

Determination of Urinary Cyclophosphamide in Oncology Nurses Handling Antineoplastic Drugs by Gas Chromatography-Mass Spectrometry

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Summary : The aim of the study was to detect exposure to antineoplastic drugs, using cyclophosphamide (CP) as the model compound, in nurses who worked in oncology departments of hospitals. Chemotherapy with antineoplastic agents is often used in the treatment of cancer. When handling antineoplastic drugs, nurses and physicians may face certain health risks. Many antineoplastic agents directly or indirectly react with DNA. Consequently, the proliferation of tumor cells is decreased. CP, one of the most commonly used antineoplastic drugs, is known to be a human carcinogen [Group 1 class according to International Agency for Research on Cancer (IARC)]. CP is known to be a model compound for the identification of potential exposure situations in the various phases of its manufacture and hospital use. A sensitive gas chromatographic method for the determination of CP in urine is used. In the present study, after liquid-liquid extraction with diethyl ether and derivatization with trifluoroacetic anhydride, CP was identified and quantified with gas chromatography-mass spectrometry (GC-MS). The urinary excretion rate ranged from 0-2.12 µg CP/ 24 h.

Keywords: Antineoplastic Drugs, Nurses, Cyclophosphamide, Gas Chromatography-Mass Spectrometry.

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Antineoplastik ilaçları uygulayan onkoloji hemşirelerinde Gaz Kromatografi-Kütle Spektrometrisi yöntemiyle İdrar siklofosfamid düzeylerinin tayini

Özet : Çalışmanın amacı, hastane ortamında antineoplastik ilaçlara potansiyel maruziyeti olduğu düşünülen onkoloji hemşirelerinde, siklofosfamid model bileşik olarak kullanarak maruziyetin boyutu tespit edilmektedir. Antineoplastik ilaçlarla yapılan kemoterapi, kanser tedavisinde sıklıkla kullanılmaktadır. Antineoplastiklere maruz kalan hemşire ve doktorlar bir çok sağlık riski ile karşılaşmaktadırlar. Antineoplastik ajanlar doğrudan yada dolaylı olarak DNA ile reaksiyona girmektedir. Sonuç olarak tümör hücrelerinin proliferasyonunu azaltmaktadırlar. CP, antineoplastik ilaçlar arasında en yaygın kullanılan ve insan karsinogeni olan bir ilaçtır (IARC'e göre Grup 1, insan karsinogeni). CP'nin hastane ortamı ve üretimin çeşitli aşamalarında potansiyel maruziyeti tanımlamada model bileşik olduğu bilinmektedir. İdrarda CP tayini için duyarlı bir kromatografik yöntem mevcuttur. Sunulan çalışmada dietileter ile sıvı-sıvı ekstraksiyon ve trifloroasetikasit ile türevlendirilmeden sonra Gaz Kromatografi-Kütle Spektroskopisi (GC-MS) ile CP'nin izolasyonu ve kantitatif tayini yapılmıştır. İdrar CP atılımı 0.2-2.12 µg CP/24 saat'dir.

Anahtar kelimeler: Antineoplastik ilaçlar, Hemşireler, Siklofosfamid, Gaz Kromatografi - Kütle Spektroskopisi

INTRODUCTION

Many widely used chemotherapeutic agents are known to be mutagens and carcinogens in animals, humans and in patients treated with therapeutic doses¹. Cyclophosphamide (CP) is widely used in cancer chemotherapy, mostly in combination with other antineoplastic agents, and as an immunosuppressant^{2,3}. CP is known to be a human carcinogen according to the International Agency for Research on Cancer (IARC: Group 1). CP is a well-documented reference mutagen expressing its genotoxicity when metabolically activated¹. The main metabo-

lites of CP are 4-hydroperoxycyclophosphamide, phosphoramidate mustard and acrolein. Further conversion to non-nitrogen mustard may also occur². The therapeutic antitumor activity of CP is most probably due to phosphoramidate mustard^{5,6}.

The primary source of human exposure to anticancer drugs is from their use in the therapy of cancer. However, persons employed in the manufacture, preparation and administration of the drugs to patients and in the care of patients may also be exposed. In Turkey, the major exposure group to these drugs is nurses. Nurses are exposed much

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more than physicians since they apply them to patients. Several studies have reported health hazards associated with occupational exposure to these drugs. When handling antineoplastic drugs, nursing personnel may face certain health risks. There is sufficient evidence that several antineoplastic drugs are carcinogenic to humans and CP, one of the alkylating antineoplastic agents, is one of them (Group 1)⁵⁻⁹. Thus CP is used as an important indicator for many antineoplastic drugs. We determined urinary CP excretion rate in oncology nurses and tried to evaluate possible risk to them.

Materials and Methods

Subjects

Our study group (n=24) consisted of female nurses handling antineoplastic drugs. Administration of drugs to patients is performed by nurses. Nurses prepare and apply antineoplastic drugs for at least two months, 15 times a week. All nurses applied safety precautions, including the use of a vertical laminar air-flow hood, protective gowns, and latex gloves in Oncology and İbn-i Sina Hospitals in Ankara, Turkey. We previously reported a similar study. In this current study, nurses explained that they had improved safety precautions, in particular; they used very protective vertical laminar air-flow hood (*safety cabinet*) (Three of them did not use the safety cabinet without explanation). The nurses expressed that since they were curious about their exposure level after using the cabinet, they voluntarily wanted to be involved in this study.

Table 1 shows the general characteristics of nurses and Table 2 shows the CP excretion rates and use of protective equipment while preparing and applying antineoplastic drugs. Each person was interviewed and a questionnaire was completed. Twenty-five percent of the nurses did not use a mask; all of the nurses wore both glove and gown. However they claimed to use the same gloves throughout the day.

Preparation of urine samples

Total 24 h urine was collected in portions starting from the end of a work period of at least four days. No urine samples were taken from the controls

because pre-studies had demonstrated that CP could not be detected in the subjects who were not exposed to antineoplastic drugs. Collected urine samples were coded and stored at -20°C until sample preparation. Materials and reagents and the sample preparation were described by Sessink et al.⁷

Briefly, sample preparation was carried out as follows: 5 ml urine samples were extracted three times with 10 ml ethyl ether and the ether layers were combined and dried under nitrogen. The residue was mixed with 100 μl of ethyl acetate. 200 μl of trifluoroacetic anhydride was used for derivatization. After derivatization, the samples were dried under nitrogen at 30°C . 100 μl toluene was added and the samples were stored in vials at -20°C until analysis.

Calibration

Calibration curves were constructed from the analysis by standard curve samples, which were freshly prepared by adding CP to blank urine. The CP concentrations of the standard urine samples were 0.01, 0.05, 0.08, 0.2 and 0.3 $\mu\text{g}/\text{ml}$ urine.

Instrumental and analytical conditions

Gas chromatography-mass spectrometric (GC-MS) analysis was performed on HP 6890 GC-HP 5972 AMS GC-MS system. Separation was carried out on a cross-linked methyl siloxen capillary column (30 m \times 0.25 mm, film thickness 0.25 μm). The initial injector temperature was 50°C . The initial oven temperature was 50°C . After 2 mins, the temperature was increased by $200^{\circ}\text{C}/\text{min}$ to 250°C , where it remained constant for 10 mins¹⁰. Helium was used as carrier gas (column inlet pressure 8.2 psi). The interface temperature was 250°C . Electron impact (EI) was used as ionization mode. Identification was carried out by the combination of retention time [CP and iphosphamide; (IP)] and MS spectrum. Retention times of derivatized CP and IP were 15.88 and 15.54, respectively. Quantification of the N-trifluoroacetyl derivatives was performed on the selected-ion fragment m/z 213 for CP and m/z 211 for IP, which was abstracted from the full-scan spectra. (signal/noise > 4). The limit of detection was 0.005 $\mu\text{g}/\text{ml}$. For quantification, the peak height ratio of CP/IP was calculated. Quantification of the trifluoroacetyl derivatives was carried out by refer-

ence to calibration curves constructed from the analysis of freshly prepared reference urine samples containing CP dissolved in blank urine.⁷

Table 1. General Characteristics of Nurses

Parameter	Nurses
<i>n</i>	24
Age (mean±SD)	32.41±6.25
Duration of exposure (mean±SD, years)	5.09±4.05
Range	0.2-15
<i>Smoking habits</i>	
Non-smokers	10
Smokers	14

Table 2. CP Excretion Rate in Oncology Nurses in Relation to Several Parameters of Exposure

No	Smoking habits (S/NS)	Frequency of handling antineoplastics (times/week)	Use of cabinet/mask/glove/gown	Urinary CP excretion rate (µg/24 h)
1	S	25	+/-/+	0.07
2	NS	30	+/-/+	0.05
3	NS	15	+/-/+	ND
4	S	25	+/+	1.22
5	S	25	+/-/+	2.12
6	NS	25	+/+	0.07
7	S	20	-/+	0.13
8	S	20	+/+	0.07
9	NS	75	+/+	0.05
10	NS	100	-/+	0.03
11	S	100	-/+	0.02
12	NS	25	+/+	0.23
13	S	25	+/+	0.03
14	S	25	+/+	0.04
15	S	20	+/+	ND
16	S	25	+/-/+	0.05
17	S	25	+/+	ND
18	S	25	+/-/+	0.34
19	NS	25	+/+	ND
20	NS	25	+/+	0.01
21	S	15	+/+	ND
22	NS	25	+/+	ND
23	NS	25	+/+	0.21
24	S	25	+/+	0.07

S: smokers, NS: non-smokers, ND: not detected, CP: cyclophosphamide.

Results

As indicated in Table 1, the present study group (n=24) consisted of female nurses handling antineoplastic drugs. Table 2 shows the CP excretion rates and other related data of the nurses including the use of protective equipments and frequency of handling antineoplastic agents (times/week). According to their questionnaires, all of the nurses wore gloves and gowns; however, they claimed to use the same gloves during the whole day and did not take them off during other activities (e.g. drinking, eating, smoking, etc). Obviously, there is no sense in using gloves during handling antineoplastic drugs under these conditions. When preparing and applying these antineoplastic drugs, chemical-barrier face and eye protection (especially for sprays or aerosols of antineoplastic agents) must be provided. However, nurses did not apply such a face and eye protection in this study. Cabinet and mask were used by 87.5% and 75% of the nurses, respectively (3 did not use the cabinet without any explanation). They used surgical masks during preparation and application. However, surgical masks are not appropriate since they do not prevent aerosol inhalation. The safety cabinet should be cleaned according to the manufacturer's instructions. In the present study, nurses explained that they had no information about the cleaning of safety cabinets (cleaning, regularly changing HEPA filters, etc.), and thus did not regularly check cleanliness of the safety cabinets.

The excretion rates ranged from 0 to 2.12 µg CP/24 h. In our previous study, we found that CP excretion rates were much higher¹¹. This difference may be a result of safety cabinet use because nurses in the previous study did not use the safety cabinet, but, did use the other protective equipment. In the present study, nurses used vertical safety cabinets. We obtained reduced CP levels since exposure to drugs by inhalation may have been decreased by safety cabinet use.

There is no correlation between CP excretion rates and frequency of handling antineoplastic agents. Nurses handling CP showed a urinary excretion of

CP. Nurses with a nondetectable CP excretion rate took all preventive measures or did not handle CP (expressed as a ND, nondetectable in Table 2).

Table 3. Urinary CP Excretion in Smoker and Non-Smoker of Nurses Applying Antineoplastic Agents

	n	Urinary CP excretion rate Mean±SD (mg/24 h)
Smokers	11	0.30±0.61*
Non-smokers	7	0.07±0.09

*p<0.0001 (Unpaired t test)

Table 4. Urinary CP Excretion According to Using of Safety Cabinet and Mask

	n	Urinary CP excretion rate Mean±SD (mg/24 h)
Safety Cabinet (-)	3	0.06±0.06
Safety Cabinet (+)	21	0.22±0.5
Mask (-)	6	0.30±0.61
Mask (+)	18	0.07±0.09

P>0.05 (Unpaired t test)

We also compared the levels of CP excretion in smoker versus to non-smoker nurses. The levels of CP excretion in smokers (0.30±0.61) were approximately four times higher than in non-smokers (0.07±0.09, p<0.0001, Table 3). These data suggest that there was a significant contamination through the glove causing oral exposure. Our results showed that urinary CP levels in nurses who used safety cabinets were higher than in nurses who did not use safety cabinets. This result was opposite of expected data; however, could be due to the small size used for statistical analysis. We also compared nurses according to mask usage and our results showed that mask usage may prevent exposure (Table 4).

Discussion

In our study, exposure of oncology nurses to at least one antineoplastic agent was assessed from levels of urinary CP. In urine samples of 24 exposed nurses the CP excretion rate was found to range from 0-2.12 µg CP/24h. Most of the nurses handling antineoplastic drugs used gloves, mask and gowns, and

drugs were prepared in laminar vertical flow hoods. Our results of the analysis of CP in urine demonstrate that when the nurses were handling CP (and other antineoplastic drugs) this particular compound (CP) was observed in urine. CP is absorbed by dermal, oral and inhalation routes. Nurses were exposed to oral CP from contaminated hands through dermal penetration, which is one of the absorption routes. The CP levels of nurses who smoke were about four times higher than the CP levels of those who did not. (p<0.0001, Table 3). We found smoking to be a confounding factor in our study. We did not find a statistically significant difference between groups in terms of taking protective measurements (p>0.05, Table 3). Our results demonstrated a significant decrease in the levels of CP excretion compared to our earlier study. It can be suggested that the use of the protective vertical laminar air-flow hood had an active protective effect in this study. All nurses wore gloves; however, they claimed to use the same gloves during the whole day and did not take them off during other activities (e.g. drinking, eating, smoking etc.). Obviously, there is no sense in using gloves during the handling of antineoplastic drugs under these conditions. Frequent changing of the gloves would prevent dermal permeability and decrease exposure. The possibility of a carcinogenic hazard for nurses handling antineoplastic agents has been discussed in several publications^{5,12}. Pyy et al⁸. detected CP on the HEPA filters of flow hoods used in antineoplastic drug preparation, demonstrating aerosolization of the drug. Sessink et al¹³. found CP excretion rates in urine samples of six pharmacy technicians ranged from 0.2 to 19.4 mg. CP was found in the urine of technicians who did (n=3) and did not (n=3) prepare CP. No CP was detected in the urine of four technicians or the control urine samples. In another study, results of Sessink et al¹⁴. were similar to those of our study. They analyzed 35 urine samples and in 35 samples of eight workers CP was detected in a range of 0.1-2.9 µg /24h. Ensslin et al¹⁵. determined via GC the urinary excretion of the unmetabolized substances in hospital personnel occupationally exposed to cytostatic drugs. They found that excretion of CP ranged from 35 to 38 µg /24h (mean 11.4

µg/24h) urine and ifosphamide from 5 to 12.7 µg /24h (mean 9 µg /24h) urine. Sessink et al¹⁶. investigated the occupational exposure to CP, IP, 5-fluorouracil (5-FU), and methotrexate (MTX) of 25 pharmacy technicians and nurses. CP or IP was detected in the urine of eight of them, ranging from less than 0.01 to 0.5 µg.

In our previous study, we investigated genotoxic activity of antineoplastic agents in oncology nurses. For this reason, we analyzed urinary CP excretion using GC-MS and assessed genotoxic effect in peripheral lymphocytes and in exfoliated buccal epithelial cells using micronucleus (MN) frequencies. Urinary CP excretion was detected in 20 nurses (range from 0.02-9.14 µg CP/24h) and MN frequencies were higher in the exposed group compared to the control group¹¹. Grummt et al.¹⁷ found a significant increase in frequencies of structural chromosome aberrations of persons occupationally exposed to antineoplastic drugs without adequate protection compared to an adequate control population (3.3±0.1 vs 0.6±0.1, respectively). Sister chromatid exchanges (SCEs) in peripheral blood lymphocytes and mutagenicity of urine (Ames test) were measured in a group of 21 nurses occupationally handling antineoplastic drugs and in a group of 21 unexposed controls by Barale et al¹⁸. They detected no differences in SCE frequencies and in urinary mutagenic activity between exposed and unexposed groups. Ensslin et al¹⁹. quantified CP, IP, and platinum by the determination of urinary concentration to evaluate the risk borne by hospital pharmacy personnel exposed to antineoplastic agents. CP was found in two urine samples (5 and 9 µg/l urine). In all samples, the IP concentration was below the detection limit. Urinary platinum concentration was comparable with that of the non-exposed control group (4.35±5.6 versus 2.3±10.4 µg/g creatinine).

Ündeğer et al²⁰ assessed DNA damage in nurses handling antineoplastic drugs by the alkaline Comet assay. They found that the DNA damage observed in the lymphocytes of the nurses was significantly higher than in the controls (p<0.0001). In another study, the frequencies of chromosome aberrations,

SCEs and MN in blood lymphocytes were compared among six non-smoking female pharmacists before and after one year of working with cytostatic drugs. They found a significant difference among groups²¹. In contrast, Stiller et al.²² did not find a statistically significant difference between the controls and the persons handling cytostatic drugs.

To minimize the risk to these nurses several safety recommendations were issued. In response to numerous inquiries, OSHA (Occupational Safety and Health Administration) published guidelines for the management of antineoplastic agents in the work place in 1986. Approximately four years later, the European Society of Clinical Pharmacy (1990) recommended the usage of laminar down flow (Class II) safety hoods, latex surgical gloves of sufficient thickness, gowns, surgical masks, and hair covers for employees handling agents. Rooms for constitution of antineoplastic agents should be exclusively used for this purpose. An outdoor exhaust system for the safety cabinets is recommended.

All possible precautions should be taken to avoid exposure to antineoplastic agents, including proper protective clothing and a monitored, negative-pressured working environment with vertical laminar flow cabinet.

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