G_2 repair and evaluation of the cytogenetic damage induced by low doses of X-irradiation during G_0 in human lymphocytes

JV PINCHEIRA[1], IH LOPEZ[2], GO TAPIA[1], MH NAVARRETE[3] and CC OYARZUN[2]

[1] Departamento de Biología Celular y Genética, Facultad de Medicina, Universidad de Chile, Santiago, Chile.
[2] Departamento de Seguridad Radiológica, Comisión Chilena de Energía Nuclear, Santiago, Chile.
[3] Departamento de Biología, Facultad de Ciencias, Universidad Autónoma, CSIC, Madrid, España

In the present study two cytogenetic parameters were used to evaluate the DNA damage induced by low doses (1 up to 40 rad) of X-ray irradiation in G_0 human lymphocytes. These parameters were the frequency of chromosomal lesions in G_2 and the length of this cell cycle phase.
The frequency of chromosomal lesions in G_2 was determined by scoring the number of chromosomal aberrations in G_0 irradiated lymphocytes post treated with two inhibitors of G_2 repair mechanisms : caffeine and 3-aminobenzamide. A dose-dependent increase in chromosomal aberrations yield was detected in G_0 lymphocytes X-ray irradiated with or without post treatment with these two DNA repair inhibitors during G_2. Nevertheless, the dose response in this latter condition was higher than the one detected in control cells, indicating that the increase of irradiation dose in G_0 lymphocytes produces an increment in the number of DNA lesions arriving to be repaired in G_2.
The analysis of the dose-response relationships for G_2 length showed an statistically significant X-ray dose-dependent increase (G_2 delay) from 2,5 up to 40 rad and a positive correlation between G_2 delay and the frequency of chromosomal lesions in G_2.
These results suggest that the DNA lesions induced by low doses of X-irradiation in G_0 lymphocytes may be higher than that detected by the standard method (control conditions) and may be responsible for an increase in G_2 length.
We propose, therefore, that an analysis of these two cytogenetic parameters can improve the evaluation of the DNA damage induced by low doses of X-rays irradiation in G_0 cells.

***Key terms**: chromosomal aberrations; G_2 duration; G_2 repair; human lymphocytes; X-irradiation at low doses.*

INTRODUCTION

Exposure of cells to ionizing radiations leads to the induction of a spectrum of lesions in DNA, including single and double strand breaks, DNA-DNA and DNA-protein crosslinks and damage to bases and sugar residues (reviewed in Ward, 1975; Hagen, 1990; Michalik, 1992).

Under normal conditions, most of this

Correspondence to: Dra Juana Pincheira, Departamento de Biología Celular y Genética, Casilla 70061, Santiago 7, CHILE. Fax: (56-2) 737-3158.

DNA damage will be removed by cellular repair mechanisms, but persistent or incorrectly repaired lesions may result in mutations, chromosome aberrations or cell death (Teoule, 1987; Bender *et al*, 1988; Swarts, 1990).

In human lymphocytes, the dependence of the yield of chromosome aberrations upon high doses of X- or γ-rays has been extensively studied (for references see Bender *et al*, 1988). However, experimental data concerning the cellular response to low doses (below 30 rad) are relatively scarce (Luchnik and Sevankaev, 1976; Takahashi *et al*, 1982; Pohl-Rüling *et al*, 1983; Fabry *et al*, 1985; Lefrancois *et al*, 1989).

The evaluation of genetic effects induced by low doses of ionizing radiation is a relevant aspect in radioprotection and biological dosimetry. However, differences in total chromosomal aberrations yield, as well as in the shape of the dose response curve for dicentric frequency have been reported. Such differences, have been related to an altered kinetics of aberration production due to DNA repair mechanisms activity (Takahashi *et al*, 1982; Pohl-Rüling *et al*, 1983).

In human and other mammalian cells, repair of DNA damage induced by ionizing radiation is accomplished by complex enzymatic repair systems (Tuschl *et al*, 1980; Bender and Moore, 1991; Bases *et al*, 1992), which eliminate the bulk of the DNA damage, so that only a fraction of it actually results in chromosomal aberrations. If these DNA repair mechanisms are inhibited following irradiation, a number of DNA lesions will remain unrepaired and consequently an increase of chromosome aberrations may be detected in metaphases (Kihlman and Natarajan, 1984; Palitti *et al*, 1984; Kihlman and Andersson, 1985).

In human lymphocytes, it has been demonstrated that the inhibition of G_2 repair mechanisms by caffeine or 3-aminobenzamide (3-AB) increases the chromosomal aberrations frequency induced by DNA damaging chemicals and radiations (Natarajan *et al*, 1982; Zwelling *et al*, 1982; Jelmert *et al*, 1992; Antoccia *et al*, 1992; Pincheira *et al*, 1993).

Therefore, the estimation of chromosomal aberrations yield in irradiated G_0 lymphocytes stimulated to proliferate and post treated with caffeine plus 3-AB during G_2 may result in an improvement of the evaluation of chromosomal damage induced by low doses of irradiation in G_0 cells.

Otherwise, it is well accepted that elimination of DNA damage requires not only functional repair mechanisms but, also, available time to carry out this process. It has been demonstrated that the exposure of mammalian cells to ionizing radiations leads to an inhibition or delay in the progression of cells through the cell cycle (Olivieri and Micheli, 1983; Bates and Lavin, 1989; Pincheira *et al*, 1991; Zaider and Minerbo, 1993). Such effect occurs at any stage of the irradiated cell cycle, and in addition to the irradiated stage, it always involves the G_2 phase (Mitchell *et al*, 1979; Zaider and Minerbo, 1993). The lengthening of G_2 (G_2 delay) allows time for DNA repair to proceed and may be correlated to the number of DNA lesions that should be repaired during this phase (Rao, 1980; Rowley and Leeper, 1985; Sabatier and Dutrillaux, 1988). Therefore, it can be expected that low doses of irradiation in G_0 might be able to induce a certain G_2 delay.

The present report proposes that measurements of chromosomal lesions in G_2 and length of this cell cycle phase may be used to improve the evaluation of cytogenetic damage induced by low doses of X-ray irradiation in human G_0 lymphocytes.

MATERIAL AND METHODS

Human subjects

The total sample included 30 healthy donors (20 males and 10 females, aged 18-48 years) from the Blood Bank of the Hospital JJ Aguirre Hospital.

Blood samples from 10 individuals were employed to analyse the effects of caffeine and 3-AB, either alone or in combination, upon chromosomal aberration frequency. To perform this analysis, 5 ml of blood sample were separated from each individual sample (20 ml) and used in control cultures. The remaining blood sample (15 ml) was irradiated at 40 rad and used to set up 4

TABLE I

Chromosomal aberrations frequency in human lymphocytes X-ray irradiated in G_0 (40 rad) and post-treated with caffeine (CAFF) and/or 3-aminobenzamide (3-AB) during G_2

TREATMENTS			ABERRATIONS / 100 METAPHASES						
X-RAY	CAFF	3-AB	NMA	ctb	csb	dic-r	gaps	T Aberr*	p <
–	–	–	1010	1.1	0.0	0.0	0.9	1.1 ± 0.33	
40 rad	–	–	1010	4.7	1.8	2.2	0.4	8.7 ± 0.88	
40 rad	5 mM	–	1010	6.2	3.0	2.1	0.6	11.3 ± 0.99	0.026
40 rad	–	3 mM	1010	4.9	1.3	3.0	0.1	9.1 ± 0.90	0.379
40 rad	5 mM	3 mM	1010	8.7	3.3	2.9	0.6	14.9 ± 1.20	0.001

NMA, number of metaphases analysed; **ctb**, chromatid breaks; **csb**, chromosome/isochromatid breaks; **dic-r**, dicentric plus centric ring chromosomes; **T Aberr**, total aberration yield.
Data from 10 experiments performed with blood samples obtained from 10 human subjects.
* Means ± SE of values obtained in the total analysed metaphases.
Effects of caffeine and 3-AB treatments, alone or in combination, on chromosomal aberrations yield assessed by one-tailed Z-tests.

lymphocytes cultures used in the analysis of the G_2 repair inhibitory effect of caffeine and 3-AB, as specified in Table I.

The blood samples obtained from the other 20 individuals were employed to perform the analysis of the effect of X-ray irradiation doses on chromosomal aberrations frequency and G_2 length, in lymphocyte cultures with or without caffeine plus 3-AB treatment during G_2. Each blood sample (40 ml) was split into 7 syringes (5 ml). One of them was used to set up 5 cultures corresponding to the controls (unirradiated lymphocytes) and the other 6 syringes were irradiated at 1, 2.5, 5, 10, 20 or 40 rad, and used to set up 30 lymphocytes cultures (5 cultures from each syringe).

X-ray irradiation and culture conditions.

Irradiation of whole blood samples were performed at 1, 2.5, 5, 10, 20 or 40 rad, using a Phillips X-ray machine (220 KV, 15 mA, 1.6 mm Cu filter, exposure 30 rad/min). The irradiation was carried out 2 h before starting cultures.

Each culture consisted in 0.5 ml of whole blood added to 5 ml of Ham's F-10 medium with 10% bovine serum and phytohaemagglutinin (Gibco), and was incubated for 48 h at 37°C. During the last 2 h of incubation, metaphase cells were blocked by colcemide addition at a final concentration of 5×10^{-7} M.

Post-irradiation treatments with caffeine and 3-AB.

Caffeine (Merck) and 3-aminobenzamide (Sigma) stock solutions were prepared in Ham's F-10 medium, filtered, sterilized and stored at 4°C. For treatments during G_2, 5 mM caffeine and 3 mM 3-AB (final concentration), alone or in combination, were added 2 h before harvesting.

Chromosome preparations and scoring

Air-dried preparations and Giemsa staining for chromosome analysis were carried out according to standard methods. In each experiment, a minimum of 100 metaphase plates from coded slides, were scored for unstable chromosomal aberrations, *i.e.*, chromatid and isochromatid breaks, dicentric and ring chromosome and gaps. The frequencies of this latter type of aberrations were excluded from the total chromosomal aberrations yield.

G_2 length estimation

The determination of G_2 length was carried out by the technique of autoradiography, in which an asynchronous population of cells was pulse labeled with a radioactive compound, such as tritiated thymidine ([^{3}H]dThd), and the percentage of mitosis was scored as a function of time (Mitchell *et al*, 1979).

The experimental procedure designed to analyse the dose effect of irradiation in the G_2 length of G_0 irradiated lymphocytes was the following : Whole blood cultures under control or X-ray irradiated conditions (1, 2.5, 5, 10, 20 or 40 rad) were treated with ([^{3}H]dThd) (specific activity 25 Ci/mmol, from the Radiochemical Center, Amersham, U.K.) to a final concentration of 1 µCi/ml of medium, during 3, 4, and 5 h before harvesting. In the last 2 h of incubation, cells in metaphase were blocked by colcemide (TC arresting solution, Difco) at a final concentration of 5 x 10^{-7} M.

Air dried preparations for autoradiography were dipped in NTB-2 nuclear track emulsion (Kodak), diluted 1:1 with distilled water, dried and placed in the dark at -20°C. After 4 days, the film was developed with Kodak D-19 developer, fixed with ultrarapid fixer (Kodak) and stained with 4% Giemsa. Scoring of labeled metaphases in a minimum of 300 metaphase plates from coded slides, corresponding to each culture, was carried out independently by 2 persons. To estimate the mean value of G_2 length, we considered the ([^{3}H]dThd) incubation time at which 50% of labeled metaphases were detected in each experiment.

Statistical analyses.

Since our interest was to test the effectivity of the inhibition of G_2 repair mechanisms by caffeine and 3-AB to detect the chromosome damage in G_2, the results obtained –under control or irradiated conditions– were expressed as mean ± standard errors (SE) of values obtained from the total number of metaphases analysed.

The effects of 5 mM caffeine and 3 mM 3-AB treatments, alone or in combination, on the total aberrations yield and on the frequency of each of the different types of aberrations scored –in control and in irradiated lymphocytes– were assessed by one tailed Z-tests.

Results corresponding to the analysis of the effect of X-ray irradiation dose on the G_2 length are presented as means ± SE of values obtained in the total analysed individuals. The statistical significance of the increase of G_2 length (G_2 delay) produced by the increasing of X-ray irradiation dose was assessed by linear correlation analysis and Student's *t*-test.

RESULTS

Effect of caffeine and 3-aminobenzamide (3-AB) on chromosomal aberrations frequency.

Table I shows the effect of 5 mM caffeine or 3 mM 3-AB treatments during G_2 on chromosomal aberrations yield in G_0 lymphocytes, X-ray irradiated at 40 rad. The frequency of chromosomal aberrations in lymphocytes treated with caffeine alone was higher than that detected in lymphocytes cultures post-treated with 3 mM 3-AB alone. However, the combined treatment with both inhibitors enhanced the frequency of the aberrations yield induced by the X-ray irradiation in G_0 ($p < 0.0001$), the increase in the number of chromatid breaks being the most pronounced effect.

Dose effect relationship in lymphocytes X-ray irradiated in G_0 and post-treated with caffeine and 3-AB during G_2

Table II summarizes the mean values of the various types of chromosomal aberrations detected in G_0 lymphocytes, X-ray irradiated from 1 to 40 rad (A) and post-treated with 5 mM caffeine plus 3 mM 3-AB during G_2 (B).

An X-ray dose-dependence of total aberrations yield was detected both in lymphocytes cultures treated with caffeine plus 3-AB, as well as in control conditions (untreated cultures). The average of total aberrations yield at each dose range in the former situation was higher than in the latter one ($p < 0.0001$). In both conditions, the increase in chromosomal damage was mainly given by the increase of chromatid breaks frequency, whereas at higher doses (10 to 40 rad) increases in chromosome-type aberrations (chromosome breaks, dicentric and centric rings) were also detected. The analysis of the dose-response relationship for the total aberrations yield, under control and treated conditions (Caff plus 3-AB), showed a good fit to a dose-response curve, where the chromosomal aberrations frequency were

TABLE II

Chromosomal aberrations frequency in human lymphocytes X-ray irradiated in G_0 (1 to 40 rad) and post-treated with 5 mM caffeine (CAFF) plus 3 mM 3-aminobenzamide (3-AB) during G_2

	X-RAY dose	ABERRATIONS / 100 METAPHASES						
	(rad)	NMA	Ab M	ctb	csb	dic-r	gaps	T Aberr*
A.	0.0	3036	1.2	1.20	0.03	0.03	0.15	1.3 ± 0.20
	1.0	2024	3.1	2.62	0.15	0.00	0.29	2.8 ± 0.36
CONTROL	2.5	2024	4.3	3.70	0.40	0.00	0.36	4.1 ± 0.45
CONDITIONS	5.0	2025	5.6	4.74	0.54	0.10	0.29	5.4 ± 0.50
	10.0	2023	6.9	5.10	1.19	0.19	0.39	6.5 ± 0.54
	20.0	2020	8.0	5.89	1.14	0.74	0.49	7.8 ± 0.60
	40.0	2970	9.8	6.28	1.85	2.10	0.60	10.2 ± 0.55
B.	0.0	2023	4.8	4.35*	0.30**	0.00	0.45	4.7 ± 0.47
	1.5	2020	6.4	5.64*	0.40	0.00	0.54	6.0 ± 0.53
+ CAFF	2.5	2026	8.1	6.91*	0.80**	0.00	0.60	7.7 ± 0.59
+ 3-AB	5.0	2023	9.2	8.26*	0.84	0.00	0.40	9.1 ± 0.63
TREATMENTS	10.0	2021	10.0	8.36*	1.43	0.30	0.40	10.1 ± 0.67
	20.0	2020	10.7	8.50*	1.75	1.25	0.30	11.5 ± 0.71
	40.0	2630	15.1	8.88*	2.80**	2.80**	0.60	14.5 ± 0.69

NMA, number of metaphases analysed; **Ab M**, abnormal metaphases; **ctb**, chromatid breaks; **cbs**, chromosomal/isochromatid breaks; **dic-r**, dicentric plus ring chromosomes; **T Aberr**, total chromosome aberrations; gaps excluded.
Data averaged from results obtained in blood samples from 20 human subjects.
* Means ± SE of values obtained in the total sample of metaphases.
Differences between control conditions *vs* caffeine plus 3-AB treatment at each X-ray irradiation dose: $p < 0.0001$ (one-tailed Z-test). Effect of inhibitors treatment on the frequency of each of the chromosomal aberrations scored. * $p < 0.0001$; ** $p < 0.05$ (one-tailed Z-tests).

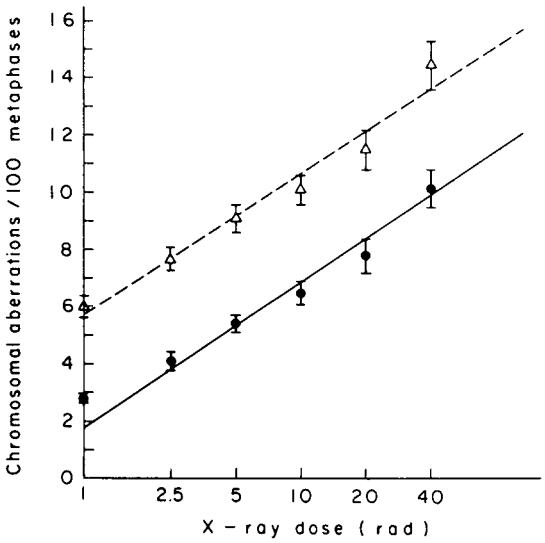

Fig 1. Percentage of total chromosomal aberrations yield plotted against logarithm of X-ray irradiation dose (rad) in G_0 irradiated lymphocytes under control conditions (filled circles) and post-treated with 5 mM caffeine plus 3 mM 3-AB during G_2 (open triangles). Continuous line, linear equation for control conditions [y = 2.24 + 1.582 x (log dose); r = 0:991; $p < 0.001$ (Student's *t*-test)]. Dashed line, linear equation for irradiated post-treated lymphocytes [y = 2.155 + 5.688 x (log dose); r = 0.982; $p < 0.001$ (Student's *t*-test)]. Vertical lines, standard errors.

plotted against the logarithm of the X-ray irradiation dose (Fig 1).

In relation to the effect of caffeine plus 3-AB treatment on the frequency of the different types of chromosomal aberrations (Table II B), the statistical analysis showed a significant increment in chromatid breaks frequency (ctb) in irradiated G_0 lymphocytes, post-treated with these inhibitors, at each of the assayed irradiation doses ($p < 0.0001$). The increase in the frequency of chromosome-type aberrations (csb and dic-r) was significant mainly at the higher doses studied ($p < 0.009$ and $p < 0.0465$, respectively). However, a p-value at the limit of significance was also detected for the csb increase at 2.5 rad ($p < 0.0495$).

G_2 length in lymphocytes X-ray irradiated in G_0

Table III shows the results corresponding to the mean values of G_2 length in lymphocytes under control conditions and X-ray irradiated

TABLE III

G_2 length (h) in human lymphocytes X-ray irradiated in G_0

X-RAY (rad)	INDIVIDUALS C-224	C-228	C-246	C-248	C-249	C-251	C-252	C-254	C-339	C-340	MEAN ± SE (h)
0	3.70	3.85	4.00	3.80	4.05	3.70	3.80	4.05	3.95	3.90	3.88 ± 0.04
1.0	3.80	3.95	4.05	3.90	4.15	3.80	3.90	4.15	4.02	3.95	3.97 ± 0.04
2.5	4.05	4.00	4.15	4.05	4.25	3.90	3.95	4.25	4.10	4.00	4.07 ± 0.04*
5.0	4.10	4.05	4.20	4.15	4.30	4.05	4.10	4.35	4.20	4.10	4.16 ± 0.02*
10.0	4.30	4.30	4.40	4.30	4.40	4.30	4.30	4.45	4.30	4.20	4.33 ± 0.02*
20.0	4.50	4.50	4.50	4.40	4.50	4.40	4.40	4.55	4.40	4.35	4.45 ± 0.02*
40.0	4.65	4.60	4.65	4.50	4.65	4.50	4.50	4.65	4.50	4.35	4.57 ± 0.03*

Means ± SE of values obtained from sample of 10 human subjects. * $p < 0.05$ (Student's *t*-test)
Correlation coefficient between X-ray dose and mean value of G_2 duration, $r = 0.9055$; $p < 0.001$ (Student's *t*-test).

in G_0 at 1 to 40 rad, in a sample of 10 healthy donors. In control conditions, some variation in G_2 length was detected among individuals. However, in irradiated cells, a dose-dependent increase of G_2 length (G_2 delay) was detected in each individual sample.

The statistical analysis of the influence of G_0 irradiation on the G_2 delay detected at each X-ray irradiation dose showed **p** values < 0.05 from 2.5 rad to 40 rad. The correlation coefficient between X-ray doses and average of G_2 length ($r = 0.9055$) was highly significant ($p < 0.001$) (Student's *t*-test).

In addition, assuming that the G_2 delay is related to the number of DNA lesions arriving to this cell cycle phase, we also analysed the relationship between G_2 length values and total chromosomal aberrations frequencies in G_2, detected by the inhibition of G_2 repair, at each X-ray irradiation dose. As pointed out in Figure 2, the mean G_2 length values (expressed in minutes) and the total aberrations yield in G_2, in X-ray irradiated lymphocytes at from 1 to 40 rad and post-treated with 5 mM caffeine plus 3 mM 3-AB, showed a linear relationship. The correlation coefficient ($r = 0.984$) was statistically significant ($p < 0.001$) (Student's *t*-test).

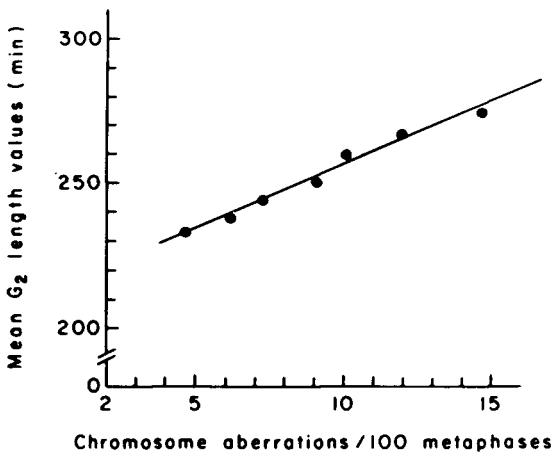

Fig 2. Correlation between mean values of G_2 length (minutes) and chromosomal aberrations in G_2. Total aberrations yield in irradiated lymphocytes post-treated with 5 mM caffeine plus 3 mM 3-AB (Table IIB). $r = 0.984$; $p < 0.001$; Student's *t*-test.

DISCUSSION

Results presented in Table I show that, in G_0 irradiated lymphocytes, 5 mM caffeine treatment during G_2 enhanced the frequency of chromosomal aberrations, whereas no significant increase of the aberrations yield was detected after 3 mM 3-AB treatment alone.

Potentiation effects of caffeine on the yield of induced chromosomal aberrations have been demonstrated by several authors, including our group (reviewed by Kihlman, 1977; Kihlman and Natarajan, 1984; Karsdon *et al*, 1989; Pincheira *et al*, 1994). These effects have been explained by: (i) interference with DNA-repair mechanisms and (ii) acceleration of the passage of cells blocked at G_2 to mitosis (Kihlman and Andersson, 1985; González-Fernández *et al*,

1985; Tanzarella *et al*, 1986; Pincheira *et al*, 1993).

In relation to 3-AB effects, it is generally accepted that 3-AB inhibits DNA repair by preventing the ligation of DNA strand breaks (Durkacs *et al*, 1980; Shall, 1994; Satoh and Lindahl, 1992) and by inducing a cell-cycle progression block at the late S/G_2 phase (Das, 1987). Thus, the mode of action of 3-AB may be explained by an stabilization of DNA damage in G_2.

The above effects would be responsible for a number of DNA strand breaks not detected as chromosomal aberrations in mitosis and hence may explain our results when the 3-AB treatment was given alone.

Results presented in Table I also show a pronounced enhancement of chromosomal aberrations after combined treatment with caffeine and 3-AB during G_2. According to Das *et al* (1984) and Das (1987), the modes of action of caffeine and 3-AB are different. Thus, the enhancement of the induced DNA damage by these two DNA repair modifiers would be due to the interaction of the effects produced by each inhibitor alone. It means that, when a combined treatment with 3-AB and caffeine is given, one effect produced by caffeine (reversion of G_2 delay) would dominate over the effect produced by 3-AB, and the lesions stabilized by 3-AB would be forced to go unrepaired to mitosis, increasing the chromosomal aberrations yield.

Therefore, results presented in Table I suggest that the inhibition of G_2 repair mechanisms by 5 mM caffeine plus 3 mM 3-AB might be used as a measurement of the DNA lesions arriving to be repaired in G_2 in lymphocytes X-ray irradiated in G_0.

Evaluation of DNA damage induced by low doses of X-ray irradiation in G_0 lymphocytes

It is generally assumed that the most adequate criterion to evaluate the cytogenetic damage induced by X-ray exposure in G_0 cells is the sum of dicentric and centric rings. However, as far as we know, this index has been proved to be adequate to evaluate DNA damage induced by irradiation doses higher than 30 rad, but not for lower exposures. Data reported by Luchnik and Sevankaev (1976) have shown that, while the frequency of dicentric and centric rings observed after higher doses was higher than that detected for other chromosome aberrations, this order is reversed and chromosome after lower doses (1 to 30 rad), where chromatid aberrations predominate.

Our results (Table IIA) are in agreement with those reported by the above mentioned authors. They show a dose-dependent increase in total aberrations yield from 1 to 40 rad, an increase which is mainly given by the enhancement of the chromosome and chromatid breaks frequency.

These results also suggest that low doses of X-rays would be more efficient in producing various chromosome damages, which would arise from DNA lesions induced in G_0. They would be carried unrepaired into the S phase, where some of them would be processed into chromatid-type aberrations via replicational errors.

Since a number of DNA lesions are normally repaired in the G_2 phase, it can be expected that increasing low doses of X-ray irradiation in G_0 would also be able to induce an enhancement of the chromosomal lesions arriving to be repaired in this cell cycle phase. This expectation was confirmed by our results (see Table IIB), where a dose-dependent increase in chromosomal aberrations yield can be observed in G_0 lymphocytes X-ray irradiated (1 to 40 rad) and post-treated with caffeine plus 3-AB during G_2. In addition, the dose-response relationship for total aberrations yield in lymphocytes –irradiated only or post-treated with caffeine plus 3-AB– showed that the calculated dose effect fits rather well with a linear response, under these two conditions (Fig 1). Thus, according to these results, the total chromosomal aberrations yield detected in lymphocytes post-treated with caffeine plus 3-AB during G_2 may represent a reliable criterion to evaluate part of the DNA damage induced in G_0 irradiated lymphocytes.

X-ray irradiation in G_0 and G_2 delay

Results presented in Table III show that the mean values of G_2 length increase with the X-ray irradiation dose. This increase was statistically significant at doses higher than 1 rad. The correlation coefficient resulting

from the analysis of the dose-effect relationship indicates that this effect fits rather well with a linear response. These results support our assumption that low X-ray irradiation doses (below 30 rad) are able to induce a certain G_2 delay.

Furthermore, our analysis of the relationship between G_2 length and chromosome aberrations frequency in G_2, detected by the inhibition of G_2 repair process, show a positive correlation (r = 0.984) at exposure doses from i to 40 rad (Fig 2). Since G_2 delay would represent the time necessary for chromosomal damage to be repaired before cell progression to mitosis (Painter and Young, 1980; Olivieri and Michely, 1983; Rowley, 1985; Sabatier and Dutrillaux, 1988), this positive correlation suggests that the increase in G_2 length detected in lymphocytes irradiated in G_0 may result from the increment of DNA lesions that should be repaired in this cell cycle phase.

Therefore, we propose that the analysis of chromosome aberrations yield in G_2 and the determination of G_2 length may improve the evaluation of the cytogenetic damage in G_0 cells exposed to low doses of X-ray irradiation.

ACKNOWLEDGEMENTS

This study was partially supported by projects CCHEN 120411201; FONDECYT 1030958; DGICYT PB94-0167; and N 1 of the "Acuerdo de Cooperación Científica y Tecnológica" between CSIC (España) and the University of Chile.

We thank Professors A Ramos and G Hoecker for facilities provided (use of an X-ray equipment). The authors also wish to thank the medical staff from the Blood Bank of the Hospital JJ Aguirre, for providing blood samples, and Mrs AM Palma and Mr H Coloma, for their technical assistance.

REFERENCES

ANTOCCIA A, PALITTI F, RAGGI T, CATENA C, TANZARELLA C (1992) The yield of fission neutron-induced chromatid aberrations in G2-stage human lymphocytes: effect of caffeine, hydroxyurea and cytosine arabinoside post-irradiation. Intl J Radiat Biol 62: 563-570

BASES R, FRANKLIN WA, MOY T, MENDEZ F (1992) Enhanced excision repair activity in mammalian cells after ionizing radiation. Intl J Radiat Biol 62: 427-441

BATES PR, LAVIN MF (1989) Comparison of γ-radiation-induced accumulation of ataxia telangiectasia and control cells in G_2 phase. Mutation Res 218: 165-170

BENDER MA, AWA AA, BROOKS AL, EVANS HJ, GROER PG, LITTLEFIELD LG, PEREIRA C, PRESTON RJ, WACHHOLZ BW (1988) Current status of cytogenetic procedures to detect and quantify previous exposure to radiation. Mutation Res 196: 103-159

BENDER MA, MOORE RC (1991) DNA polymerase mediates increase in exchange production by X-radiation in human lymphocytes moving from G_0 to G1. Mutation Res 250: 319-324

DAS SK (1987) Further evidence for a different mode of action of caffeine and benzamide on mammalian cells. Mutation Res 192: 69-72

DAS SK, LAU CC, PARDEE AB (1984) Comparative analysis of caffeine and 3- aminobenzamide as DNA repair inhibitors in Syrian baby hamster kidney cells. Mutation Res 131: 71-79

DURKACZ BW, AMIDIJI O, GRAY DA, SHALL S (1980) (ADP ribose)n participates in DNA excision repair. Nature (London) 283: 593-596

FABRY L, LEONARD A, WAMBERSIE A (1985) Induction of chromosome aberrations in G_0 human lymphocytes by low doses of ionizing radiations of different quality. Radiat Res 103: 122 - 134

GONZALEZ-FERNANDEZ A, HERNANDEZ P, LOPEZ-SAEZ JF (1985) Effect of caffeine and adenosine on G_2 repair: mitotic delay and chromosome damage. Mutation Res 149: 275-281

HAGEN U (1990) Molecular radiation biology: future aspects. Radiat Environ Biophys 29: 315-332

JELMERT O, HANSTEEN I, LANGARD S (1992) Enhanced cytogenetic detection of previous *in vivo* exposure to mutagens in human lymphocytes after treatment with inhibitors of DNA synthesis and DNA repair *in vitro*. Mutation Res 271: 289-198

KARSDON J, VAN RIJN J, BERGER H, NATARAJAN AT (1989) Increased frequency of spontaneous and X-ray induced chromosomal aberrations in lymphocytes from neonates and the influence of caffeine and *in vitro* study. Mutation Res 226: 13-19

KIHLMAN BA (1977) Caffeine and Chromosome. Amsterdam: Elsevier

KIHLMAN BA, NATARAJAN AT (1984) Potentiation of chromosomal alterations by inhibitors of DNA repair. In: COLLINS A, DOWNES CS, JOHNSON RT (eds) DNA repair and its inhibition. Nucleic Acid Symposium, Ser 13. Lancaster: IRL Press. pp 319-339

KIHLMAN BA, ANDERSSON HC (1985) Synergistic enhancement of the frequency of chromatid aberrations in cultured human lymphocytes by combinations of inhibitors of DNA repair. Mutation Res 150: 313-325

LUCHNIK NV, SEVANKAEV AV (1976) Radiation induced chromosomal aberrations in human lymphocytes. I. Dependence on the dose of gamma-rays and an anomaly at low doses. Mutation Res 36: 363-378

LEFRANCOIS D, AL ACHKAR W, AURIAS A, COUTURIER J, DUTRILLAUX AM, DUTRILLAUX B, FLURY-HERARD A, GERBAULT-SEREAU M, HOFFSCHIR F, LAMOLIATTE E, LOMBARD M, MULERIS M, PRIEUR M, RICOUL M, SABATIER L, VIEGAS-PEQUIGNOT E (1989) Chromosomal aberrations induced by low-dose - irradiation. Study of

R-banded chromosomes of human lymphocytes. Mutation Res 212: 167-172

MICHALIK (1992) Model of DNA damage induced by radiation of various qualities. Intl J Radiat Biol 62: 9-20

MITCHELL JB, BEDFORD JS, BAILEY SM (1979) Dose - rate effects on the cell cycle and survival of S3 Hela cells. Radiat Res 79: 520-536

NATARAJAN AT, CSUKAS I, DEGRASSI F, VAN ZEELAND AA (1982) Influence of inhibition of repair enzymes on the induction of chromosomal aberrations by physical and chemical agents. Progr Mutation Res 4: 47-59

OLIVIERI G, MICHELI A (1983) Mitotic delay and repair in human lymphocytes. Mutation Res 122: 65-72

PAINTER RB, YOUNG BR (1980) Radiosensitivity in ataxia telangiectasia: A new explanation. Proc Natl Acad Sci USA 77: 7315-7317

PALITTI F, DEGRASSI F, DE SALVIA R, TANZARELLA C, FIORE M (1984) Potentiation of induced sister chromatid exchanges and chromatid-type aberrations by inhibitors of DNA synthesis and repair in G_2. In: TICE RR, HOLLAENDER A (eds) Sister chromatid exchanges: 25 years of experimental Research. New York: Plenum Press. pp 313-318

PINCHEIRA J, LOPEZ-SAEZ JF (1991) Effect of caffeine and cycloheximide during G_2 prophase in control and X-ray irradiated human lymphocytes. Mutation Res 251: 71-77

PINCHEIRA J, GALLO C, BRAVO M, NAVARRETE MH, LOPEZ-SAEZ JF (1993) G_2 repair and aging: influence of donor age on chromosomal aberrations in human lymphocytes. Mutation Res 295: 55-62

PINCHEIRA J, RODRIGUEZ M, BRAVO M, NAVARRETE MH, LOPEZ-SAEZ JF (1994) Defective G_2 repair in Down syndrome: effect of caffeine, adenosine and niacinamide in control and X-ray irradiated lymphocytes. Clin Genet 45: 25-31

POHL-RULLING J, FISCHER P, HAAS O, OBE G, NATARAJAN AT, VAN BUUL PPW, BUCKTON KE, BIANCHI NO, LARRAMENDY M, KUCEROVA M, POLIKOVA Z, LEONARD A, FABRY L, PALITTI S, SHARMA T, BINDER W, MUKHERJEE RN, MUKHERJEE U (1983) Effect of low-dose acute X-irradiation on the frequencies of chromosomal aberrations in human peripheral lymphocytes *in vitro*. Mutation Res 110: 71-82

RAO PN (1980) The molecular basis of drug-induced G_2 arrest in mammalian cells. Mol Cell Biochem 29: 47-57

ROWLEY R (1985) Is G_2-arrest and active cellular response to irradiation? Intl J Radiat Biol 48: 811-820

ROWLEY R, LEEPER DB (1985) Cell cycle age dependence for radiation-induced G_2 arrest: Evidence for time dependent repair. Radiat Res 103: 326-336

SABATIER L, DUTRILLAUX B (1988) Effect of caffeine in Fanconi anemia. I. Restoration of a normal duration of G_2 phase. Hum Genet 79: 242-244

SATOH MS, LINDAHL T (1992) Role of poly (ADP ribose) formation in DNA repair. Nature 356: 356-358

SHALL S (1984) Inhibition of DNA repair by inhibitors of nuclear ADP ribosyl transferase. In: COLLINS A, DOWNES CS, JOHNSON RT (eds) DNA repair and its inhibition. Nuclei Acid Symposium, Ser 13. Lancaster: IRL Press. pp 143-191

SWARTS SG, NELSON GB, WALLEN CA, WHEELEN KT (1990) Radiation-induced cytotoxicity, DNA damage and DNA repair: implications for cell survival theory. Radiat Environ Biophys 29: 93-102

TAKAHASHI E, HIRAI M, TOBARI I, UTSUGI T, NAKAI S (1982) Radiation-induced chromosome aberrations in lymphocytes from man and crab-eating monkey. Mutation Res 94: 115-123

TANZARELLA C, DE SALVIA R, DEGRASSI F, PALITTI F, ANDERSSON HC, HANSSON K, KIHLMAN BA (1986) Effect of post-treatment with caffeine during G_2 on the frequencies of chromosome-type aberrations produced by X-rays in human lymphocytes during G_0 and G_1. Mutagenesis 1: 41-44

TEOULE R (1987) Radiation-induced DNA damage and its repair. Intl J Radiat Biol 51: 573-589

TUSCHL H, ALTMANN H, KOVAC R, TOPALOGLOU A, EGG D, GUNTHER R (1980) Effects of low-dose radiation on repair processes in human lymphocytes. Radiat Res 81: 1-9

WARD JF (1975) Molecular mechanisms of radiation-induced damage to nucleic acid. In: LETT JT, ADLER HI (eds) Advances in Radiation Biology. Vol 5. New YorK: Academic Press. pp 182-239

ZAIDER M, MINERBO GN (1993) A mathematical model for cell cycle progression under continuous low-dose-rate irradiation. Radiat Res 133: 20-26

ZWELLING LA, KERRIGAN D, POMMIER Y (1982) Inhibitors of (poly-adenosine diphosphoribose) synthesis slow the resealing rate of X-ray-induced DNA strand breaks. Biochem Biophys Res Commun 104: 897-902