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Urinary cyclophosphamide excretion and micronuclei frequencies in peripheral lymphocytes and in exfoliated buccal epithelial cells of nurses handling antineoplastics

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Abstract

In this study, urinary cyclophosphamide (CP) excretion rate, as well as micronuclei (MN) in peripheral lymphocytes and in buccal epithelial cells were determined for 26 nurses handling antineoplastics and 14 referents matched for age and sex. In urine samples of 20 out of 25 exposed nurses CP excretion rate was found in a range of 0.02–9.14 μg CP/24 h. Our results of the analyses of CP in urine demonstrates that when the nurses were handling CP (and other antineoplastic drugs) this particular compound was observed in urine. The mean values (±SD) of MN frequencies (%) in peripheral lymphocytes from the nurses and controls were 0.61 (±0.32) and 0.28 (±0.16), respectively ($p < 0.01$). The mean value (±SD) of MN frequency (%) in buccal epithelial cells of nurses was 0.16 (±0.19) and also mean MN frequency in buccal epithelial cells for controls was found to be as 0.08 (±0.08), ($p > 0.05$). Age, sex and smoking habits have not influenced the parameters analyzed in this study. Handling time of antineoplastics, use of protective equipment and handling frequency of drugs have no effect on urinary and cytogenetic parameters analyzed. No correlation was found between the urinary CP excretion and the cytogenetic findings in nurses. Neither could we find any relationship between two cytogenetic endpoints. Our results have identified the possible genotoxic damage of oncology nurses related to occupational exposure to at least one antineoplastic agent, which is used as a marker for drug handling. As a whole, there is concern that the present handling practices of antineoplastic drugs used in the several hospitals in Ankara will not be sufficient to prevent exposure. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Antineoplastic drug; Occupational exposure; Micronucleus; Buccal cell; Lymphocyte; Cyclophosphamide excretion rate

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1. Introduction

Many antineoplastic drugs are mutagens, carcinogens and teratogens. Considering the mechanisms of the antineoplastic drugs that are used, it is not surprising that many persons involved in health care especially nurses are worried about the effects that these drugs may have on their health [1]. Most of the Turkish Health Care personnel involved in preparation and administration of antineoplastics do not use any self protective measures. Previous studies in Turkish Health Care personnel suggest that handling antineoplastic drugs involves a potential risk [2–4], however, the magnitude of this risk depends on the extent of exposure. Therefore, studies employing indicators for assessing exposure and biological effects are strongly recommended.

To monitor the biological uptake in exposed workers, methods for the detection of specific antineoplastic drugs or their metabolites in urine of exposed workers have been developed for cyclophosphamide (CP), *cis*-platinum (Cis), iphosphamide (IP), methotrexate (MT) and 5-fluorouracil (5-FU) [5–11]. Several previous studies have been carried out to monitor the biological effects of antineoplastic drugs in nurses. Methods for the detection of chromosomal aberrations [11,12], DNA strand breaks [13], sister chromatid exchanges [14–16] or micronuclei (MN) in peripheral lymphocytes [17] and in buccal epithelial cells [18] were applied. The objectives of the present study were; (1) to investigate whether oncology nurses are occupationally exposed to antineoplastic drugs especially cyclophosphamide by measuring the urinary concentrations of CP; (2) to investigate the genotoxic effects associated with occupational exposure to antineoplastics by analyzing MN frequencies in peripheral lymphocytes and in exfoliated buccal epithelial cells; and (3) to study a possible relationship between 1 and 2.

2. Materials and methods

2.1. Subjects

The exposed group ($n = 26$) consisted of female nurses handling antineoplastic drugs. We considered as exposed persons, individuals involved in preparation and administration of antineoplastics for at least 1 year, 4 or 5 times a day, with minimal protection working in several hospitals of Ankara. Most of the nurses do all activities other than administration of antineoplastic agents in the same area. No safety-hoods are used. The tables on which preparations have occurred are cleaned by liquid detergents regularly. A more general cleaning of the work environment is carried out every 1–2 months. In these departments in total about 30 g of CP is prepared and administered daily. The control group ($n = 14$) comprising mostly of nurses, and secretaries (all female) was not occupationally exposed to known genotoxic agents. Informed consent was obtained after the objective of the study was fully explained. Each person was interviewed and a questionnaire was filled in. The questions covered a detailed occupational, medical, family and dietary history including variables known to influence cytogenetic endpoints. Table 1 presents the distribution of the main characteristics of both groups, exposed and control. Fifty percent of the nurses handling antineoplastics wore both gloves and masks, however, they claimed to use the same gloves and masks during the whole day. About 23% of the workers did not wear any glove or mask. Recent illnesses prior to the study and use of oral contraceptives were not significantly different in either group. Exposed nurses had subjected to slightly more radiodiagnostic examinations, but not statistically significant. Six nurses in the

Table 1
General characteristics of nurses and controls

Parameter	Nurses	Controls
n	26	14
Age (mean ± SD, years)	29.84 ± 6.23	28.14 ± 6.62
Duration of exposure (mean ± SD, years)	5 ± 4.45	–
(Range)	(1–19.5)	–
	% (*n*)	% (*n*)
Smoking habits		
Non-smokers	42 (11)	78 (11)
Smokers	58 (15)	22 (3)
1–10 cigarettes/day	33 (5)	67 (2)
11–20 cigarettes/day	67 (10)	33 (1)

exposed group and one control subject had been vaccinated against Hepatitis B at least 1 month before the study. In addition to CP, IP, Cis, 5-FU, etoposide (EP), doxorubicin (DO), MT and vincristine (VC) were the most frequently prepared and administered cytostatic drugs.

2.2. CP in urine

Total 24-h urine was collected in portions starting from the end of a spell of duty of at least 4 days. No urine samples were taken from the controls. The amounts of urine excreted and the excretion periods were registered. The urine samples were stored at −20°C and sent to the laboratory in Nijmegen, The Netherlands for the determination of CP. Materials and reagents and the sample preparation procedure are described elsewhere [5]. Since the analysis procedure of CP is further optimized, a description is given below.

2.2.1. GC-MSMS analysis of CP

Analysis were performed on a Varian Saturn 4D GCMS controlled by a Compag Prolinea 4/50 personal computer. The on-column injection mode was used. A total of 1 ml aliquots was injected on a 30-m DB-5ms column (J&W Scientific, Folsom, CA, USA) with 0.25 mm internal diameter and 0.25 mm film thickness by means of a 8200 CX autosampler (Varian). The column was connected to a deactivated fused silica retention gap (Varian, Houten, The Netherlands). The initial injection temperature was 110°C. After 1 min, the temperature was increased by 180°C/min to 280°C. After 3 min, the temperature was decreased to the initial temperature by cooling down with compressed air. The initial oven temperature was 110°C. After 1 min, the temperature was increased by 10.0°C/min to 290°C where it remained constant for 7 min. Helium was used as carrier gas (column inlet pressure 14.0 psi). The interface temperature was 290°C. The manifold (iontrap) temperature was 230°C. The iontrap was operated in the MSMS-mode. Ion fragment $m/e =$ 307, the most abundant ion after initial EI-ionisation, was trapped as the parent-ion. In the second stage an excitation amplitude of 46 V (nonresonant) and an excitation-RF of 79.4 amu were applied to produce daughter-ions. Identification was carried out by the combination of retention time and MSMS-spectrum. Daughter-ion $m/e = 212$ was used for quantification. The ratio of the peak areas of derivatized CP and IF (internal standard) was calculated and compared to freshly prepared calibration curves of CP in reference urine in a range of 0.5–20 mg/l. The limit of detection was 0.1 ng/l.

2.3. MN in peripheral lymphocytes

Blood samples were taken from each study subject. For nurses, the work sample was collected in the morning of a regular working day by venipuncture into heparinized tubes. The cells were cultured for 72 h in RPMI 1640 medium (Sigma) containing 25% fetal calf serum (Seromed), 100 U/ml penicillin, 100 μg/ml streptomycin and 1.5% (phytohemagglutinin; Gibco) cytochalasin B (Cyt B, Sigma) was added at the 48th h of cultures at a final concentration of 5 μg/ml to obtain binucleated cells [19]. Samples of the cultures were harvested 24 h after the addition of Cyt B. Trisodium citrate (1.25%) was used for mild hypotonic effect. Slides were stained with Giemsa. MN were counted in 1000 binucleated cells with well-preserved cytoplasm using magnification of 1000×. All the slides were scored blindly by the same person.

2.4. MN in exfoliated epithelial cells

Exfoliated epithelial cells from the buccal mucosa were collected by scraping the right and left cheeks with a damp wooden spatula after washing out the mouth with water. The cells were then smeared on clean slides, dried in the air and fixed in alcohol: acetic acid (3:1 v/v). Slides were stained by the Feulgen reaction and then counterstained with Fast green. Criteria of scoring were described by Sarto et al. [20] and Tolbert et al. [21]. One thousand cells were analyzed for each individual. All the slides were scored blindly by the same person.

2.5. Statistical analysis

Differences between study groups for categorical variables were evaluated by the chi-square test and

Fisher's exact test. Other comparisons between groups were evaluated by Student's *t*-test and Mann–Whitney *U*-test. Pearson and Spearman rank correlations were used as appropriate. All *p*-values are two-tailed.

3. Results

As indicated in Table 1, the two groups were carefully matched for age and sex. Among the nurses, smoking was more representative compared to the controls. Table 2 shows the CP excretion rates and other relevant data of the nurses involved in the preparation and administration of antineoplastic agents and involved in the cleaning of waste due to the handling of these drugs. The excretion rates ranged from 0 to 9.14 μg CP/24 h. No relation was observed between the excretion rate and the kind of activity, neither a correlation was observed with the frequency of handling cytostatics or the years of working with these agents. The higher CP excretion rates were observed among nurses handling CP. However, nurses handling other antineoplastic agents

Table 2
CP excretion rate in oncology nurses in relation to several parameters of exposure

No.	Handling antineoplastics (preparation/ administration/ waste clearing after administration)	Smoking habits (cigarettes/day)	Handling time of cytostatics (years)	Frequency of handling antineoplastics	Use of glove/ mask	Antineoplastics mostly frequently handled	Urinary CP excretion rate (μg/24 h)
1	+/+/−	1–10	5	5 times/day	+/+	IP, AD, EP	0.16
2	+/+/+	1–10	2	3 times/day	+/−	IP, EP, VC	ND
3	+/+/−	11–20	1.5	5 times/day	−/+	CP, ET, AD	9.14
4	+/+/+	1–10	2	1.5 times/day	+/+	AD, HX, Cis	ND
5	+/+/−	11–20	6	5 times/day	−/−	CP, 5-FU, Cis	3.51
6	+/+/−	NS	4	once a day	+/+	CP, IP, Cis	0.11
7	+/+/+	11–20	10	5 times/day	−/−	AD, Cis, CP	2.00
8	+/+/+	11–20	2	1.5 times/day	+/+	AD, MT, IP	ND
9	+/+/−	NS	3	4–5 times/day	+/+	Cis, 5-FU, AD	ND
10	+/+/−	NS	2.5	3–4 times/day	+/+	Cis, AD, IP	ND
11	+/+/+	NS	3	5 times/day	+/−	AD, 5-FU, CP	3.93
12	+/+/+	1–10	11	5 times/day	+/−	AD, Cis, MT, 5-FU, CP	0.68
13	+/+/+	11–20	3	4–5 times/day	−/−	IP, EP	0.35
14	+/+/+	NS	4	2–3 times/day	−/−	IP, EP, VC	0.06
15	+/+/+	NS	5	4–5 times/day	+/−	AD, CP, Cis	0.06
16	+/+/−	NS	3	3 times/day	+/+	CP, VC, AD	0.09
17	+/+/+	NS	5	5 times/day	+/+	CP, AD, IP	0.03
18	+/+/−	11–20	6	5 times/day	−/−	CP, 5-FU, Cis	1.27
19		1–10	2–3	2–3 times/day			–*
20	+/+/+	11–20	2	9 times/day	+/+	Cis, EP, CP, IP, AD	2.31
21	+/+/+	NS	3	9 times/day	+/+	CP, HX, AD, Cis, EP	1.07
22	+/+/−	11–20	6	4–5 times/day	+/+	Cis, 5-FU, IP	0.02
23	+/+/+	NS	12	15 times/day	+/+	Cis, VP, IP	0.97
24	+/+/+	11–20	19.5	20 times/day	+/−	5-FU, CP, IP, EP, Cis	4.30
25	−/+/+	11–20	15	20 times/day	−/−	5-FU, CP, IP, EP, Cis	0.08
26	+/+/+	NS	2	5–6 times/day	+/+	Cis, 5-FU, IP	0.31

*Not analyzed.
ND: not detected.
Abbreviations: 5-FU, 5-fluorouracil; CP, cyclophosphamide; MX, methotrexate; AD, adriamycin; EP, etoposide; VC, vincristine; Cis, *cis*-platinum; IP, iphosphamide.

than CP also showed a urinary excretion of CP. Nurses with a nondetectable CP excretion rate did not handle CP. No relationship was observed between the CP excretion rates and the use of gloves/masks.

The mean frequencies of micronucleated lymphocytes for the exposed and control groups are shown in Table 3. The overall mean frequency of micronucleated cells in the exposed group was significantly increased compared to control ($p < 0.01$; Table 3). There was also a significant difference between nonsmoking controls and nonsmoking exposed nurses ($p < 0.01$). The mean frequency of MN for smoking subjects in both groups were higher than in the nonsmoking subjects, however, the difference was not significant ($p > 0.05$). The mean frequencies of MN in buccal cells observed in the exposed and control subjects are shown in Table 4. We found a (statistically nonsignificant) trend in the increased number of MN in buccal cells of oncology nurses as compared with controls ($p > 0.05$; Table 4). Smokers and nonsmokers do not differ significantly with respect to the incidence of MN in both groups ($p > 0.05$). With respect to the analysis of the data we have looked for possible correlations. The following was concluded: (a) no correlations were observed between the amounts of CP excreted in urine and the MN frequencies of buccal cells and the MN frequencies of lymphocytes in the nurses. (b) There is no correlation between the MN frequencies of buccal cells and MN frequencies of lymphocytes for the exposed and control group. (c) Neither could we find any relationship with cytogenetic end-points and handling time of antineoplastics, handling frequency of drugs and use of protective equipment.

Table 3
MN frequencies in peripheral blood cells of nurses and controls

Groups	N	MN frequency in peripheral blood cells (%) (X ± SD)	Min–max
Controls	13*	0.28 ± 0.16^{a}	0.00–0.50
Smokers	3	$0.33 \pm 0.21^{b,d}$	0.10–0.50
Nonsmokers	10	$0.26 \pm 0.16^{c,d}$	0.00–0.40
Nurses	23*	0.61 ± 0.32^{a}	0.10–1.20
Smokers	13	$0.61 \pm 0.32^{b,e}$	0.20–1.20
Nonsmokers	10	$0.61 \pm 0.33^{c,e}$	0.10–1.10

[a] $p < 0.01$; [b] $p > 0.05$; [c] $p < 0.01$; [d] $p > 0.05$; [e] $p > 0.05$.
*MN data for only one subject in controls and three subjects in nurses are not available.

Table 4
MN frequencies in buccal cells of nurses and controls

Groups	N	MN frequency in buccal cells (%) (X ± SD)	Min–max
Controls	14	0.08 ± 0.08^{a}	0.00–0.30
Smokers	3	$0.07 \pm 0.05^{b,d}$	0.00–0.10
Nonsmokers	11	$0.08 \pm 0.09^{c,d}$	0.00–0.30
Nurses	25*	0.16 ± 0.19^{a}	0.00–0.80
Smokers	14	$0.18 \pm 0.21^{b,d}$	0.00–0.80
Nonsmokers	11	$0.13 \pm 0.16^{c,e}$	0.00–0.40

[a] $p > 0.05$; [b] $p > 0.05$; [c] $p > 0.05$; [d] $p > 0.05$; [e] $p > 0.05$.
*MN data for only one subject is not available.

4. Discussion

In the present study, nurses handling antineoplastic drugs and a control group were monitored for MN in peripheral lymphocytes and in buccal epithelial cells. Exposure of oncology nurses to at least one antineoplastic agent was assessed from levels of urinary CP. In urine samples of 20 out of 25 exposed nurses a CP excretion rate was found in a range of 0.02–9.14 μg CP/24 h. Hirst et al. [22] found that CP excretion rates in urine samples of two nurses were in a range of 0.35–9.08 μg/24 h. Two exposed nurses in this study did not wear any protective clothing and handled the CP vials in their routine manner. In urine samples of three out of 11 Dutch exposed workers, mainly hospital nurses and Dutch pharmacy technicians, CP excretion rate was found in a range of 0.1–0.5 μg/24 h. Higher levels were detected in the urine of eight out of 11 Czech exposed workers, a range of 0.1–2.9 μg/24 h [23]. Most of the workers handling antineoplastic drugs wore gloves, masks and special clothes and the drugs were prepared in laminar vertical-flow hoods in that study. Our results of the analyses of CP in urine demonstrate that when the nurses were handling CP (and other antineoplastic drugs) this particular compound was observed in urine. No confounding due to smoking was observed, because in urine of both smokers and nonsmokers CP was detected. As de-

scribed no safetyhoods were used. Also the cleaning procedures differ from the procedures applied in the studies described by Sessink et al. [7,24]. Anyway, the higher CP excretion rates were observed among nurses handling CP, suggesting a direct relation between handling of CP and occupational exposure as measured by the CP excretion rate. On the other hand, nurses handling other antineoplastic agents than CP showed also a urinary excretion of CP. This suggests a possible role for other factors than the handling of CP itself. The absence of a relationship between the CP excretion rates and the use of gloves or masks is remarkable. A more extended occupational hygiene study will be needed to get more insight in the causes of exposure.

Literature studies dealing with cytogenetic changes in peripheral lymphocytes of persons handling antineoplastics are contradictory. In some studies no increase in cytogenetic effects was observed [25–31]. On the other hand, others have found an association between handling antineoplastic drugs and occupational exposure of workers [4,12,17, 23,32–34]. None of these studies except two [23,25] gave exposure data on specific antineoplastic agents. It is known that several factors affect the frequency of MN, including sex, smoking habits, age and also technical differences (time of sampling, composition of culture medium, culture time with and without cytochalasin [35]). Age, sex and smoking habits have not influenced the MN frequencies in the present study. Our result demonstrates that occupational exposure to antineoplastics leads to a significant induction of cytogenetic damage in peripheral lymphocytes after controlling possible confounding factors. Several antineoplastic drugs induce significantly increased MN from chromosomal breakage/rearrangement (adriamycin, CP), breakage (MT), or loss of whole chromosomes due to spindle impairment (VC) [36]. Nonsignificant MN increases in human peripheral lymphocytes of workers exposed to CP were described in the study of Sorsa et al. [30]. Thiringer et al. [33] found no elevation in MN among nurses working with antineoplastic drugs. A significant increase in MN in peripheral lymphocytes of oncology nurses was found in studies of Yager et al. [37], Anwar et al. [17], and Kevekordes et al. [34]. Comparison of results obtained from different studies may not easily be done, because, there are differences in potential exposure (e.g., handling practices, extent and quantities handled) or methodological limitations.In the field of cytogenetics, Stich and Rosin [41] were the first to propose adaptation of the MN test to exfoliated cells as a measure of chromosome damage in epithelial tissues. Whereas lymphocytes must be stimulated; epithelial cells do not need to be stimulated; MN in exfoliated cells reflect genotoxic events that occurred in the dividing basal layer 1–3 weeks earlier [38]. Elevation in the number of MN in exfoliated cells indicates an increased risk for cancer of the oesophagus, urinary bladder, cervix [39,40] and oral cavity [41].

Regarding the MN levels in buccal epithelial cells, we were unable to demonstrate any significant difference between exposed and control subjects. It should be emphasized that although the mean frequency among nurses (0.16%) appears to be double that in control subjects (0.08%), it was not statistically different.

In our study, we could not find any significant difference between smokers and nonsmokers in both groups. Stich and Rosin [38] have found the frequency of micronucleated buccal cells elevated only in those subjects smoking at least one package of cigarettes per day and also consuming at least 150 ml of ethanol daily. Fontham et al. [42] have demonstrated significantly elevated frequencies for smokers compared to nonsmokers regardless of the amount smoked. Machado-Santelli et al. [18] do not find any significant effect of smoking with respect to the incidence of buccal MN in oncology nurses. Our results are not comparable with previous findings due to the differences on smoking intensity of subjects, number of cells analyzed, sensitivity of the test, scoring criteria, etc. Apart from above considerations, there is no reasonable explanation for the lack of influence of smoking on the frequency of micronucleated cells. Smoking habits and age are not the confounding factors in our study. This observation is consistent with earlier results of several authors [18,40].

Machado-Santelli et al. [18], however, found a significant increase in MN in buccal cells of nurses handling antineoplastics. Sarto et al. [43] also observed significant increases in MN in buccal cells of seven patients undergoing antiblastic chemotherapy for the first time. An explanation for our findings

may be that efficiency of MN in buccal cells to detect possible chromosomal damage due to exposure to antineoplastics is lower than that of MN in lymphocytes, in line with Sarto et al. [43].

In addition, contrary to the proposal of Machado-Santelli et al. [18], buccal mucosa cells might not be the direct target of exposure through inhalation of the antineoplastic drugs due to the inadequate protection. However, considering the limitations of the MN assay in buccal cells, occupational exposure to antineoplastic drugs cannot be totally excluded. In the present study, no correlation was found between the urinary CP excretion and the cytogenetic findings in nurses. In agreement with Sessink et al. [23], while urinary CP excretion reflects current exposure, MN frequencies are able to detect recent exposures [34]. We did not find a correlation between the MN frequencies of peripheral lymphocytes and MN frequencies of buccal cells, probably due to differences in kinetics of MN occurrence or differences in exposure of the target cells.

Our results, as well as other studies [2–4,22], have identified the possible genotoxic damage of oncology nurses related to occupational exposure to at least one antineoplastic agent, which is used as a marker for drug handling. As a whole, there is concern that the present handling practices of antineoplastic drugs used in the several hospitals in the Ankara will not be sufficient to prevent exposure.

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