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G_2 repair and chromosomal damage in lymphocytes from workers occupationally exposed to low-level ionizing radiation

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Abstract

The effect of the G_2 repair of chromosomal damage in lymphocytes from workers exposed to low levels of X- or γ-rays was evaluated. Samples of peripheral blood were collected from 15 radiation workers, 20 subjects working in radiodiagnostics, and 30 healthy control donors.

Chromosomal aberrations (CA) were evaluated by scoring the presence of chromatid and isochromatid breaks, dicentric and ring chromosomes in lymphocytes with/without 5mM caffeine plus 3mM-aminobenzamide (3-AB) treatment during G_2.

Our results showed that the mean value of basal aberrations in lymphocytes from exposed workers was higher than in control cells ($p< 0.001$). The chromosomal damage in G_2, detected with caffeine plus 3-AB treatment was higher than the basal damage (untreated conditions), both in control and exposed populations ($p< 0.05$). In the exposed workers group, the mean value of chromosomal abnormalities in G_2 was higher than in the control ($p< 0.0001$).

No correlation was found between the frequency of chromosome type of aberrations (basal or in G_2), and the absorbed dose. Nevertheless, significant correlation coefficients ($p< 0.05$) between absorbed dose and basal aberrations yield ($r = 0.430$) or in G_2 ($r = 0.448$) were detected when chromatid breaks were included in the total aberrations yield. Under this latter condition no significant effect of age, years of employment or smoking habit on the chromosomal aberrations yield was detected. However, analysis of the relationship between basal aberrations yield and the efficiency of G_2 repair mechanisms, defined as the percentage of chromosomal lesions repaired in G_2, showed a significant correlation coefficient ($r = -0.802$; $p< 0.001$).

These results suggest that in addition to the absorbed dose, the individual G_2 repair efficiency may be another important factor affecting the chromosomal aberrations yield detected in workers exposed to low-level ionizing radiation.

Key words: Chromosome aberrations ionizing radiation, G_2 repair, human lymphocytes, occupational exposure

INTRODUCTION

Several cytogenetics studies have shown an increase of chromosome abnormalities in lymphocytes from radiation workers (Evans *et al.*, 1979; Bauchinger *et al.*, 1980; Balasem *et al.*, 1992; Braselmann *et al.*, 1994) and from medical radiologists (Maznik, 1987; Bigatti *et al.*, 1988; Jha and Sharma, 1991; Barquinero *et al.*, 1993) exposed to low doses of X- or γ-rays. Similar results have also been detected in populations subjected to an increased burden in natural radiation or to an additional exposure due to the Chernobyl accident (Pohl-Rüling *et al.*, 1991; Bauchinger *et al.*, 1994; Lazutka and Dendonyte, 1995).

Dosimetric studies in populations exposed to low doses of ionizing radiation (below 50 mSV), in which the common cytogenetic markers used were of the chromosomal type

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of aberration (dicentric and ring chromosomes, translocations and chromosome breaks) have shown contradictory results. According to data reported by Evans *et al.* (1979) in nuclear dockyards and Maznik (1987) in medical radiologists, the increase of basal chromosomal aberration frequency would be correlated to the absorbed dose, whereas no correlation was found in other studies (Bauchinger *et al.*, 1980; Bigatti *et al.*, 1988; Jha and Sharma, 1991; Balasen *et al.*, 1992; Barquinero *et al.*, 1993; Braselmann *et al.*, 1994).

The absence of a dose-dependence response in populations exposed to low doses has attributed to the induction of DNA repair enzymes (Pohl-Rüling *et al.*, 1991), the half-life of lymphocytes (Lloyd *et al.*, 1980; Braselmann *et al.*, 1994), and/or several other population variables such as age, sex, and smoking history, which might influence the aberrations yield detected in exposed populations (Bender *et al.*, 1988).

In relation to the effect of DNA repair mechanism activity, it is well known that in irradiated lymphocytes most of the DNA damage induced by ionizing radiation will be removed by DNA repair mechanisms operating in G_0 and G_1 (Wolff, 1972; Vyas *et al.*, 1991; Boerrigter and Vijg, 1992; Chukhlovin *et al.*, 1995; Banáth *et al.*, 1998). However, in theory some DNA lesions can remain unrepaired or misrepaired and become committed to going through the cell cycle when the lymphocytes culture is set up. Therefore, some of the DNA damage derived from these lesions may arrive unrepaired at G_2.

In previous studies on proliferating lymphocytes, we found that the inhibition of G_2 repair mechanisms with caffeine plus 3-aminobenzamide increased the chromosomal aberrations yield induced spontaneously or by X-rays (Pincheira *et al.*, 1985). This increment, which represents the unrepaired chromosomal lesions in G_2, may allow a certain estimation of the DNA damage arriving at this stage of the cell cycle. Furthermore, differences between basal and G_2 aberration frequencies may also allow us to estimate the frequency of chromosomal damage repaired in G_2 and the efficiency of the DNA repair mechanisms during this cell cycle phase.

In order to estimate the basal and G_2 chromosomal damage in lymphocytes from workers exposed to low-level radiation, we compared the chromosomal aberration frequency in lymphocytes with and without caffeine plus 3-aminobenzamide treatment during G_2 in a sample of 35 workers exposed to low-level doses of X- or γ-rays and 30 control donors. We also analyzed the influence of factors such as age, years of employment, smoking habit, and inter-individual differences of the G_2 repair mechanism efficiency on the chromosomal damage detected in this sample of exposed workers.

MATERIAL AND METHODS

Subjects

The subjects of the sample were selected according to a questionnaire, detailing personal, medical and occupational history such as age, sex, job type, years of employment, smoking habits, viral infections, and drugs or diagnostic X-ray exposure.

The sample of exposed individuals was divided into 2 groups. Group A corresponded to 15 radiation workers (14 males and 1 female) employed at the Chilean Nuclear Energy Commission (CCHEN) and exposed to gamma and/or X-radiation (coded A-1 to A-15). Group B included 20 medical radiologists (3 physicians and 17 technicians; 11 males and 9 females) exposed to diagnostic levels of X-rays (coded B-16 to B-35). The annual dose of radiation received by each exposed worker, monitored by physical dosimeter, was below the maximum permissible occupational limit (50 mSv). For a number of reasons, we did not have access to complete records of the annual exposure doses in 12 cases from group B. The control group consisted of 30 healthy individuals (17 males and 13 females; 20-65 years of age) with no radiation history except natural background. Neither the radiation

workers nor the control group had received chemotherapeutic or cytostatic drugs.

Chromosome preparation and metaphases analysis

For chromosome analysis, standard 48-hour cultures with 0.5 ml whole blood, 4 ml of cell culture medium F-10 nutrient mixture (Gibco. BRL, USA), 0.5 ml fetal bovine serum (Gibco BRL, USA) and 0.15 ml of phytohemagglutinin (Gibco BRL, USA) were established. Colcemide (Gibco BRL, USA) at 5 x 10^{-7M} final concentration was added to the culture 2 hours before harvesting.

Caffeine (Merck) and 3-aminobenzamide (Sigma, USA) stock solutions were prepared in F-10 medium, filtered, sterilized and stored at 4°C. For treatments during G_2, 5mM caffeine and 3mM 3 amino-benzamide (3-AB) final concentration were added 2 hours before harvesting.

Four slides were prepared for each culture and stained with Giemsa. The coded slides were scored blindly under a microscope by 3 investigators. At least 303 metaphases from each culture (with and without caffeine plus 3-AB) were scored for unstable chromosomal aberrations *i.e.*: chromatid, chromosome/ isochromatid breaks, acentric fragments, dicentric and ring chromosomes. Chromatid breaks were recorded when the distal segment was dislocated from the chromosome axis or when the unstained segment was a size larger than the chromatid width. Chromosome/ isochromatid breaks were recorded when the broken piece was displayed with respect to the chromosome axis. Acentric fragments were considered along with chromosome/ isochromatid breaks. A dicentric chromosome with an acentric fragment was scored as one aberration.

Chromosome/isochromatid breaks and dicentric and ring chromosomes correspond to the chromosome type of aberrations, which result from illegitimate reuniting (misrejoining) of free ends from different DNA double-strand breaks (dsb). These dsbs arise from unrepaired or misrepaired DNA lesions induced by ionizing radiation during G_0 or G_1 and therefore are considered to be cytogenetic markers in dosimetric studies. On the other hand, chromatid breaks should not be considered in this latter type of studies because not all of this type of chromosomal abnormalities would be expected to be consequence of radiation exposure (Bender *et al.*, 1988; Natarajan *et al.*, 1993; Sachs *et al.*, 1997). However, since in the present study chromosomal abnormalities were not used as recorder of absorbed doses, chromatid breaks were also included in the total aberrations yield.

The efficiency of the DNA repair mechanisms during G_2 (G_2 RE) defined as the percentage of chromosomal lesions repaired in G_2 was calculated for each exposed workers as follows:

$$G_2\,RE = \frac{f\,CA\,(+\,In) - f\,CA\,(b)}{f\,CA\,(+\,In)} \times 100 \quad [1]$$

f CA (+In) corresponds to the chromosomal aberration frequency in lymphocytes treated with caffeine plus 3-AB during G_2 and f CA (b) to the basal aberration frequency in untreated lymphocytes (control conditions). Assuming that under the present conditions, caffeine plus 3-AB treatment is a preferential inhibitor of the G_2 repair, equation [1] is similar to the one we used (Pincheira and López-Sáez, 1991) to estimate the caffeine potentiation effect of the damage.

The one-tailed Z test was used for statistical comparison of the mean values of aberrations frequency in lymphocytes from control and exposed groups under control and treated conditions.

The statistical significance in the increase of chromosomal aberrations yield under control or treated conditions in lymphocytes from each exposed worker in relation to the mean value of the control population was tested by the chi square test (X^2).

The relationship between aberrations yield and absorbed dose, age, years of employment or G_2 repair efficiency was analyzed using linear regression analysis, Student's t test, and determination coefficient (r^2).

RESULTS

Table I shows the basal aberration frequencies and dose exposure records of each of the 35 exposed workers studied. It also shows the mean value ± SE of the chromosomal aberrations in the exposed and control groups. Since no significant differences were detected in the mean values of aberrations yields and abnormal metaphases between the two groups of exposed workers (codes A and B), the data from all 35 workers were analyzed together.

A comparison of aberrations yield between controls and exposed workers showed that the mean value of the

TABLE I

Chromosomal aberration frequency in lymphocytes from workers exposed to low levels of ionizing radiation and from control donors.

Subject Code	Total dose* mSv	Ab.M N°	Aberrations / 100 metaphases ctb	csb	dic-r	Ab.yield ± SE
A-1	58.68 [a]	12	2.97	1.32	0.33	4.62 ± 1.2*
A-2	55.73 [a]	10	2.64	0.66	0.00	3.30 ± 1.0*
A-3	16.06[a]	12	3.30	0.66	0.33	4.29 ± 1.2*
A-4	6.06[a]	6	0.99	0.99	0.00	1.98 ± 0.8
A-5	41.16[a]	10	3.63	0.66	0.33	4.62 ± 1.2*
A-6	18.30[a]	12	2.31	1.65	0.33	4.29 ± 1.2*
A-7	3.78[a]	4	0.99	0.33	0.33	1.65 ± 0.7
A-8	5.93[a]	5	1.32	0.33	0.00	1.65 ± 0.7
A-9	9.13[a]	4	0.99	0.33	0.00	1.32 ± 0.7
A-10	2.36[a]	3	0.99	0.00	0.00	0.99 ± 0.6
A-11	23.18[a]	4	1.32	0.00	0.00	1.32 ± 0.7
A-12	2.72[a]	5	1.32	0.66	0.00	1.98 ± 0.8
A-13	3.69[a]	3	0.99	0.00	0.00	0.99 ± 0.6
A-14	5.22[a]	10	2.64	0.99	0.33	3.96 ± 1.1*
A-15	24.03[a]	4	0.99	0.33	0.00	1.32 ± 0.7
B-16	0.60[a]	7	2.31	0.99	0.00	3.30 ± 1.0*
B-17	0.00[b]	8	1.98	0.99	0.00	2.97 ± 0.9*
B-18	2.74[a]	6	1.32	0.66	0.00	1.98 ± 0.8
B-19	2.49[b]	8	1.65	0.66	0.33	2.64 ± 0.9*
B-20	1.40[b]	3	1.65	0.00	0.33	1.98 ± 0.8
B-21	0.63[b]	10	2.64	1.32	0.00	3.96 ± 1.1*
B-22	0.15[b]	3	0.99	0.00	0.00	0.99 ± 0.6
B-23	0.00[b]	8	1.98	0.66	0.00	2.64 ± 0.9*
B-24	0.60[a]	6	2.31	0.00	0.00	2.31 ± 0.9
B-25	0.30[b]	5	1.32	0.66	0.00	1.98 ± 0.8
B-26	32.62[a]	10	2.31	0.66	0.33	3.30 ± 1.0*
B-27	1.17[b]	12	2.97	1.32	0.33	4.62 ± 1.2*
B-28	1.80[a]	8	1.98	0.66	0.00	2.64 ± 0.9*
B-29	12.20[a]	6	1.98	0.00	0.00	1.98 ± 0.8
B-30	40.30[a]	9	2.31	0.66	0.00	2.97 ± 0.9*
B-31	0.25[b]	6	1.98	0.00	0.00	1.98 ± 0.8
B-32	0.35[b]	10	3.63	0.33	0.33	4.29 ± 1.2*
B-33	0.57[b]	9	2.31	0.66	0.00	2.97 ± 0.9*
B-34	0.31[b]	7	1.32	0.99	0.00	2.31 ± 0.7
B-35	0.90[a]	12	3.30	0.99	0.33	4.62 ± 1.2*
Exposed group (35) 10605 metaphases	X±SE	7.34±0.5	1.99±0.13(**)	0.60±0.07(**)	0.11±0.03(**)	2.71 ± 0.15(**)
Control group (30) 3036 metaphases	X±SE	0.9±0.07	1.23±0.2	0.03±0.03	0.03±0.03	1.29 ± 0.20

Ab.M = Abnormal metaphases. (a) Total dose absorbed during the last three years prior to the study; (b) Non-available on incomplete records of dose exposure; ctb = chromatid breaks; csb = chromosome / isochromatid breaks; dic-r = dicentric and ring chromosomes; Ab. yield = Total aberrations yield (gaps excluded). 303 metaphases were scored per each individual. (*) Significantly different from the mean value of the control group, $p< 0.05$ (chi square test). (**) $p< 0.05$, (one-tailed Z test).

aberrations in this latter group was higher than in controls ($p< 0.0001$, one-tailed Z test). Furthermore, 18 of the 35 exposed workers showed a basal aberrations frequency higher than the mean value of the controls ($p < 0.05$, chi square test).

The analysis of the relationship between absorbed dose and total aberrations yield in 23 of the 35 exposed workers with complete record of annual dose exposure showed a correlation coefficient ($r=0.4302$), statistically significant ($p < 0.05$, Student's *t* test), and a determination coefficient $r^2 = 0.185$. However, no significant correlation ($r=0.204$) was found when only chromosome types of aberrations (dicentric, and ring

TABLE II

Chromosomal aberration frequency in lymphocytes treated with 5mM caffeine plus 3mM aminobenzamide during G_2 from exposed workers and control donors.

Subject Code	Total dose* mSv	Ab.M N°	Aberrations / 100 metaphases ctb	csb	dic-r	Ab.yield ± SE
A-1	58.68[a]	22	7.92	0.99	0.00	8.91 ± 1.6(*)
A-2	55.73[a]	24	7.92	1.65	0.33	9.90 ± 1.7(*)
A-3	16.06[a]	20	5.28	1.65	0.33	7.26 ± 1.5(*)
A-4	6.06[a]	12	2.64	1.98	0.00	4.62 ± 1.2
A-5	41.16[a]	18	5.94	1.32	0.33	7.59 ± 1.5(*)
A-6	18.30[a]	25	9.24	0.66	0.00	9.90 ± 1.7(*)
A-7	3.78[a]	15	3.30	2.31	0.33	5.94 ± 1.3
A-8	5.93[a]	11	3.96	0.99	0.00	4.95 ± 1.2
A-9	9.13[a]	10	2.97	1.32	0.00	4.29 ± 1.2
A-10	2.36[a]	11	3.63	0.00	0.00	3.63 ± 1.1
A-11	23.18[a]	10	3.30	0.66	0.00	3.96 ± 1.1
A-12	2.72[a]	14	3.96	0.99	0.00	4.95 ± 1.2
A-13	3.69[a]	12	2.97	0.99	0.00	3.96 ± 1.1
A-14	5.22[a]	20	5.94	1.98	0.33	8.25 ± 1.6(*)
A-15	24.03[a]	14	3.96	0.99	0.00	4.95 ± 1.2
B-16	0.60[a]	22	5.94	2.97	0.00	8.91 ± 1.6(*)
B-17	0.00[b]	21	4.95	2.31	0.00	7.26 ± 1.5(*)
B-18	2.74[a]	20	3.30	1.98	0.00	5.28 ± 1.5
B-19	2.49[b]	17	5.28	1.98	0.00	7.26 ± 1.5(*)
B-20	1.40[b]	12	4.62	0.00	0.00	4.62 ± 1.2
B-21	0.63[b]	18	3.63	2.97	0.330	6.93 ± 1.5
B-22	0.15[b]	9	2.64	0.33	0.00	2.97 ± 0.9
B-23	0.00[b]	23	6.93	1.32	0.00	8.25 ± 1.6(*)
B-24	0.60[a]	23	6.60	1.98	0.00	8.58 ± 1.6(*)
B-25	0.30[b]	14	4.29	0.33	0.33	4.95 ± 1.2
B-26	32.82[a]	19	4.62	2.31	0.33	7.26 ± 1.5(*)
B-27	1.17[b]	19	6.93	0.66	0.33	7.92 ± 1.6(*)
B-28	1.80[a]	12	4.29	0.00	0.00	4.29 ± 1.2
B-29	12.20[a]	15	4.29	1.32	0.00	5.61 ± 1.3
B-30	40.30[a]	19	5.94	0.99	0.00	6.93 ± 1.5
B-31	0.25[b]	18	6.27	0.66	0.33	7.26 ± 1.5(*)
B-32	0.35[b]	21	5.94	1.98	0.33	8.25 ± 1.6(*)
B-33	0.51[b]	17	3.96	1.98	0.00	5.94 ± 1.3
B-34	0.31[b]	18	5.94	1.32	0.00	7.26 ± 1.5(*)
B-35	0.90[a]	19	4.29	2.97	0.33	7.59 ± 1.5(*)
Exposed group (35) 10605 metaphases	X±SE	16.9±0.7	4.96±0.2	1.40±0.1	0.11±0.03	6.47 ±0.2(**)
Control group (30) 3036 metaphases	X±SE	4.5±0.2	4.29±0.38	0.30±0.09	0.06±005	4.65 ±0.38

Ab.M = Abnormal metaphases. (a) = Total dose absorbed during the last three years prior to the study; (b) Non-available or incomplete records of dose exposure; ctb = chromatid breaks; csb = chromosome/isochromatid breaks; dic-r = dicentric and ring chromosomes; Ab. yield = total aberrations yield (gaps excluded). 303 metaphases were scored per each individual. (*) Significantly different from the mean value of the control group, $p< 0.05$, (chi square test). (**) $p< 0.05$ (one-tailed Z test).

chromosomes and chromosome breaks) were included in this analysis.

Table II shows the dose exposure records and chromosomal aberration frequencies in G_2, detected in lymphocytes from exposed workers treated with 5mM caffeine plus 3mM 3-AB during G_2. It also shows the corresponding mean values ± SE on control cells. In both the exposed and control groups, the mean values of chromosomal aberrations in G_2 were significantly higher ($p < 0.0001$, one-tailed Z test) than the basal aberrations frequency detected in untreated lymphocytes (Table I).

The mean value of chromosomal aberrations in G_2 in the exposed group was higher than in the control group ($p < 0.0001$, one-tailed Z test). Nevertheless, the comparative analysis of the aberrations yield in G_2 in each exposed worker with the corresponding mean value of the control group showed that only 17 of the 35 exposed individuals exhibited a statistically significant increment in aberrations frequency ($p<0.05$, chi square test).

In 23 of 35 exposed workers with complete absorbed dose records, analysis of the relationship between the absorbed dose and chromosomal aberrations frequency in G_2 showed a correlation coefficient ($r = 0.448$) that was statistically significant ($p < 0.05$, Student's *t*-test) and a determination coefficient (r^2) of 0.20. No statistically significant correlation was detected when only chromosome types of aberrations (dicentric and ring chromosomes and chromosome breaks) were included.

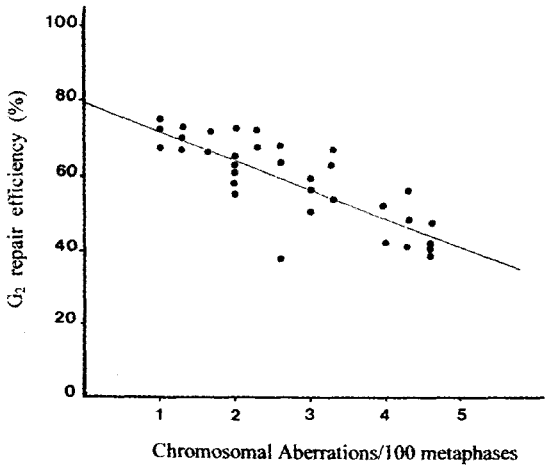

Figure 1 Correlation between the basal aberrations frequency and the G_2 repair efficiency, defined as the percentage of chromosomal aberrations repaired in G_2, in the sample of 35 exposed workers ($r = -0.803$; $p<0.001$, Student's *t* Test).

Table III summarizes the aberration yield in lymphocytes with and without caffeine plus 3-AB treatment during G_2, as well as records of age, years of employment, and the smoking habits of each exposed worker. It also shows the calculated G_2 repair efficiency (G_2 RE), defined as the percentage of chromosomal lesion repaired in G_2 for each exposed worker.

No statistically significant correlation was found between chromosomal aberrations yield (basal or in G_2) and age or years of employment ($r = 0.25$ and 0.2 respectively). As in G_2, the analysis of the effect of the smoking habit on the basal chromosomal aberrations yield showed no significant differences between smoking and non-smoking exposed workers (2.54 vs 2.85 and 6.48 vs. 6.46, mean values of the basal and G_2 aberration frequency, respectively).

The mean value of repair efficiency (G_2 RE) in the exposed group was lower (59%) than in the control group (72.3%) ($p < 0.001$ Student's *t* test). However, 13 of the 35 exposed individuals showed G_2 RE values included in the normal variation range, 11 of these latter 13 cases corresponded to exposed workers without a significant increase in the basal aberrations yield. In contrast, exposed individuals who exhibited a high basal aberration frequency (Codes: A-1; A-3; A-5; B-21; B-27; B-32 and B-35) also showed lower G_2 RE (48.1; 40.9; 39.1; 42.8; 41.6; 48.0 and 39.1 respectively).

The analysis of the relationship between G_2 RE and basal aberrations yield in the exposed group showed a linear response with a statistically significant correlation coefficient ($r = -0.802$) ($p < 0.001$, Student's *t* test). This negative value of the correlation would be expected since both variables included the basal aberrations yield. However, it should be noted that the actual correlation showed a wide range of G_2 RE for each value of the aberrations yield (Fig.

1). The determination coefficient calculated for the effect of G_2 RE ($r^2 = 0.644$) indicated that the 64.4% of the basal aberration yield variation detected in this sample of exposed workers was related to the individual G_2 repair efficiency.

DISCUSSION

Analysis of chromosomal aberrations in peripheral lymphocytes of workers occupationally exposed to low-levels of ionizing radiation have shown an increased

TABLE III

Comparison of the chromosomal aberrations yield in lymphocytes from exposed workers, with/without 5mM caffeine plus 3mM 3' aminobenzamide (3-AB) during G_2.

Subject	Age (years)	Employment	Smoking	Total dose[a]	Aberr. / 100 metaphases		RE
Code	/Sex	(years)	habit	(mSv)	Basal	+Caff. + 3-AB	(%)
A-1	51/M	19	+	58.68[a]	4.62*	8.91*	48.1
A-2	35/M	9	+	55.73[a]	3.30*	9.90*	66.7
A-3	42/M	19	-	16.06[a]	4.29*	7.26*	40.9
A-4	47/F	19	+	6.06[a]	1.98	4.62	57.1
A-5	36/M	7	-	41.16[a]	4.62*	7.59*	39.1
A-6	48/M	23	-	18.30[a]	4.29*	9.90*	56.7
A-7	43/M	22	+	3.78[a]	1.65	5.94	72.2
A-8	38/M	19	+	5.93[a]	1.65	4.95	66.7
A-9	53/M	21	-	9.13[a]	1.32	4.29	69.2
A-10	40/M	11	-	2.36[a]	0.99	3.63	72.7
A-11	44/M	19	+	23.18[a]	1.32	3.96	66.7
A-12	36/M	11	-	2.72[a]	1.98	4.95	60.0
A-13	38/M	16	-	3.69[a]	0.99	3.96	75.0
A-14	44/M	22	-	5.22[a]	3.96*	8.25*	52.0
A-15	44/M	18	-	24.03[a]	1.32	4.95	73.3
B-16	43/M	16	-	0.60[a]	3.30*	8.91*	63.0
B-17	38/F	15	+	0.00[b]	2.97*	7.26*	59.1
B-18	30/M	10	+	2.74[a]	1.98	5.28	62.5
B-19	40/M	20	-	2.49[b]	2.64*	7.26*	63.6
B-20	31/M	10	+	1.40[b]	1.98	4.62	57.1
B-21	49/F	18	-	0.63[b]	3.96*	6.93	42.8
B-22	21/F	3	-	0.15[b]	0.99	2.97	66.7
B-23	40/F	15	+	0.00[b]	2.64*	8.25*	68.0
B-24	44/F	15	+	0.60[a]	2.31	8.58*	73.0
B-25	37/F	18	-	0.30[b]	1.98	4.95	60.0
B-26	42/F	19	-	32.82[a]	3.30*	7.26*	54.5
B-27	37/M	18	-	1.17[b]	4.62*	7.92*	41.6
B-28	46/M	10	+	1.80[a]	2.64*	4.29	38.5
B-29	46/M	15	+	12.20[a]	1.98	5.61	64.7
B-30	47/M	4	-	40.30[a]	2.97*	6.93	57.1
B-31	24/M	4	-	0.25[b]	1.98	7.26*	72.7
B-32	37/F	18	+	0.35[b]	4.29*	8.25*	48.0
B-33	43/F	17	+	0.51[b]	2.97*	5.94	50.0
B-34	51/M	10	+	0.31[b]	2.31	7.26*	68.2
B-35	43/M	23	-	0.90[a]	4.62*	7.59*	39.1
Exposed group (35) X±SD	40.5±7	27.6±1.4	16(+)/19(-)	12.7±16.5	2.71±1.2	6.47±1.8	59.0±11[**]
Control group (30) X±SD	37.6±9	-	-	-	1.29±0.4	4.65±0.4	72.3±5.6

RE = Repair efficiency during G_2, defined as the percentage of chromosomal aberrations repaired in G_2 and calculated as was described in Material and Methods. (*) Significantly different from the mean value of the control group, ($p<0.05$; chi square test). (**) Student's *t* test. (a) Radiation dose absorbed during the last three years prior to the study. (b) Non-available or incomplete records of annual dose exposure. (+) Smoker (-) Non smoker.

frequency of chromosomal abnormalities (Evans *et al.*, 1979; Bauchinger *et al.*, 1980; Lloyd *et al.*, 1980; Léonard *et al.*, 1984; Maznik, 1987; Bigatti *et al.*, 1988; Jha and Sharma, 1991; Balasem *et al.*, 1992; Barquinero *et al.*, 1993; Braselmann *et al.*, 1994). In agreement with these studies, our results showed that the mean value of the basal aberrations yield in lymphocytes from workers exposed to low doses of X- or γ-rays was higher than controls (Table I). They also showed that in the exposed group, the mean value of chromosomal aberrations in G_2 detected in lymphocytes treated with caffeine plus 3AB was higher than controls (Table II). Thus, this would mean that in these exposed workers, the amount of chromosomal damage to be repaired in G_2, as well as that damage arriving unrepaired to mitosis (basal), should be higher than in controls.

Our results also showed that the frequency of chromosomal type of aberrations (dicentric and ring chromosomes and chromosome breaks) both basal as well as in G_2 were not correlated with the absorbed dose. Nevertheless, a significant correlation ($r = 0.4302$ and 0.448 respectively) was detected when chromatid breaks were included in the total aberrations yield. Under this latter condition, the determination coefficient (r^2), corresponding to the basal aberrations yield (0.18) and to the chromosomal damage in G_2 (0.20), indicated that about 20% of the aberration frequency variation may be explained by its linear relation to the absorbed dose.

Since *in vivo* exposure to low doses of radiation is a chronic event, the frequency of chromosomal damage detected in occupationally-exposed workers may also be affected by several other factors (Bender *et al.*, 1988). These include the low dose exposure (below 50 mSv), the uncertainty of dose estimation, the life-time of the lymphocytes, and other population variables such as age, years of employment, smoking habits, and alcohol consumption (Evans *et al.*, 1979; Lloyd *et al.*, 1980; Léonard *et al.*, 1984; Maznik, 1987; Tawn and Binks, 1989; Mayer *et al.*, 1989; Braselmann *et al.*, 1994; Lazutka and Dedonyte, 1995).

In relation to the effect of some of these factors, our results showed no significant correlations between age or years of employment and basal or G_2 chromosomal aberrations yield. However, the estimated determination coefficients (r^2) indicated that the age of the exposed worker could affect the aberrations yield by approximately 5% and the years of employment by about 7%. No significant effect was detected for smoking, as supported by other authors (Léonard *et al.*, 1984; Bigatti *et al.*, 1988; Barquinero *et al.*, 1993).

Another important factor that could influence the chromosomal aberration frequency in populations that are occupationally or environmentally exposed to low-level radiation may be the activation and efficiency of DNA repair mechanisms and the inter-individual differences in intrinsic radiosensitivity (Pohl-Rüling *et al.*, 1991; Barquinero *et al.*, 1993; Virsik-Peuckert *et al.*, 1997; Scott *et al.*, 1999).

DNA repair mechanisms are activated at the moment the irradiation occurs. The average repair time estimated for 60% of the double strand breaks is approximately 10 minutes, while the repair of the remaining DNA damage can take up to 2 hours (Holmerg and Gumauskas, 1985; Ward, 1991). However, in populations exposed to low-level radiation, the moment of the DNA damage induction cannot be specified, thus a correlation analysis between the activation of DNA repair mechanisms and chromosome damage would be difficult to carry out.

In relation to DNA repair efficiency, it is well known that the frequency of the chromosomal damage detected in mitosis depends on the efficiency of DNA repair mechanisms, operating in both proliferating (G_1, S, G_2) and in non-proliferating cells (G_0). Such efficiency depends on the amount of DNA damage to be repaired and the activity of the DNA repair mechanisms. This activity is determined by the availability of several molecules and gene products (multigenic pathways) involved in DNA repair pathways (Wallace, 1998;

Taylor and Lehmann, 1998). The amount of these latter elements may vary due to inter-individual differences and/or their request by the cells due to the amount and frequency of the induced DNA damage (Banáth *et al.*, 1998).

This means that factors such as low dose exposure, timing, and/or inter-individual genetic differences would have a share of the efficiency of DNA repair mechanisms which are responsible for the aberrations yield detected in workers exposed to low-level radiation. Thus, the estimation of the DNA repair efficiency, expressed as the percentage of chromosomal lesions repaired during one cell cycle phase, *i.e.*: G_2, may represent a measure of the influence that these factors may have on the frequency of chromosomal damage detected in individuals exposed to low-level ionizing radiation.

To this respect, our results are presented in Table III showing that in exposed workers, the mean value of DNA repair efficiency in G_2 (G_2 RE) was lower (59.0%) than in the control group (72.3%). They also showed that most exposed workers with high frequency of basal aberrations yield exhibited a low percentage of G_2 repair efficiency. Additionally, the analysis of the relationship between G_2 RE and the basal aberrations yield showed a significant inverse correlation coefficient ($r = -0.802$), which indicates that the higher the efficiency of DNA repair process is, the lower the frequency of chromosomal damage detected in mitosis is. Moreover, the determination coefficient calculated indicated that 64.4% of the variation on the basal aberrations yield would be explained by its lineal relation to the G_2 RE values. These results therefore support the assumption that the basal aberration frequency detected in lymphocytes from workers exposed to low-level radiation may be influenced by the variation in the efficiency of the DNA repair mechanisms among different individuals. They also indicate that the assessment of individual G_2 repair efficiency may represent important information to consider for avoiding occupational risk in workers exposed to low-level ionizing radiation.

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