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Evaluation of occupational genotoxic risk in a Brazilian hospital

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Abstract

Many therapeutic, diagnostic and prophylactic procedures used in hospitals are of potential genetic risk. An evaluation was made of genotoxic occupational risk in 42 workers from the Hospital de Clínicas de Porto Alegre, RS, Brazil, who had been occupationally exposed to lead (solder), ethylene oxide (sterilization area), antineoplastic drugs (nurses and pharmacists) or ionizing radiation. They were compared with 42 unexposed individuals. There was an increase in the frequency of binucleated cytochalasin-blocked lymphocytes with micronuclei, though it was not significant (P = 0.058). The groups exposed to antineoplastic drugs and radiation had a significant increase in micronuclei frequency (P = 0.038 and P = 0.022, respectively). The high frequencies of dicentric bridges suggest the action of clastogenics in these two groups. These results suggest that the safety procedures adopted were very important to protect workers from exposure to mutagenic agents and should be improved in the radiological and chemotherapeutical areas.

INTRODUCTION

There is increasing concern about the mutagenic and carcinogenic effects of genotoxic agents in humans occupationally or accidentally exposed (Carrano and Natarajan, 1988). Mutagenic agents are used in hospitals either for maintenance or for the diagnosis and treatment of patients. However, for professionals who are continually exposed to these agents, the risks need to be assessed in order to establish proper management.

The mutagenic effect of ionizing radiation has been studied extensively (Fenech *et al*., 1990; Balasem and Ali, 1991; Erexon *et al*., 1991) and an increase in chromosomal aberrations was observed in occupationally exposed workers when the permissible levels of radiation were exceeded (Jha and Sharma, 1991).

Chemicals, such as cytostatic agents, induce chromosomal damage, both experimentally (*in vitro*) and clinically in patients (Migliore *et al*., 1991; Gree *et al*., 1991). McDiarmid *et al*. (1992) reported a positive correlation between the time of occupational exposure to antineoplastic drugs and the frequency of sister chromatid exchanges.

Lead disturbs cell proliferation and DNA synthesis *in vivo*, and may be responsible for certain types of cancer, mainly in the kidneys (Gerber *et al*., 1980). Lerda (1992) demonstrated that lead causes chromosomal breaks. This phenomenon, which is very frequent, is the main chromosomal alteration caused by occupational intoxication with lead.

Ethylene oxide is an alkylating agent used to sterilize hospital materials and is mutagenic in microorganisms (Pfeiffer and Dunkelberg, 1980; Agurell *et al.*, 1991), plants (Jana and Roy, 1975), animals (Ehrenberg *et al.*, 1974; Generoso *et al.*, 1980, 1983) and humans (Hogstedt *et al*., 1983). Workers occupationally exposed to ethylene oxide have an increased rate of chromosomal aberrations and micronucleus frequency (Ribeiro *et al*., 1994) and a decreased repair capacity (Mayer *et al*., 1991).

Workers from the Hospital de Clínicas de Porto Alegre (HCPA) were monitored using the cytokinesis-blocked lymphocyte analysis (Fenech and Morley, 1985) to measure frequency of micronuclei, dicentric bridges and spindle anomalies. The main advantage of this method of evaluation is the reliable identification of cells that have completed only one nuclear division (Fenech, 1997). Our aim was to verify whether the safety procedures in use were enough to protect healthy workers from exposure to mutagenic agents.

MATERIAL AND METHODS

Peripheral blood samples were collected from 84 workers; 42 out of them were occupationally exposed to lead (N = 11), ionizing radiation (N = 11), ethylene oxide (N = 10) or cytostatic drugs (N = 10). The remaining 42 were controls from the same institution, matched for age, sex and smoking habits. The individuals exposed to lead were chosen based on their levels of delta-aminolevulinic acid and those exposed to ionizing radiation were chosen based on the level of radiation recorded by their dosimeters. The group exposed to cytostatic agents was formed by all individuals from this area and the individuals exposed to ethylene oxide were chosen based on their time of activity in the sterilization area. Table I shows the subjects' sex and age.

All individuals answered the personal health questionnaire published by the International Commission for Pro-

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tection against Environmental Mutagens and Carcinogens (ICPEMC) (Carrano and Natarajan, 1988).

An aliquot of blood (0.3 ml) was added to 5 ml of standard supplemented RPMI 1640 medium containing 20% fetal calf serum and phytohemagglutinin (PHA, 0.2%). The flasks were cultured at 37°C. After 44 h, 4 µg of cytochalasin B/ml (Sigma Chemical Co., St. Louis, MO, USA) was added (Fenech, 1993). After 72 h, the cells were harvested and treated with sodium citrate (1%) and then fixed in methanol:glacial acetic acid (3:1, U/U). The slides for each individual were randomly numbered and stained with Giemsa. The presence of micronuclei and other anomalies was determined in 2000 binucleated cells per individual. The slides from exposed and non-exposed subjects were evaluated in parallel by "blind" analysis. Spindle anomalies were considered when two or more nuclei in the same cell clearly showed different sizes. The presence of dicentric bridges was scored when clearly separated nuclei were linked by nuclear material. Differences between groups were assessed using a two-sample *t*-test. The influence of age, smoking and drinking habits on the frequency of micronuclei was also examined.

RESULTS AND DISCUSSION

There was a slight non-significant increase (P = 0.058) in the frequency of micronucleated lymphocytes (16.9 ± 6.9) (in 2000 analyzed cells per individual) in exposed workers compared to the controls (14.2 ± 5.8). This slight increase in the frequency of micronuclei was attributable to those exposed to cytostatic drugs and ionizing radiation. The frequency of dicentric bridges was increased in the same groups. There was no increase in spindle disorders.

The frequency of micronucleated lymphocytes in individuals exposed to antineoplastic drugs was significantly greater (P = 0.038) than in the paired controls. The frequency of dicentric bridges was also increased, although not significantly (P = 0.0545), while the frequency of spindle anomalies was similar in both groups (Table II). An increased frequency of SCE has also been found, following low level exposures in nurses working with cancer patients compared to office workers (Norppa *et al.*, 1980; Waksvik *et al.*, 1981; Sorsa *et al.*, 1982). Structural chromosomal aberrations were also significantly greater in nurses exposed to antineoplastic drugs compared to non-exposed laboratory workers and hospital clerks (Nikula *et al.*, 1984). Similarly, nurses who handled cytostatic drugs without using safety covers had more dicentric chromosomes than those who used safety covers, but there was no difference in the frequency of chromosomal breaks and SCE (Oestreicer *et al.*, 1990). Our sample consisted of nurses and pharmacists from a hospital considered to have well-established safety rules.

The group exposed to ionizing radiation had an increased frequency of micronucleated cells relative to controls (P = 0.0217), and also a non-significantly higher frequency of dicentric bridges (P = 0.1172). The spindle anomalies were similar in both groups (Table III). Among hospital workers, such as physicians, nurses and technicians, who are exposed to very low levels of X- or γ-rays, there is an increased frequency of chromosomal aberrations, including dicentrics (Bigatti *et al.*, 1988). In our assay, dicentric chromosomes were indicated by the frequency of bridges. Medical workers exposed to diagnostic levels of X-rays show a higher frequency of dicentric and acentric chromosomes compared to normal controls (Jha and Sharma, 1991). The frequency of micronuclei did not show a correlation with the radiation doses recorded by the dosimeters. This result is in agreement with the results reported by Barquinero *et al*. (1993) in a study with 26 workers with an accumulated dose range from 2.3 to 131.7 mSv. As pointed out by Barquinero *et al*. (1993), it is difficult to establish dose-effect relationships for low radiation levels.

The lead-exposed group showed a similar micronuclei frequency and dicentric bridges to that of the con-

Table I - Age and sex of the hospital workers studied.

Group	Exposed			Controls		
	Age (years) mean ± SD	Sex		Age (years) mean ± SD	Sex	
		M	F		M	F
Cytostatic drugs	29.8 ± 6.0	1	9	28.9 ± 4.3	1	9
Lead	38.8 ± 7.2	10	1	36.0 ± 8.7	10	1
Ethylene oxide	35.8 ± 6.5	10	0	35.9 ± 7.8	10	0
Ionizing radiation	40.4 ± 7.7	6	5	39.9 ± 8.8	6	5
Total	36.4 ± 7.8	27	15	35.3 ± 8.4	27	15

M: Male; F: female.

Table II - Frequency of binucleated lymphocytes with micronuclei (MN) and other nuclear anomalies in 2000 cells analyzed per hospital worker potentially exposed to cytostatic drugs, and their respective controls.

Cytostatic drugs				Controls			
Subject	MN	SA	DB	Subject	MN	SA	DB
659	35	29	11	744	04	41	02
692	30	83	06	555	17	64	03
624	27	52	13	207	09	61	02
889	23	61	16	917	22	75	08
707	18	52	03	778	09	37	04
013	18	35	04	015	10	43	03
625	13	65	03	454	07	75	01
603	11	28	09	186	15	36	03
629	09	83	0	407	11	70	0
014	08	50	01	713	11	39	01
Mean	19.2*	53.8	6.6	**Mean**	11.5	54.1	2.7
SD	9.3	19.8	5.4	**SD**	5.2	16.4	2.2

MN - Micronuclei; SA - spindle anomalies; DB - dicentric bridges; SD - standard deviation; *P < 0.05 compared to controls.

trols (Table IV). Lead-exposed individuals had a significant increase in the frequency of spindle disorders, with no parallel increase in micronuclei frequency. Previous studies have also demonstrated a genotoxic effect of lead (Gerber *et al.*, 1980; Lerda, 1992). Based on the low levels of aminolevulinic acid in our subjects (Table IV), there apparently was no dangerous exposure to lead in these individuals. Levels of aminolevulinic acid higher than 15 mg/l can be considered dangerous.

Although other studies have demonstrated a significant relationship between the frequency of genetic alterations and the degree of exposure to ethylene oxide (Lerda and Rizzi, 1992; Ribeiro *et al.*, 1994), we observed no such relationship (Table V), thus confirming the effectiveness of the safety procedures adopted.

There was no significant correlation between the genetic abnormalities detected and non-occupational factors such as age, sex and smoking or drinking habits, although the lack of influence of the latter on the micronuclei frequency could be related to the fact that these were not frequent habits in our subjects.

The concomitant analysis of dicentric bridges and spindle disorders when determining the micronucleus frequency does not involve much extra work, and may serve as a reference to the type of mutagen (clastogenic or aneugenic). Based on our results, we suggest clastogenic activity in the individuals with an increased frequency of micronuclei.

Workers handling antineoplastic drugs can be exposed through inhalation of aerosolis, transdermal absorption and accidental ingestion. An increase in genetic damage in workers occupationally exposed to antineoplastic drugs has been associated with careless handling (Grummt *et al.*, 1993; Machado-Santelli *et al*., 1994; Sorsa and Anderson, 1996; Undeger *et al*., 1999). In our study, pharmacists and nurses used safety covers and followed the guidelines for working with antineoplastic drugs. Based on the results of the analysis of genetic damage by the micronuclei assay, the group of workers handling antineoplastic drugs was advised to modify their work schedule to reduce exposure time.

The safety procedures adopted were very important to protect workers from exposure to mutagenic agents and should be improved in the radiological and chemotherapeutical areas. The cytokinesis-blocked micronucleus assay appears to be useful for monitoring populations chronically exposed to genotoxic agents.

Table III - Frequency of binucleated cells with micronuclei (MN) and other nuclear anomalies in 2000 cells analyzed per hospital worker potentially exposed to ionizing radiation, and their respective controls.

Ionizing radiation					Controls			
Subject	mSv	MN	SA	DB	Subject	MN	SA	DB
166	2.4	25	43	08	771	19	57	01
504	12.9	24	57	13	855	06	46	0
675	23.6	23	33	04	426	15	43	08
262	5.1	22	51	08	590	21	76	14
557	2.9	22	45	16	484	17	85	03
059	8.9	21	50	17	745	18	74	03
364	7.4	21	67	0	126	17	43	10
560	5.4	20	57	12	027	21	55	18
755	4.2	19	61	14	839	12	64	0
135	1.4	19	44	16	581	23	46	09
396	3.6	14	72	0	548	05	56	0
Mean	7.1	20.9*	52.7	10.2	**Mean**	15.8	58.6	6.0
SD	6.4	3.0	11.4	5.7	**SD**	5.9	14.5	6.2

mSv, Radiation dose in millisieverts. For other abbreviations see Table II.
*$P < 0.05$ compared to controls.

Table IV - Frequency of binucleated cells with micronuclei (MN) and other nuclear anomalies in 2000 cells analyzed per hospital worker potentially exposed to lead, and their respective controls.

Lead					Controls			
Subject	δ-ala	MN	SA	DB	Subject	MN	SA	DB
946	5.5	24	81	03	242	06	69	05
295	4.5	23	80	03	807	07	41	01
282	5.5	22	37	05	352	19	74	09
327	8.0	19	54	07	762	19	43	04
969	5.0	17	74	07	538	06	73	03
329	5.0	17	72	07	720	18	65	07
162	4.5	16	61	02	666	23	33	10
021	5.0	12	82	06	068	14	35	08
096	4.5	11	60	08	360	23	40	11
509	7.0	09	50	04	272	17	50	04
637	4.5	09	50	04	476	24	35	07
Mean	5.4	16.3	63.7	5.1	**Mean**	16.0	50.7	6.3
SD	1.1	5.5	15.1	2.0	**SD**	6.9	16.3	3.1

δ-ala, Delta-aminolevulinic acid levels in urine (mg/l). For other abbreviations see Table II.

Table V - Frequency of binucleated cells with micronuclei (MN) and other nuclear anomalies in 2000 cells analyzed per hospital worker potentially exposed to ethylene oxide, and their respective controls.

Ethylene oxide				Controls			
Subject	MN	SA	DB	Subject	MN	SA	DB
339	18	93	02	338	16	53	09
685	16	44	12	751	08	66	09
842	15	53	11	816	16	46	21
113	11	46	04	160	10	54	08
177	10	45	08	333	14	61	06
519	09	36	05	293	18	46	11
686	08	58	09	650	15	44	02
841	08	50	07	448	08	35	04
674	08	34	02	893	19	44	06
827	05	50	03	245	08	51	06
Mean	10.8	50.9	6.3	**Mean**	13.2	50.0	8.2
SD	4.2	16.5	3.6	**SD**	4.3	9.0	5.2

For abbreviations see Table II.

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RESUMO

Vários procedimentos terapêuticos, diagnósticos e profiláticos usados em hospitais apresentam um risco genético. Para avaliar o risco genotóxico ocupacional, 42 trabalhadores do Hospital de Clínicas de Porto Alegre, RS, Brasil, ocupacionalmente expostos a chumbo (uso de soldas), óxido de etileno (área de esterilização), drogas antineoplásicas (enfermeiros e farmacêuticos) e radiação ionizante foram comparados com 42 indivíduos não expostos. A análise de linfócitos binucleados apresentou um aumento estatisticamente não significativo (P = 0.058) na freqüência de micronúcleos. Quando analisados separadamente, os grupos expostos a drogas antineoplásicas e radiação ionizante apresentaram um aumento estatisticamente significativo (P = 0.038 e P = 0.0217, respectivamente) na freqüência de micronúcleos. As freqüências de pontes dicêntricas e anomalias de fuso sugerem a ação de clastogênicos nestes dois grupos. Fatores como idade, sexo e hábitos de fumo e álcool não apresentaram correlação com as alterações genéticas observadas. Estes resultados sugerem que os procedimentos de segurança adotados foram muito importantes para proteger os trabalhadores da exposição a agentes mutagênicos e que estas medidas devem ser melhoradas nas áreas de radiologia e quimioterapia.

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