The environmental measurements can be useful to check the effectiveness of aspiration and air exchange systems ^{10,11}.

The current trend in developed countries is the use of a safety device, also known as closed-system device, which prevents the release of drugs into the environment during preparation and administration. Studies have shown that the introduction of this device, in conjunction with the use of BSCs, appeared to contain surface contamination of ANDs ¹².

The objective of the present study was to evaluate surfaces and materials before and after cleaning procedures and to evaluate the effectiveness of the closed-system in reducing contamination in sites of preparation and administration of cyclophosphamide, utilizing gas chromatography coupled to mass spectrometry with a previous solid phase extraction (SPE-GC/MS).

MATERIALS AND METHODS

Study Design

For collecting data about the workplace, the work procedures, the personnel involved and the drugs used in all hospitals, a standardized questionnaire ¹¹ was used, which was the basis to choose the sampling strategy.

To measure the contamination at the sites of preparation and administration of antineoplastic drugs, sampling was carried out after the first and third weeks of cyclophosphamide use, before and after cleaning procedures, in four hospital centers, certifying that cyclophosphamide was manipulated in amount of at least 6 g, according to hospital's routine. Only in Center II the study was conducted in two phases.

In Phase 1 of the study the manipulation of cyclophosphamide was done according to a traditional technique, preparation using an ampoule and/or vial, needle and syringe and transfer of the drug from the vial and/or ampoule with a syringe or needle. In Phase 2 the Center II staff had been using the closed-system device (Phaseal[®], purchased from Carmel Pharma) for several months before the samples collection, which allowed them to become adept with a new technique.

In all hospitals centers, contamination was evaluated on surfaces at the preparation sites, which had contamination probability. Wipe samples were taken from safe airflow hood (60 x 60 cm), from the floor adjacent to the airflow hood (70 x 70 cm) and from the external area to airflow hood (60 x 60 cm). Door handles, gloves and infusion bags (25 x 25 cm) also have been analyzed in this study. The surfaces were washed with 20 mL of 0.03 M NaOH, and afterwards wiped dry with two sheets of absorbent tissues (Kleenex professional wipes), which were then stored in coded, 125 mL plastic screw top containers. Uneven surfaces were sampled by applying the sodium hydroxide solution to the paper and wiping the surface.

The collected samples were quickly sent to the laboratory conditioned in polystyrene boxes packed with dry ice and kept at -20 °C prior to analysis.

Materials

Chemicals

Cyclophosphamide (CP) (ACRÓS-USA[®]; purity > 97%); trifluoroacetic anhydride (SIGMA-USA; purity > 99%). Other chemicals were of the highest purity available. SPE disposable cartridges (Chem Elut[®], diatomaceous earth, 300 mg) were purchased from Varian[®] (USA).

Analytical conditions

The analyses were performed with a Hewlett Packard 6890 GC coupled to a 5972-A MS both controlled by a Hewlett Packard Vectra XM® series 3 5/9- personal computer. The analytical column used was a Hewlett Packard HP5-MS®, 30 m, 0.25 mm internal diameter and 0.25 µm film thickness- connected to a deactivated fused silica retention gap (HP Retention Gap 5 m, 0.53 mm internal diameter) and 1 µL of the cleaned sample was injected in the on-column mode; the initial injector temperature was 80 °C. After 1 min, the injector temperature was increased at 75 °C/min to 300 °C, remaining constant. The initial oven temperature was 70 °C. After 1 min, the temperature was increased at 15 °C/min to 250 °C, where it remained constant for 3 min and was then increased at 30 °C/min to 300°C, and held constant for 5 min. Helium (He) was used as carrier gas (column inlet pressure 82 kPa). The interface temperature was 310 °C.

Initially, CP identification was carried out in SCAN mode, with full spectra (50 to 350 m/z) from the tenth to the fifteenth minute of the chromatographic run. From the full spectra, ion fragments 307 and 309 were selected for analysis in the SIM mode. Finally, the analyte was identified by the presence of ions 307 and 309 and by the retention time of N-trifluoroacetylated CP.

Quantification was performed on the 307 selected ion fragment and the peak area of the N-trifluoroacetylated derivative CP was calculated. A calibration curve was traced ranging from 0.01 to 2 ng of CP (corresponding to the amount injected into the GC-MS), treated as samples and injected before each set of samples, in duplicate.

Sample treatment

The following is a modification of a procedure by Sessink *et al.*¹³, previously described by Martins *et al.*¹⁴. The original technique utilized liquid-liquid extraction for the clean-up step, whereas in the present work, solid phase extraction with a modified form of a diatomaceous earth packed into a polypropylene cartridge was used.

Thirty milliliters of 0.03 mol L⁻¹ NaOH were added to the sample vial, together with 20 mL of previously collected NaOH solution. After sonication for 90 min and 10 min agitation, the sample was centrifuged at 3000 rpm for 10 min. To the supernatant (1 mL), 1 mL of TRIS buffer (0.2 mol/L; pH 4.5) was added. One milliliter of this mixture was placed on the extraction column. After 5 min, the column was eluted with 5

mL of ethyl acetate and the extract was dried in an evaporator centrifuge under vacuum. Ethyl acetate (50 µL) and trifluoroacetic anhydride (50 µL) were added and mixed. The vial was closed for derivatization at 70 °C for 20 min. After cooling at room temperature, the sample was dried with the evaporator centrifuge under vacuum, and 50 µL of toluene were added. The sample was then sonicated for 3 min and stored at -20 °C prior to analysis.

Confidence parameters

Validation was performed according to FDA, 1996, FDA, 2001 and IUPAC, 2002¹⁵⁻¹⁷ and the parameters below were evaluated.

Linearity was tested by examination of a plot of residuals produced by linear regression of the responses on the amounts of the analyte in a calibration set, between 0.01 and 2 ng, in six replicates for each level. A calibration curve was generated for each analytical run, in duplicate, and it consisted of a blank and six non-zero samples covering the expected range, including quantification limit (LOQ). When CP mass was greater than the upper limit of the range, the sample was diluted.

The quantification limit (LOQ) was determined by comparing the measured signals of samples with known low concentrations of analyte to those of blank samples thereby establishing the minimum amount of analyte that could be reliably quantified, with a signal-to-noise ratio of 10:1.

Bias and recovery were determined by five replicate analyses of samples after the addition (spiking) of a known mass of the analyte in the LOQ (0.01 ng), middle (0.2 ng) and high level (2 ng). The results were compared with those obtained when the analyte was spiked after the clean-up procedure of the sample. No certified materials are available for ANDs, so that standard addition method should be used for evaluating the reliability of the method.

Precision was determined with five replicate analyses of samples containing known amounts of the analyte, using the LOQ (0.01 ng), middle (0.2 ng) and high level (2 ng), during a single analytical run (repeatability) and between-runs (intermediate precision).

Long-term stability was determined by examination of three aliquots of each of the low (0.01 ng) and high level (2 ng), stored under -20 °C for fifteen and thirty days. The levels of stability of samples were compared to the mean of determined values for the samples from the first day of long-term stability testing.

Statistical analysis

The InStat® software package was used for statistical calculations. The Kruskal-Wallis and Mann-Whitney tests were used for multiple comparisons between samples.

RESULTS

Confidence Parameters

Table 1 demonstrates the results obtained after the validation procedure. The method described produced a linear curve in the range of 0.01 to 2 ng, with a correlation coefficient of 0.9939. The dynamic range evaluated was able to quantify CP in the majority of samples (85.7%).

Samples

For this study, in Phase 1, 143 samples were collected from 4 distinct centers: 62 collected from different surfaces, 39 pairs of gloves and 42 infusion bags. After analysis, a total of 115 samples (80.5%) showed the presence of cyclophosphamide in concentrations ≥ 0.01 ng, which is the limit of quantification of this method (Table 2).

In phase 2, 33 samples were collected at Center II, including 16 samples from different surfaces, 9 from gloves and 8 from infusion bags. Cyclophosphamide could only be quantified in 9 (27.3%) of the 33 samples. Table 2 shows that in all surfaces the results were lower. Application of the Mann-Whitney test revealed a significant difference ($p < 0.0001$) in cyclophosphamide concentrations before and after the use of the device.

Application of the Kruskal-Wallis test revealed no significant difference (p value was 0.2010) when the median concentrations of cyclophosphamide at the safe airflow hood surface, gloves and infusion bags were compared.

No significant differences were observed before and after cleaning procedures (Mann-Whitney test: center I: $p = 0.2403$; center II: $p = 0.9356$; center III: $p = 0.9999$; center IV: $p = 0.6307$). The centers weren't capable of effectively decontaminate the surfaces resulting, sometimes, in surface cumulative contamination. Figure 1 and Table 3 show cyclophosphamide concentrations on surfaces before and after cleaning procedures.

Parameter	Result
Limit of Quantification (LOQ)	0.01 ng
Dynamic Range	0.01-2 ng
correlation coefficient (\pm standard error)	0.9939 (\pm 5904.7)
regression equation (\pm standard error)	$y = 1345.6 (\pm 77.4) x + 1603.9 (\pm 4348.8)$
Bias	86-118%
Recovery	98.9%
Precision (range)	repeatability: 5.0-7.5% intermediate precision: 6.5-19%
Stability	30 days at -20°C

Table 1. Confidence parameters of the proposed method for cyclophosphamide determination in wipe test by GC/MS after solid phase extraction.

	Floor adjacent to airflow hood (ng/cm ²)	Safe airflow hood (ng/cm ²)	Door handles (ng)	External area to airflow hood (ng/cm ²)	Gloves (ng)	Infusion bags (ng/cm ²)
CENTER I	0.1-0.3	0.1-1	NQ*-1100	0.1-0.7	NQ*-3640	0.3-3
CENTER II						
Phase 1**	0.1-0.5	0.2-0.3	135-1170	0.1-0.5	NQ*- 104000	0.8-36
Phase 2	0.2	0.1	NQ*	NQ*	NQ*	NQ*
CENTER III	NQ*-0.2	0.1-4	NQ*-240	NQ*-2	NQ*-5000	NQ*-0.1
CENTER IV	0.1-3	0.7-53	NQ*-76	NQ*-0.5	NQ*-141000	NQ*-533

Table 2. Cyclophosphamide levels (range) in wipe test and gloves collected in four distinct hospitals centers (phase 1, n = 143 samples; phase 2- center II, n =33). *NQ = not quantified for this method (below LOQ). ** Phase 1 = the drug was prepared using the traditional technique and Phase 2 = the Center II utilized the closed-system to prepare the drug.

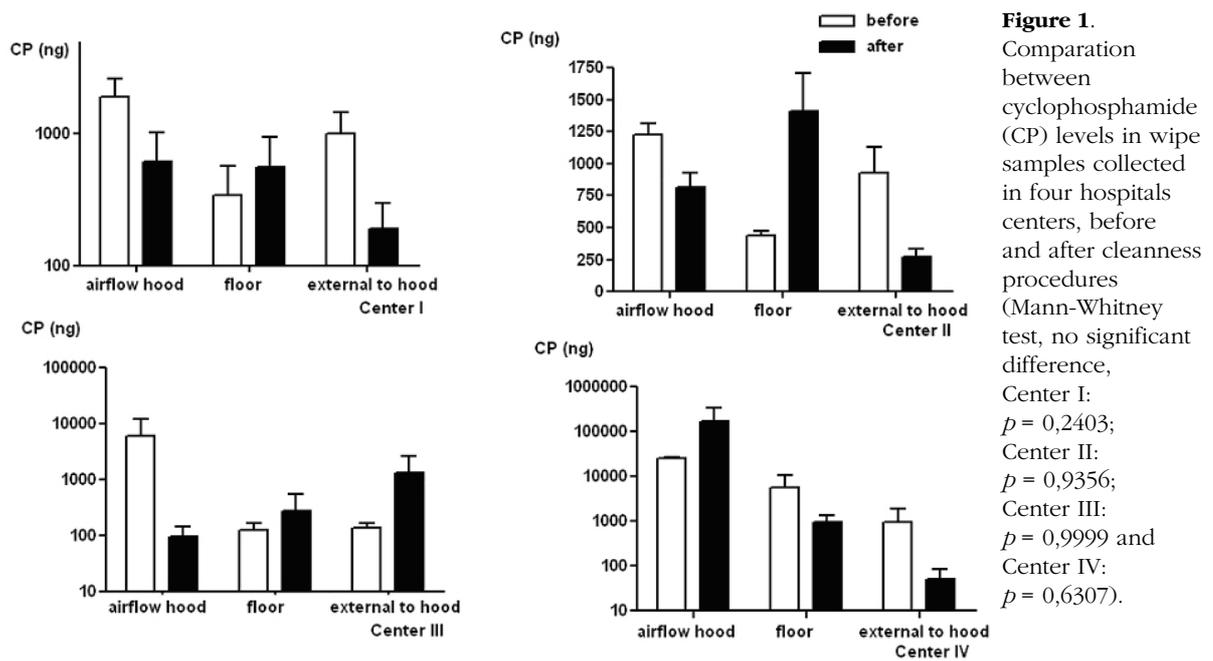


Figure 1. Comparison between cyclophosphamide (CP) levels in wipe samples collected in four hospitals centers, before and after cleanliness procedures (Mann-Whitney test, no significant difference, Center I: $p = 0,2403$; Center II: $p = 0,9356$; Center III: $p = 0,9999$ and Center IV: $p = 0,6307$).

	Before cleanness	After cleanness
Floor adjacent to airflow hood (ng/cm ²)	*NQ- 3	0.1-0.5
Safe airflow hood (ng/cm ²)	0.1- 4	0.1-53
Door handles (ng)	*NQ- 688	*NQ- 1170
External area to airflow hood (ng/cm ²)	*NQ- 2	*NQ-0.4

Table 3. Cyclophosphamide levels (range) in wipe test collected in four distinct hospitals centers, before and after cleanness procedures (n=62). *NQ= not quantified for this method.

DISCUSSION

In order to assess occupational exposure to ANDs, surface, biological e environmental monitoring should be carried out. Since even very low exposure levels may result in a health hazard, highly sensitive and specific analytical methods are required ¹⁸.

In this study, the presence of cyclophosphamide on different surfaces and materials was determined based on the method proposed by Sessink *et al.* ¹³, with the technique used to identify the analyte being similar to that employed by other investigators, i.e., gas chromatography coupled to mass spectrometry ^{2,19}. Most of the several methods for CP determination in wipe samples use extraction procedures using liquid-liquid extraction with ethyl acetate ^{13,18,20}. The samples, in this study, were extracted with a solid phase, using a diatomaceous earth, which reduces the occupational risk from solvent exposure, an important characteristic when a method is in routine.

In Table 1 is possible observed that the validated SPE-GC/MS method, in the present study,

can be considered satisfactory, according to the guidelines adopted ¹⁵⁻¹⁷.

No significant difference when the median concentrations of cyclophosphamide at the biological safety hood surface, gloves and infusion bags were compared showed CP contamination on several surfaces of infusion bags that may have been contaminated from gloves utilized by health care workers, in the chemotherapy handling sites. On the other hand, this can also increase the risk of exposure in other areas of a hospital. Sessink *et al.* ¹³ detected CP in the urine of pharmacy technicians and nurses didn't directly involve in the preparation and administration of this drug.

Minoia *et al.* ²⁰, Connor *et al.* ²¹ and Minoia *et al.* ²² reported values between 10 to 96610 ng cyclophosphamide/cm² in wipe tests collected in several hospital departments. This shows the wide range of concentrations that can be determined in different surfaces, even in places where access is not restricted. All this leads to the conclusion that it is imperative to monitor the contamination of surfaces such as drug racks

and door handles, where the probability of contact is great and is not restricted to those professionals involved in drug preparation.

According to Connor *et al.* ¹², significant reduction in the levels of antineoplastic drugs is observed when the Phaseal device is used. These authors collected samples from different surfaces before and after using of this system and analyzed the samples by GC/MS/MS. The detection limit of their method was 0.1 ng mL⁻¹ and even so drugs weren't quantified in many samples after the use of the closed system.

Nygren *et al.* ²³ compared the efficiency of a closed system in minimizing the environmental concentrations of antineoplastic drugs and concluded that the use of the traditional technique, an open system for the preparation and administration of drugs may result in significantly higher environmental concentrations.

The use of a closed-system device for the preparation and administration of antineoplastic drugs appeared to be able to contain the contamination inside and outside of airflow hood. In spite of, the sensitivity of the detection technique and the apparent inefficiency of the routine cleaning procedures revealed unexpected results.

Cleaning of the BSC should be performed at the end of a session of preparation activities, such as at the end of a shift or day of work, depending on the volume of hazardous drug preparation. Traditionally, a cleansing agent followed by ethanol has been used to clean and disinfect work surfaces; however, ethanol does not deactivate hazardous drugs. Many drug manufacturers recommend sodium hypochlorite (bleach solution) to inactivate cytotoxic drugs. Sodium thiosulfate solution inactivates the bleach, thus reducing the potential for corrosion of work surfaces. Oxidation from the bleach followed by nucleophilic substitution from sodium thiosulfate result in the chemical degradation and mutagenic inactivation of many commonly used chemotherapy drugs ²⁴.

The question of whether exposure can be diminished by a reduction in handling is difficult to answer. Normally, it is reasonable to assume a positive correlation between use and exposure and currently, no recommended exposure limits (RELs), permissible exposure limits (PELs), or threshold limit values (TLVs®) have been established for antineoplastics drugs ^{25,26}.

A balance must be achieved to continue the use of these beneficial drugs in patients, while assuring the health of personnel administering

them. Much of the new guidance revisits the long standing elements of a comprehensive safe handling program and reminds us that the risk remains and our vigilance is required, but that a harmonized safe handling approach has been adopted that assures minimal risk to workers who provide lifesaving therapies to their patients ²⁷.

CONCLUSIONS

In summary from our findings it can be concluded that surfaces and materials contamination was found in all hospitals during the traditional technique for preparation and administration of cyclophosphamide and a significant reduction in contamination when a closed-system was used. However, some values were considered unexpected, especially those obtained from samples collected after the cleaning surfaces.

Although the results of the present study are not sufficient to determine the true dimension of contamination of the work environment with antineoplastic drugs, they support previous findings and may contribute to future studies by supporting the use of the present method for monitoring occupational exposure with these agents.

The exposure evaluation by means of environmental and biological monitoring, health monitoring, the adoption of information programs, training of staff and the use of environmental and personal protective equipments are some of the measures recommended to control exposure and to protect the health of staff who are in routine contact with antineoplastic drugs in workplace.

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