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Biomonitoring of nurses handling antineoplastic drugs

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Abstract

The micronuclei analysis in exfoliated cells of the buccal cavity was employed in the cytogenetic monitoring of nurses handling antineoplastic drugs. The group under study consisted of 25 subjects who showed a marked increase in micronucleated cells as compared with the control group (Chi-square = 15.12, with one degree of freedom, $P < 0.001$).

Key words: Antineoplastic drug

1. Introduction

Cytogenetics methods have been extensively used for the biological monitoring of populations exposed to mutagenic and carcinogenic agents. The micronucleus assay can detect both clastogenic agents and spindle poisons (Evans, 1988) Micronuclei are acentric fragments or complete chromosomes which, during a mitotic cycle, have failed in attaching to the mitotic spindle and are excluded from the nucleus (Heddle et al., 1983). In this approach, the micronucleus assay in exfoliated cells (Stich et al., 1982) provides a useful tool in order to evaluate the genetic risk of occupationally exposed individuals. This assay provides a good estimation of the frequency in which chromosomal aberrations occur and it is faster and simpler than the study of cells in culture. Furthermore, the evaluation of the genetic damage may be done on the target or directly exposed cell.

Among the individuals occupationally exposed to mutagenic and carcinogenic agents, the group handling antineoplastic drugs deserves special attention. Previous studies by Falk et al. (1979) show an association between contact with cytostatic drugs and increased urinary mutagenicity, by biological monitoring of hospital personnel in oncology units. By using various kinds of tests,

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several groups have later reported both positive and negative findings of mutagenicity among nurses and pharmacists handling antineoplastic drugs. Significant increases in structural chromosome aberrations and in gap frequencies in lymphocytes of nurses handling anti-neoplastic drugs were reported by, respectively, Nikula et al. (1984) and Waksvik et al. (1981). An increased frequency of sister chromatid exchanges was also observed in exposed nurses related to control by Norppa et al. (1980), Waksvik et al. (1981) and Goloni-Bertollo et al. (1992), whereas other groups did not confirm this result (Kolmodin-Hedman et al., 1983; Stiller et al., 1983; Barale et al., 1985; Benhamou et al., 1988). Straight comparison of the data from different studies is difficult, since the protective equipment used by nurses varies from one hospital to another.

In this study, a group of 25 nurses handling antineoplastic drugs was biomonitored through micronucleus assay.

2. Material and methods

2.1. Individuals examined

Fifty normal individuals were divided in two sub-groups: control and exposed. The control subgroup, comprising nurses, physicians, secretaries and drivers, was not occupationally exposed to known genotoxic agents. The exposed subgroup consisted of nurses handling antineoplastic drugs (vincristine, vinblastine, aracytosine C, 5-fluorouracyl, cyclofosfamide, cisplatin, methotrexate, bleomycin, mitomycin and adriablastin). We considered as exposed only individuals who regularly make up dilutions of the drugs, independent of any other kind of manipulation such as load or perform injections.

Each individual filled out a questionnaire concerning to smoking habits, diseases, use of drugs, coffee and alcohol consumption, and exposure to other genotoxic agents (chemicals or radiation). The questionnaire of the exposed subgroup also included questions on the use of protective equipment (gloves, masks, safety hoods, gowns, eyeglasses)

2.2. Cytological preparations

The exfoliated cells from oral cavity were obtained by scraping gently the right and left cheeks with a cleaned slide glass according to Stich et al. (1982), with modifications suggested by J.A. Soares-Vieira and G.J.F. Gattas (personal communication). The slides were submersed in 8 ml of saline solution (NaCl, 0.9%) and carried immediately to the laboratory. Cells were spun down from the suspension (10 min, 1200 rpm) and the pellet was fixed in methanol/acetic acid (3:1), twice every 5 min. The final cell suspension was dropped on clean, moist and cool glass slides. After 24 h they were stained using Feulgen and Rossenbeck (1924) plus fast green method. Criteria of scoring were described by Sarto et al. (1987) and Tolbert et al. (1992). The slides were coded prior to score to avoid bias and blind read. One trained person scored all the slides and some of them were reanalyzed by a second person. 1000 cells were analyzed for each individual.

2.3. Statistical analysis

We have low frequency data which suggest the Binomial to Poison approximation. In order to compare the two sub-groups, using this approximation we built conditional test as prescribed by Charkravarti et al. (1967). Since the conditional distribution is binomial, the use of the Chi-square test for adherence is adequate, whenever the total frequency of aberrations is not very high. On the other case, we would use the exact binomial probabilities to obtain the significance level.

3. Results

Well-spread cell preparations are obtained from exfoliated cells of buccal mucosa when the Soares-Vieira Gattas modification of the technique of Stich et al. (1982) is used. Micronucleated cells are easily identified in these preparations, and the observation of the criteria established by Sarto et al. (1987) avoids mistaking them with other nuclear abnormalities (Fig. 1).

The comparison of the mean age of the control group (34.60 ± 1.54 years) to that of the exposed group (32.08 ± 1.29 years), using the Student's *t*-test, did not show any significant difference. Other factors such as alcohol, coffee and medicine consumption were not considered important, as no significant association was observed among them.

Smoking habits were evaluated by defining the total exposure obtained from the total number of cigarettes per day multiplied by number of years. The control group was slightly more exposed, even though the number of smokers was the same in both groups. Within the exposed (e) group and control (c) group we compared the frequency of micronuclei in smokers versus non-smokers ($\chi_e^2 = 0.0682$, $P > 0.8$; $\chi_c^2 = 1.39$, $P > 0.2$, both with one degree of freedom). Smokers and non-smokers do not differ significantly with respect to the incidence of micronuclei. Then we compared the exposed and control groups without reference to the habit of smoking.

The mean frequency of micronuclei observed in the subgroup exposed was 0.344%, significantly different (Chi-square 15.12, with one degree of freedom, $P < 0.001$) from that of the control, 0.168% (1000 cells/individual were analyzed). Tables 1 and 2 show the results obtained in the control and exposed groups, respectively.

Table 1
Characteristics and frequencies of micronucleated cells in control group

Subject No.	Age (years)	Sex (m/f)	Cigarettes smoking		Micronucleated cells (%)
			No./day	Duration (years)	
1	24	m	0	0	0.0
2	45	f	0	0	0.2
3	34	f	0	0	0.0
4	45	f	0	0	0.0
5	43	m	0	0	0.8
6	34	f	0	0	0.0
7	37	f	20	19	0.3
8	28	f	0	0	0.4
9	34	f	5	20	0.1
10	54	m	20	40	0.1
11	33	f	0	0	0.1
12	35	f	0	0	0.0
13	28	f	0	0	0.4
14	34	f	30	12	0.2
15	48	f	0	0	0.1
16	31	f	5	10	0.0
17	25	f	0	0	0.2
18	32	m	0	0	0.3
19	27	f	15	10	0.2
20	30	f	0	0	0.0
21	41	f	0	0	0.3
22	30	f	0	0	0.0
23	32	f	0	0	0.1
24	34	f	30	12	0.2
25	27	m	0	0	0.2
mean	34.6				0.168
Std. error	±1.51				±0.036

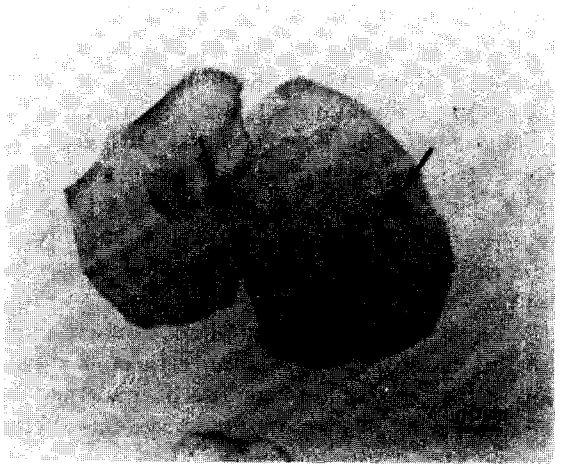

Fig. 1. Buccal mucosa cells stained by Feulgen and fast green, showing a micronucleus (arrow).

Furthermore, it is important to take into consideration that only one nurse used a safety hood to handle antineoplastic drugs, whereas 21 out of 25 nurses used gloves. 19 out of 25 used masks, and two individuals did not have routine self-protective behavior at all.

4. Discussion

Among the most commonly used antineoplastic drugs, there are clastogenic and aneugenic agents (IARC, 1975; 1976; 1981; 1982; Prejeant and Montgomery; 1984), which require protection equipment to avoid genotoxic risks to the individuals that manage these agents. The group of nurses analyzed did not follow the adequate rec-

Table 2
Characteristics and frequencies of micronucleated cells in exposed group

Subject No.	Age (years)	Sex (m/f)	Cigarettes smoking		Micronucleated cells (%)	Exposition	
			No./day	Duration/years		h/day	years
1	34	f	0	0	0.5	7	1
2	32	f	20	10	0.0	7	6
3	30	f	10	16	0.0	7	1
4	22	m	0	0	0.6	3	1
5	28	m	0	0	0.0	3	3
6	25	m	20	5	1.0	1	3
7	32	m	20	10	0.0	3	3
8	30	f	0	0	0.1	1	3
9	30	f	0	0	0.4	1	6
10	31	f	0	0	0.6	1	3
11	42	f	0	0	0.5	7	3
12	25	f	0	0	0.3	7	3
13	25	f	0	0	0.9	7	3
14	25	f	0	0	0.3	7	6
15	35	f	0	0	0.1	5	3
16	28	f	15	10	1.0	7	3
17	36	f	5	15	0.7	1	6
18	28	f	0	0	0.2	5	3
19	42	f	0	0	0.0	5	3
20	35	f	0	0	0.6	1	3
21	35	f	0	0	0.1	3	1
22	37	f	0	0	0.2	7	3
23	50	m	0	0	0.0	5	3
24	28	f	0	0	0.3	7	3
25	37	f	15	12	0.2	7	3
mean	32.1				0.344		
Std. error	±1.29				±0.065		

ommendations for the management of those drugs, and so the biomonitoring becomes of great value. Our data lead us to conclusion that the individuals exposed to the drugs presented higher level of genetic damages. However, it was not possible to demonstrate differences among the highly exposed subgroup (determined by the number of hours per day multiplied by years of drug handling). These data are consistent with those of McDiarmid et al. (1992), who did not find any relation between duration or exposure and the level of sister chromatid exchanges in lymphocytes of pharmacists that handle antineoplastic drugs.

Our data are consistent with those of Norppa et al. (1980), Waksvik et al. (1981), Goloni-Bertollo et al. (1992) and Thiringer et al. (1991) as they found higher sister chromatid exchange rates in lymphocytes of nurses that handle antineoplastic drugs, as well as mutagenicity observed in their urine through the Ames test in *Salmonella typhimurium* (Falk et al., 1979 and Thringer et al., 1991). No dose-response relationship was observed for any parameter in that study. Once the analysis procedures used for the biomonitoring were different, comparison of absolute values was not possible.

We may consider that the buccal mucosa cells are the direct target of exposure through inhalation of the drugs due to the use of inadequate or the absence of protection. Other systems such as lymphocytes, represent cells indirectly exposed. This could explain that the frequency of micronuclei in lymphocytes of the exposed nurses is not

statistically significant when compared with the controls (Thiringer et al., 1991). Sorsa et al. (1988) refer to a statistically non-significant trend in the increased number of micronuclei in binucleated lymphocytes of workers in the production of cyclophosphamide drug as compared with control.

Maybe the sister chromatid exchange in lymphocytes is a more sensitive test, although this kind of aberration probably has a lower genotoxicity potential. These observations emphasize the importance of the micronuclei assay in the detection of genetic damage of cells directly exposed to the agents which are analyzed in the biomonitoring program.

Sarto et al. (1987) concluded that the micronuclei assay on exfoliated cells of human buccal mucosa is, indeed, very sensitive and is able to detect the effect of low doses of environmental carcinogens (e.g., smoking 20 cigarettes/day). We were not able to detect higher frequency of micronuclei in smokers compared to non-smokers, probably due to the small number of smokers in each subgroup.

Concerning the data of Thiringer et al. (1991), it is important to emphasize the working conditions of the individuals analyzed: 91% of the nurses reported they always used a safety hood, whereas the remainders used it between 76 and 99% of the time. In the present study, among the 25 individuals exposed, only one used the safety hood. Surgical masks and gloves may be considered as insufficient protective equipment.

In conclusion, our results demonstrate the genetic risks to which individuals that handle antineoplastic drugs are exposed if the safety requirements are not fulfilled. Simultaneous use of adequate hoods, gloves and garments would provide appropriate working conditions which are imperative to the health of the individuals that handle antineoplastic drugs.

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