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Short report

Sister chromatid exchange studies for monitoring DNA damage in lymphocytes of malignant lymphoma patients under cytostatic therapy

Semra Şardaş[1], Feriha Erdoğan,[2] Orhan Seyfi Şardaş,[2] Mustafa Çengel[3] and Ali Esat Karakaya[1]

[1]Department of Toxicology, Gazi University, Faculty of Pharmacy, 06330, Ankara, Turkey. Fax: (+90) 312 223 5018. [2]Department of Haematology and Oncology, Ankara University, Faculty of Medicine, Ankara, Turkey. [3]Turkish State Railways Hospital, Ankara, Turkey.

Sister chromatid exchange (SCE) frequencies were studied in lymphocytes from 45 patients with malignant lymphoma. Fifteen patients were untreated when studied. The mean SCE frequency for these patients was 8.70 ± 0.99 per mitosis. The mean score for 35 controls was 4.37 ± 1.19. SCE mean scores were significantly higher in the untreated patients than in the controls ($p < 0.001$). Nine patients were treated with radiotherapy alone. The mean SCE frequency (6.80 ± 0.87) they demonstrated was significantly lower ($p < 0.01$) than that found in untreated patients. Twelve patients received cyclophosphamide 1 month before the study was started. They demonstrated a mean SCE frequency (12.00 ± 1.31) significantly higher ($p < 0.05$) than that found in patients who had received regimens that did not contain cyclophosphamide (9.72 ± 1.32). From these findings we suggest that untreated patients with malignant lymphoma have elevated SCE frequencies, which may be further increased by chemotherapeutic agents.

Key words: Cytostatic drugs, malignant lymphoma, sister chromatid exchanges.

Introduction

The association of chromosomal structural changes with the development, and in some cases apparently the initiation, of neoplasms has stimulated studies on sister chromatid exchange (SCE) incidence. These studies have been particularly done in leukemias and lymphomas, where relatively ready access is available to proliferating materials that can be cultured over two cell cycles to give harlequin-stained chromosomes for SCE analysis. In addition, many cytostatic drugs used in cancer chemotherapy cause chromosomal damage in lymphocytes of patients.[1,2] This human chromosomal damage can be detected in the form of structural abberations, SCE or micronuclei in cultured lymphocytes. Frequencies of SCE significantly higher than in controls were found in patients under treatment with cytostatic agents.[3,4] Several studies have reported health hazards associated with professional exposure to these drugs;[5,6] however, these observations were not confirmed by others.[7,8]

The inconsistency may depend on differences in the individual pattern of exposure and on the role of other agents potentially able to induce a similar effect, i.e. smoking, drinking, dietary habits, chemicals and radiations.[9,10] The objective of this study was to determine the SCE frequencies in lymphocytes from newly diagnosed, untreated adults with malignant lymphoma and from similar patients exposed to radiotherapy or intensive chemotherapy, compared with unexposed controls.

Materials and methods

Materials

Peripheral lymphocytes were examined from 45 patients. Of these, 19 were with Hodgkin's disease and 26 were with non-Hodgkin's lymphoma (14 nodular, 12 diffuse). Patients and controls did not differ in age (31.5 mean age). Fifteen patients were evaluated at diagnosis. Nine were studied 6 weeks after termination of radiotherapy since response to the test is severely reduced in peripheral lymphocytes immediately following irradiation. Twelve patients were studied after cyclophosphamide chemotherapy and nine patients were studied after different chemotherapy regimens (adriamycin, bleomycin, CCNU regimens).

Correspondence to S Şardaş

In addition, peripheral lymphocytes were studied from 35 controls who had no current or underlying disease and no viral illness during the preceding 12 months. All subjects were given a detailed questionnaire to provide as much information as possible about the factors that may potentially confound the analysis of SCE. Smoking is perhaps the most important confounding factor in the interpretation of SCE frequencies. Among patients, only seven were ex-smokers and consumed less than 5 cigarettes/day and stopped smoking at the start of their diagnosis. For our purposes, subjects consuming less than 5 cigarettes/day were considered to be non-smokers and therefore all controls were selected from non-smokers.

SCE analysis

Venous blood from controls and patients was drawn into heparinized tubes from each subject and placed in culture on the same day. The culture medium contained TC 199 Medium (Gibco, Grand Island, NY) supplemented with 20% fetal calf serum (Gibco), 2% phytohemaglutinin (Sigma, St Louis, MO), 5 μg/ml 5 bromodeoxyuridine (Sigma), 150 U/ml penicillin and 150 μg/ml streptomycin. The cultures were incubated in the dark at 37°C for 72 h. During the last 3 h of incubation, 0.5 μg/ml colchicine (Gibco) was added to the culture. Microscope slides were prepared by a conventional method and stained by the fluorescence plus Giemsa technique of Perry and Wolff.[11]

SCEs were analyzed in 30 cells containing 46 chromosomes in each preparation, and the mean SCE frequency was calculated as SCEs per cell of each subject. SCEs were scored by two independent investigators.

Results

Table 1 shows the mean number of SCE/cell and the standard deviation (SD) of the total exposed and control subjects. There is a significant difference ($p < 0.001$) in the SCE frequencies between the 35 controls (4.37 ± 1.19) and the 15 untreated patients with malignant lymphoma (8.72 ± 0.99). The data were analyzed by Wilcoxon–Mann–Whitney tests.

The mean SCE score of the total 30 treated patients (9.50 ± 0.3) was similar to that of untreated patients. When the influence of the type of treatment on the mean SCE score was examined, SCE scores in patients who had received radiotherapy were lower ($p < 0.01$) than those in untreated patients. SCE scores in 21 patients who had recent exposure to chemotherapy (1 month) were close (10.86 ± 2.1) to those in untreated patients. When the influence of specific chemotherapy regimens was evaluated (Table 1), 12 patients who were on daily cyclophosphamide treatment had apparently elevated SCE scores (12.00 ± 1.31). All nine patients treated with adriamycin, bleomycin, CCNU regimens had SCE scores (9.72 ± 1.32). In these patients, the SCE score was higher than in untreated patients (9.72 versus 8.72), but the difference was not significant ($p < 0.15$).

Table 1. Mean number of SCE/cell among patients with lymphoma and controls

Parameters	No. of subjects studied	Mean SCE ± SD
Untreated lymphoma patients	15	8.72 ± 0.99
Treatment modality		
cyclophosphamide alone	12	12.00 ± 1.31
adriamycin–bleomycin–CCNU regimens	9	9.72 ± 1.32
total chemotherapy regimen	21	10.86 ± 2.1
radiotherapy alone	9	6.8 ± 0.87
Total treated patients	30	9.50 ± 0.3
Controls	35	4.37 ± 1.19

Discussion

The mean SCE values of untreated patients with malignant lymphoma were significantly higher than those from controls. Patients receiving cyclophosphamide at the time of study had the highest SCE means. Patients who were treated with radiotherapy alone had SCE means that were lower than those of untreated patients.

According to patient data published previously, cyclophosphamide, adriamycin and cisplatin have been observed to cause increased urinary mutagenicity.[12–14] The patient studies have also provided evidence about the toxicokinetic parameters to be considered in urine mutagenicity analyses, especially the proper timing of sample taking in relation to treatment. The high SCE scores observed in patients with lymphoma are very similar to the results of Kurvink *et al.*[15] which suggests an innate permanent modification, perhaps involving DNA replication or repair, or a secondary response to the disease.

In conclusion, SCE frequencies were higher in untreated lymphoma patients than in controls. Treatment with certain chemotherapeutic agents appeared to elevate further SCE means. SCE studies of patients prior to treatment, after the initiation of specific chemotherapeutic regimens and after cessation of treatment are needed.

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