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Clinical report

Cytogenetic analysis in peripheral lymphocytes of cancer patients treated with cytostatic drugs: results from an EC Collaborative Study

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Many of the cytostatic drugs commonly used in cancer chemotherapy treatments have been shown to be genotoxic *in vivo* and *in vitro*. We present a cytogenetic collaborative study on 13 cancer patients treated with different antitumor agents. For comparison we also carried out a cytogenetic analysis on 14 healthy untreated controls. The frequency of sister chromatid exchanges and structural chromosome aberrations in peripheral blood lymphocytes of the cancer patients was determined prior to the treatment, just after it and 3–7 weeks later. The results obtained show clear differences between the basal levels of cytogenetic alterations in cancer patients, even though the mean value is higher in this group than the basal levels of the group of healthy individuals. Treatment with cytostatics increases the frequency of both cytogenetic biomarkers analyzed, which declined to values similar to those initially observed several weeks after the treatment. Our data are in qualitative and quantitative agreement with other results previously found by other authors.

Key words: Cancer patients, chromosomal aberrations, cytostatic treatment, human lymphocytes, sister chromatid exchanges.

Introduction

It is well known that many of the antineoplastic drugs commonly used in cancer therapy have mutagenic and carcinogenic properties, and, as a consequence, their use can cause cytogenetic damage in cancer patients.[1–4] A significant number of cancer patients treated with such agents develop secondary malignancies unrelated to the original neoplasia[5,6] and these secondary processes may be a consequence of the genetic damage caused by the chemotherapeutic treatment.

The genotoxic risk of antineoplastic agents has been detected both in patients undergoing chemotherapeutic treatment,[7–10] and in personnel occupationally exposed in industry and hospitals.[3,11–13] This genotoxicity has been evaluated on the basis of increased levels of mutagens in the urine of exposed persons or increased frequencies of cytogenetic damage.

Cytogenetic methods with human peripheral lymphocytes have been extensively used in biomonitoring studies of human populations exposed to genotoxic agents. Both chromosomal aberrations (CA) and sister chromatid exchanges (SCE) are commonly used assays for biomonitoring human populations exposed to genotoxic environmental chemicals. The first EC Research Program on the Biomonitoring of Human Populations had, among its aims, the calibration of cytogenetic biomarkers between laboratories, and its relation to molecular damage detection techniques such as DNA and protein adducts. One of the needs was to establish collaborative studies between different laboratories to harmonize protocols and scoring criteria. In this context, we present the results obtained in a cytogenetic study carried out with the participation of three European laboratories on the genotoxic effects that treatments with different cytostatic drugs have on the peripheral blood lymphocytes of cancer treated patients as well as its persitence over time. The genetic endpoints recorded have been CA and SCE. Lymphocyte cultures were set up with blood from the different donors collected before chemotherapy, just after treatment and several weeks after the last treatment.

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Materials and Methods

Population

The study was performed with 13 cancer patients treated with different antineoplastic agents at Cardiff and/or Swansea Hospital (UK). Eight individuals were treated with 10 mg/day of melphalan for 5 days (myeloma patients: codes ET, AP, AT, DJ, JA, SR, HS and TH), two were treated with 20 mg/day of chlorambucil for 3 days (CCL patient: code GD; and myeloma variant: code RF), two received a treatment of cisplatin (ovarian cancer: EH and KF), and only one patient (code ML) received a simultaneous treatment with bleomycin, etoposide and cisplatin.

Lymphocyte cultures were also made from 14 healthy donors used as reference controls.

Blood samples

Heparinized blood samples were obtained from the 13 cancer patients before the treatments (pre-treatment sample), just after the treatment (post-treatment) and 3–7 weeks later (follow-up sample). The pre-treatment sample of each individual acts as its own control.

In the case of the control group, only one blood sample was obtained.

Cell cultures

Cell cultures were set up at Swansea, UK, where the slides were mounted, and scored at Bellaterra, Spain (CA) and Patras, Greece (SCE). The protocols used are as follows.

CA. Cultures were set up by adding 0.8 ml of whole blood to 10 ml of chromosome medium (RPMI) containing 20% fetal calf serum, antibiotics and L-glutamine. Lymphocytes were stimulated by 1.5% phytohemagglutinin. Cultures were incubated at 37°C for 48 h. Colcemid was added 2 h before fixation to arrest cells in metaphase. Cells were collected by centrifugation, resuspended in hypotonic solution (KCl 0.56%) for 15–20 min at 37°C and fixed in 3:1 methanol:glacial acetic acid. Slides were air dried and stained with Giemsa.

One hundred metaphases were scored, whenever possible, for each donor on coded slides.

SCE. Cultures were set up by adding 0.8 ml of whole blood to 10 ml of chromosome medium (RPMI) containing 20% fetal calf serum, 24 μM BrdUrd, antibiotics and L-glutamine. Lymphocytes were stimulated by 1.5% phytohemagglutinin. Cultures were incubated at 37°C in complete darkness for 72 h. Colcemid was added 2 h before fixation to arrest cells in metaphase. Cells were collected by centrifugation, resuspended in hypotonic solution (KCl 0.56%) for 15–20 min at 37°C and fixed in 3:1 methanol:glacial acetic acid. Slides were air dried and stained for 15 min in a solution of 0.5 mg Hoechst 33258 per ml in Sorensen's buffer pH 6.8. The slides were then washed, dried, mounted with the buffer under a coverslip and exposed to daylight for 48 h. The slides were then stained for 10 min in a 3% Giemsa solution made in the Sorensen's buffer.

Statistical analysis

Differences between groups were evaluated by the Mann–Whitney *U*-test for CA and Student's *t*-test for SCE. The χ^2 test was used to compare the number of cells with aberrations within individuals. The comparison of each individual sample over time was performed using the Wilcoxon matched pairs test for CA and the *t*-test for the dependent samples for SCE.

Results and discussion

The cytogenetic results obtained in the present study are indicated in Table 1 (cancer patients) and in Table 2 (healthy controls). In some cases the cytogenetic study was not able to be carried out in all three samples due to the low stimulation of the cultured lymphocytes.

From these values the existence of a wide variability before treatment in the baseline frequency of CA among cancer patients is evident, their mean value being much higher than in the control group. Thus, the control population presents a mean frequency ($\pm$SE) of 2.34 ± 0.05 aberrations/100 cells (range from 0 to 6), while the baseline frequency in the group of cancer patients was statistically higher ($p < 0.001$, *U*-test) showing 12.27 ± 2.74 aberrations/100 cells (range from 0 to 46). This wide variability in the CA frequency between cancer patients has been reported by several authors[14] and increased CA frequencies before treatments have been reported for different patients with different types of tumors,[15,16] and have been related to non-specific chemotherapy or diagnostic X-ray irradiation.

Table 1. Cytogenetic biomarkers in cancer patients

Code	Sex	Age (years)	Sample	CA					SCE	
				no. of cells	chromatid type	chromosome type	aberrations(%)	cells with aberrations(%)	no. of cells	SCE/cell (mean ± SE)
ET	woman	72	pre-	74	3	6	12.2	6.7	25	14.00 ± 1.38
			post-	100	6	3	9.0	7.0	18	18.61 ± 1.48[b]
			follow-up	—	—	—	—	—	25	18.68 ± 1.32[b]
AP	man	67	pre-	100	2	0	2.0	2.0	25	13.88 ± 1.30
			post-	200	12	10	11.0	8.0	25	17.60 ± 1.08[b]
			follow-up	26	3	1	15.4	15.4[a]	25	14.00 ± 1.45
AT	man	75	pre-	100	2	2	4.0	3.0	25	12.56 ± 1.05
			post-	100	14	4	18.0	15.0[a]	25	19.24 ± 1.18[b]
			follow-up	100	3	3	6.0	5.0	25	14.36 ± 1.18[b]
DJ	woman	65	pre-	100	4	1	5.0	5.0	25	10.76 ± 0.84
			post-	100	4	1	5.0	5.0	17	21.23 ± 1.18[b]
JA	man	73	pre-	76	4	2	8.0	8.0	25	17.88 ± 1.27
			post-	27	0	1	3.7	3.7	—	—
			follow-up	34	2	0	5.9	5.9	25	19.69 ± 1.52
SR	woman	75	pre-	100	10	23	33.0	24.0	25	24.68 ± 1.57
			post-	56	10	16	46.4	30.3	—	—
			follow-up	—	—	—	—	—	25	28.36 ± 1.80
HS	man	70	pre-	100	9	6	15.0	12.0	25	25.68 ± 1.74
			post-	85	10	9	22.3	17.6	—	—
			follow-up	100	6	3	9.0	8.0	25	24.04 ± 1.45
TH	man	76	pre-	129	3	9	9.3	7.7	25	17.68 ± 1.08
			post-	100	7	2	9.0	8.0	25	22.88 ± 1.27[b]
			follow-up	100	3	2	5.0	5.0	25	17.92 ± 1.11
GD	man	54	pre-	87	5	2	8.0	8.0	23	7.91 ± 0.63
			post-	87	3	2	5.7	4.6	—	—
			follow-up	35	4	3	20.0	16.7	—	—
RF	man	65	pre-	100	4	7	11.0	9.0	25	15.80 ± 0.94
			post-	—	—	—	—	—	25	15.72 ± 0.69
			follow-up	100	1	0	1.0	1.0	25	15.80 ± 1.12
EH	woman	71	pre-	100	3	26	29.0	19.0	25	10.72 ± 0.72
			post-	100	6	39	45.0	27.0	25	26.60 ± 1.16[b]
			follow-up	100	1	45	46.0	26.0	25	12.04 ± 0.56
KF	woman	72	pre-	—	—	—	—	—	25	7.56 ± 0.69
			post-	31	0	0	0.0	0.0	25	16.20 ± 0.85[b]
			follow-up	100	3	1	4.0	4.0	25	12.92 ± 0.95[b]
ML	man	48	pre-	100	4	7	11.0	7.0	25	9.88 ± 0.48
			post-	—	—	—	—	—	25	19.28 ± 0.66[b]
			follow-up	100	5	4	9.0	9.0	25	15.96 ± 0.80[b]
Mean ± SE			pre-		4.61 ± 0.74	7.67 ± 2.41	12.27 ± 2.74	10.28 ± 2.48		14.54 ± 1.60
			post-		6.91 ± 1.68	9.01 ± 3.85	15.92 ± 4.83	11.74 ± 2.94		19.71 ± 1.14
			follow-up		5.08 ± 1.20	7.04 ± 4.29	12.13 ± 4.16	9.70 ± 2.40		17.61 ± 1.49

We calculated the total of aberrations by assuming that one exchange implies two breaks
[a] $P < 0.05$, χ^2.
[b] $P < 0.05$, t-test.

Table 2. Cytogenetic biomarkers in control donors

Code	Sex	Age (years)	CA					SCE	
			no. of cells	Chromatid type	chromosome type	aberrations (%)	cells with aberrations (%)	no. of cells	SCE/cell (mean ± SE)
AOHOM	man	56	100	1	1	2.0	2.0	25	9.00 ± 0.59
AAPAP	woman	50	100	0	3	3.0	2.0	25	8.56 ± 0.67
ANMNL	man	28	100	0	1	1.0	1.0	25	8.76 ± 0.66
ANOCH	man	38	100	1	0	1.0	1.0	25	11.20 ± 0.75
ANDCE	man	33	100	0	1	1.0	1.0	25	11.44 ± 0.75
ASDSP	man	41	100	0	0	0.0	0.0	25	12.00 ± 0.74
MEO	woman	45	70	1	1	2.8	2.8	25	12.68 ± 0.75
EM	woman	26	100	0	0	0.0	0.0	25	16.40 ± 0.83
KD	woman	23	100	4	2	6.0	5.0	25	10.96 ± 0.79
SSWSS	woman	62	100	3	2	5.0	5.0	25	10.84 ± 0.69
MDCCI	man	47	100	3	1	4.0	4.0	25	9.80 ± 0.61
MAEAM	man	45	100	3	1	4.0	4.0	25	12.24 ± 0.85
AF	woman	35	100	2	0	2.0	2.0	25	11.28 ± 0.74
MB	woman	50	100	0	1	1.0	1.0	25	12.04 ± 0.73
Mean ± SE				1.32 ± 0.38	1.03 ± 0.24	2.34 ± 0.50	2.20 ± 0.46		11.23 ± 0.53

We calculated the total of aberrations by assuming that one exchange implies two breaks

The study of SCE in the blood lymphocytes of the same patients reveals a similar patern to that observed for CA. Thus, in the sample taken before the treatment, a wide variability in SCE frequency was detected and these values are higher than those observed in the group of controls where a mean of 11.23 ± 0.53 SCE/cell was found.

The analysis of the CA frequency just after treatment, and also in the follow-up, shows a wide variability and, when these values were compared with their own control (pre-treatment), no specific patern was observed, since increases and decreases were detected in CA frequencies among patients for both post-treatment samples. Nevertheless, a tendency to obtain higher values was detected as measured in the mean frequency that increases just after treatment. Such increases in the CA frequency related to chemotherapy treatment have also been reported previously.[10,14,17-18]

The study of the persistence of CA several weeks after the last treatment indicated a tendency to lower values observed just after treatment. This decline reached a mean value similar to that observed in the pre-treatment. Recovery of induced CA in lymphocytes of cancer patients after the cessation of chemotherapy has been observed,[16,19] although long-term increases in CA frequency have also been reported.[20-22]

In a similar way, the analysis of the SCE frequency just after the treatment indicates a significant increase in the SCE values that reaches a mean of 19.71 ± 1.14 SCE/cell. For this cytogenetic endpoint, this increase was observed in cultures of all the patients scored. Other authors have previously reported increased frequencies of SCE baseline in peripheral blood lymphocytes of patients receiving anticancer therapy.[14,23-25]

The analysis of the SCE frequency several weeks after treatment (follow-up study) indicates that SCE levels declined to 17.61 ± 1.49, but were still higher than those observed before treatment. Decline to SCE baseline has been demonstrated previously[25,26] and it may be the result of repair of induced lesions, replacement of damaged lymphocytes from a pool of imbalanced precursors or a combination of processes.[25]

Taking into account the high variability observed among patients, the statistical analysis to evaluate the effects of the treatment and its persistence was only done with those individuals from whom the three blood samples were available, in which a study of matched values was carried out. In this study, the CA values ($n = 7$) obtained were 10.76 (pre-), 16.38 (post-) and 15.33 (follow-up), thus, although it shows the tendency previously indicated, the observed differences are not significant. Nevertheless, in the SCE study ($n = 8$), although similar paterns were observed, i.e. 12.76 (pre-), 19.52 (post-) and 15.21 (follow-up), all these differences were statistically significant.

The increases in the frequency of the genetic end-points analyzed confirms data reported previously by other authors for this type of genetic damage or for micronuclei in binucleated peripheral blood lymphocytes[22] and oral cavity cells.[10] Nevertheless, a lack of response was obtained when micronuclei in hair root cells[10] or mutation induction at the *hprt* locus of peripheral lymphocytes[27] were used as genetic end-points. The decrease in the frequency of genetic damage detected in the follow-up study could probably be due to the elimination of the damaged cells, dilution into the bloodstream or the inability of these cells to complete mitotic division; although types and mechanisms of action of the chemotherapeutic agents, their doses, sampling time, cell kinetics and the individual sensitivity are factors to have in mind to explain this decline. When comparing the CA and SCE values individually, no significant correlation was found ($p = 0.15$), indicating that different mechanisms act on the induction of such biomarkers of genetic damage.

From this study two conclusions can be observed. On one hand, the importance of the longitudinal design of particular populations. Therefore, in the study of individuals suffering from specific diseases and treated with chemical compounds, such as cytostatics, the possible existence of increased basal frequencies as a consequence of the illness should be taken into account. On the other hand, the fact that the alterations induced by the treatments can decline over time could modify the results of a defined study, according to when the follow-up sample was obtained.

In spite of the general decrease observed following the cessation of chemotherapy, this study has shown increases in the frequency of genetic damage induced by antineoplastic drugs, suggesting the existence of an increased risk of the appearance of second malignancies related to chemotherapeutic treatment, as has been previously reported.[28]

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