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Chromosomal Aberrations, Sister Chromatid Exchanges, and Urinary Thioethers in Nurses Handling Antineoplastic Drugs

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In this study we examined the structural chromosomal aberrations (CA) and sister chromatid exchanges (SCE) of 12 nurses handling moderate quantities of antineoplastic drugs. Urinary thioethers were also measured to assess the potential exposure to alkylating drugs. Two control groups with similar mean age and smoking habits and working in the same hospital were also studied: 1) control clerks and 2) control nurses. Our study did not show any clear increase of chromosomal damage in exposed nurses as compared to controls. However, CA in control nurses were significantly increased ($p = 0.05$) with respect to control clerks. The results of baseline urinary excretion of thioethers were statistically higher ($p < 0.02$) in exposed subjects than in control clerks.

Key words: SCE, mercapturic acid, biological markers, occupational exposures

INTRODUCTION

It is well known that many widely used antineoplastic drugs can be mutagenic, teratogenic, and carcinogenic in experimental systems, and second tumors were observed in patients undergoing some antineoplastic therapies [IARC, 1982]. The original observation of Falk et al. [1979] that nurses handling antineoplastic drugs had increased excretion of metabolites in an active mutagenic or premutagenic form proved indirectly that these nurses could adsorb microdoses of drugs. The next surveys, reviewed by Sorsa et al. [1985], used both dose test (urinary mutagenicity) and/or effect test (chromosomal aberrations, sister chromatid exchanges), but conflicting results were reported. The review concluded that handling antiblastic drugs involves a potential risk but the magnitude of this risk depends on the extent of exposure; therefore, studies employing various indicators for assessing exposure and biological effects are recommended.

We studied a group of 12 nurses handling moderate quantities of antineoplastic drugs who had worked in the past years without flow hoods and only in the last few

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months had adopted complete protective measures. The goal of the study was to understand if a real genotoxic hazard were present. The structural chromosomal aberration (CA) and sister chromatid exchange (SCE) tests in lymphocytes were performed to evaluate accumulated and unrepaired chromosomal damage. In order to assess the potential current exposure to alkylating drugs, assay of urinary thioethers was also performed. Some studies had shown indeed an increased excretion of these metabolites in exposed nurses [Jagun et al., 1982; Bayhan et al., 1987].

MATERIALS AND METHODS

Working Environment and Examined Subjects

The preparation of antineoplastic drugs in the oncology department in which this study was carried out was done in a small room with frequent traffic by 12 nurses (exposed group) who used hand gloves and surgical masks. Only 4 months before blood sampling for this study, a horizontal laminar flow hood (recycling environmental air) was introduced. The average length of working in the oncology department was 8.5 ± 6.6 y (range = 2–22 y); 4 nurses worked in the outpatient department and 8 in the wards, without substantial differences in handled quantities among individuals. The quantity of antiblastic drugs used in the department during the 12 months before blood sampling was calculated; the monthly averages were: cyclophosphamide 65.4 g, 5-fluorouracyl 62.0 g, dacarbazyn 7.5 g, methotrexate 3.5 g, etoposide 2.9 g, epi-doxorubicin 2.5 g, cis-platinum 2.0 g, doxorubicin 1.5 g, teniposide 0.8 g, peptichemio 0.4 g, mitomycin C 0.2 g, bleomycin 0.2 g, actinomycin D 0.2 g, and vinblastine 0.1 g; in total, about 8,000 vials of antiblastic were prepared per y.

Two control groups of 12 subjects each, with similar mean age, sex ratio, and smoking habits, and working in the same hospital, were also selected in the study: 1) hospital clerks (control clerks), who had never been exposed to known genotoxic agents; and 2) nurses working in surgical and medical wards who had never handled antineoplastic drugs (control nurses). Blood samplings of exposed and controls were drawn in the same period, coded by the physicians of the oncology department and sent to our laboratory where they were coded again (double blind reading).

Cytogenetic Methods

Chromosomal aberrations (CA). For each blood sample, two replicate cultures were set up by adding 300 μl of whole blood to 5 ml complete culture medium containing 25% of fetal bovine serum and PHA M (Chromosome medium 1A, Gibco, New York, USA). The lymphocytes were cultured for 48 h and the metaphases blocked during the last 3 h with colchicine (Sigma, St. Louis, USA) for a final concentration of 0.1 μg/ml. At the end of the culture, the 2 tubes were processed together. The metaphasic chromosomes were prepared as described elsewhere [Sarto et al., 1982]; CA were classified as cells with breaks of chromatid or chromosome type and cells with exchanges (only one chromatid type exchange was found); gaps were also analyzed but not reported.

Sister chromatid exchanges (SCE). 5-bromodeoxyuridine (Sigma, St. Louis, USA) was added at the beginning of cultures of 72 h at a final concentration of 30 μg/ml. SCE were counted in 30–50 metaphases per subject; in total 554 cells were analyzed in the exposed group, 580 in control nurses, and 402 in control clerks.

TABLE I. Distribution by Age, Sex, and Smoking Status of Subjects and Controls in Study of Nurses Handling Antineoplastic Drugs

	No. of subjects	Age (y), M ± sd	Sex ratio, M/F	No. of smokers
Exposed nurses	12	36 ± 10	3/9	3
Control clerks	12	34 ± 9	3/9	3
Control nurses	12	33 ± 9	4/8	3

Differential staining of sister chromatid, obtained by a fluorochrome plus Giemsa technique, and criteria of scoring have been detailed elsewhere [Sarto et al., 1987].

Mercapturic Acid Assessment

Urinary thioether (mercapturic acid) excretion was determined in the 12 exposed nurses. The control group consisted of 8 of the 12 control clerks. Thioethers were measured according to Vainio et al. [1978] with minor modification [Rizzi et al., 1987]; briefly, thioethers were determined with the usual colorimetric method, varying the pH medium to obtain a higher sensitivity. To avoid misleading results due to diet and other effects [Aringen and Lidums, 1988], a strict control of these was maintained during the study. The urine samples were collected immediately before the exposure at 7 A.M. (first sample) of the first day of the working week (Monday), at 8 P.M. of the same day (second sample), and at 7 A.M. of the next day (third sample), before a new workshift. This protocol was applied because, from previous clinical [Trevisan et al., 1985] and experimental data [Trevisan et al., 1988], the peak of excretion of thioethers appeared between the eighth and twelfth h after the beginning of exposure. Data were expressed in mmoles of −SH groups for mmoles of creatinine and they were compared with the first control group.

Statistical Methods

The various parameters measured in exposed and control groups were studied by using the nonparametric Mann-Whitney U test; the probability of occurrence was two-tailed.

RESULTS

Some findings of the examined groups are shown in Table I.

In Figure 1, the SCE frequencies in each subject and the averages in the 3 examined groups are reported. The mean frequency of SCE in the exposed group (10.24) is slightly increased with respect to control nurses (8.91) and control clerks (9.48), but the differences are not statistically significant. Within each group there are no statistically significant differences between the 3 smokers and the 9 nonsmokers.

Table II reports the averages of chromatid breaks, chromosome breaks, and exchanges in the 3 groups. Control nurses present an increase of chromatid and chromosome-type aberrations with respect to exposed and control clerks. The difference between chromosome-type aberrations of control nurses and control clerks is statistically significant ($U = 37$; $p = 0.05$); no other significant difference is found between exposed and control groups and between the 2 control groups for the various types of CA.

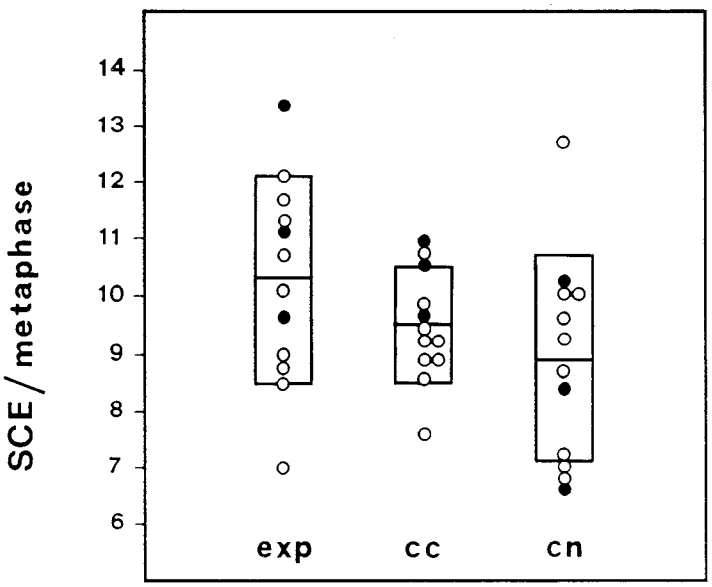

Fig. 1. SCE frequencies in each exposed and control subject and averages of the three examined groups. Solid circles = smokers, open circles = nonsmokers. exp = exposed, cc = control clerks, cn = control nurses.

TABLE II. Mean Frequencies and Ranges (in Parentheses) of Chromatid Breaks, Chromosome Breaks, and Exchanges in Study of Nurses Exposed to Antineoplastic Drugs and Control Groups

	No. of metaphases analyzed	% of metaphases with aberrations, M ± sd (range)		
		Chromatid breaks	Chromosome breaks	Exchanges
Exposed nurses	1,194	0.50 ± 0.67 (0–2.0)	0.92 ± 1.16 (0–4.0)	0.08 ± 0.29 (0–1.0)
Control clerks	1,140	0.67 ± 1.15 (0–4.0)	0.25 ± 0.45 (0–1.0)	0.17 ± 0.39 (0–1.0)
Control nurses	1,193	1.08 ± 1.24 (0–3.0)	1.00 ± 0.95 (0–3.0)	0.08 ± 0.29 (0–1.0)

Figure 2 summarizes the data on mercapturic acid excretion in each exposed and control subject. The averages of thioethers in control clerks were 0.11 ± 0.04, 0.10 ± 0.01, and 0.10 ± 0.03 in the first, second, and third samples respectively; in the exposed nurses, these were 0.16 ± 0.05, 0.20 ± 0.07, and 0.15 ± 0.04 in the first, second, and third samples, respectively. The baseline (first sample) excretion of thioethers is statistically increased in exposed subjects with respect to controls ($U = 13$; $p<0.02$). While in control clerks the mean excretion of thioethers is similar in the third sample, in exposed subjects, the second sample is 25% and 33% increased as compared to the first and second samples respectively, but the differences are not statistically significant, the data being very skewed. There is no significant difference between smokers and nonsmokers.

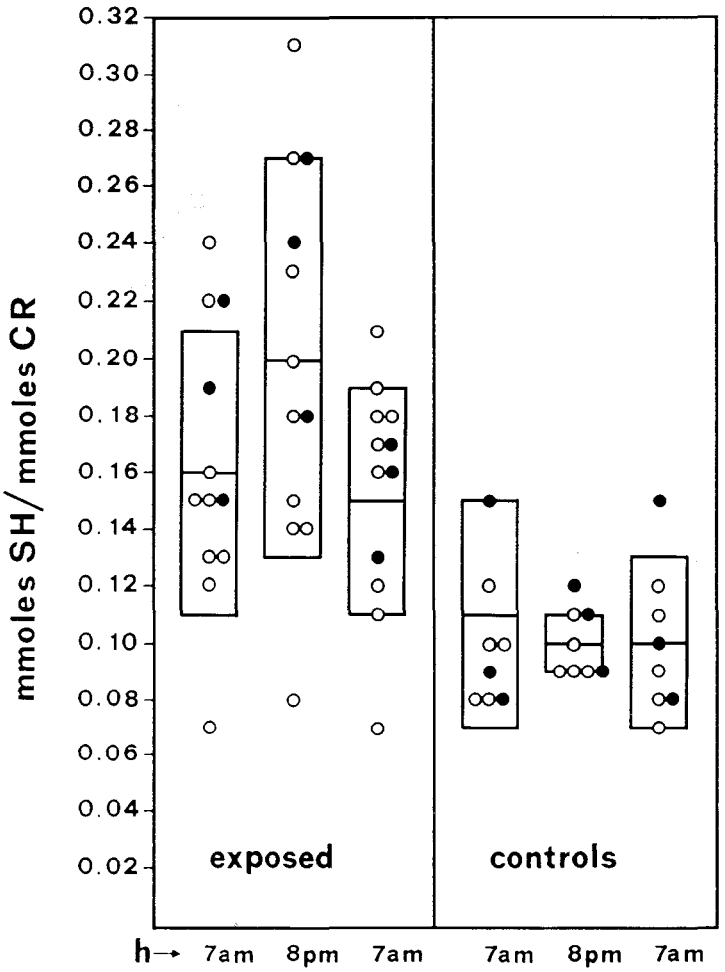

Fig. 2. Urinary thioether (mercapturic acid) values in each exposed and control subject and averages (± sd) of the 2 examined groups. The samples were collected on Monday (at 7 A.M. and 8 P.M.) and on Tuesday (at 7 A.M.). The mean value of thioethers of the first sample is statistically increased in exposed with respect to controls ($U = 13$; $p < 0.02$). Solid circles = smokers, open circles = nonsmokers.

DISCUSSION

A few studies have been published on chromosome damage among nurses handling antineoplastic drugs. Norppa et al. [1980] found that 20 nurses exposed to antineoplastics presented a significant increase of SCE in lymphocytes only as compared to a group of office workers but not as compared to other hospital nurses. No information on the quantity of antineoplastics handled was available in this study. Waksvik et al. [1981] studied SCE and CA frequencies in a first group of 10 nurses with an average exposure to antineoplastics of 2,150 h and in a second group of 10 nurses with an exposure of 1,078 h. Only the first group of nurses showed significantly higher frequency of SCE than an office personnel control group; nurses differed from control group only when gaps were compared. Nikula et al. [1984] reported a sig-

nificant increase of chromosome-type aberrations in 11 oncology nurses, whose average time of exposure had been 2,800 h, compared to hospital clerks and laboratory technicians. Barale et al. [1985] observed no increase of SCE in a group of 21 exposed nurses as compared with other nurses not exposed to antineoplastics; the mean quantity of drugs handled in the last 2 weeks was 171 g (range = 2–1,054 g).

The present study shows no clear chromosomal damage in nurses handling antineoplastics who had been using complete hygienic measurements (gloves, masks, flow hood) during the last months. The mean quantity of drugs handled by exposed nurses reported in the present study was modest (11.5 g in the last 2 weeks) if compared with that used by the group studied by Barale et al. [1985]. Some authors (Norppa et al., 1980; Waksvik et al., 1981] reported slight increases of SCE in a control group of hospital nurses with respect to hospital clerks. We find that chromosomal aberrations in control nurses are significantly increased as compared to control clerks. It is possible that sanitary hospital staff is not completely devoid of exposure to genotoxic agents such as ionizing radiation, anaesthetic gases, and sterilizing agents.

The baseline urinary excretion of thioethers was statistically increased in exposed as compared to control clerks. This finding could suggest that a low but chronic exposure to alkylating agents could stimulate the metabolic pathway of conjugation with GSH and hence an increase of mercapturic acid excretion. Jagun et al. [1982] found a 65% increase of thioethers in pre-exposure samples of exposed subjects with respect to controls. The same group found an important increase (more than 4-fold) of thioethers following a work shift with antineoplastics, which was not observed in our study. The slight increase of thioethers after exposure in the nurses of the present study confirms that the group was subject to such a low exposure that a genotoxic effect could not be revealed. Findings in reported studies and our investigation suggest that handling antineoplastic drugs may or may not be hazardous depending on the hygienic measurements adopted and the quantities of drugs handled. In general, if personnel wear gloves and masks and work in adequately ventilated flow hoods, the risk of contact with drugs is minimal.

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