Serial Measurement of Sister Chromatid Exchanges in the Peripheral Lymphocytes of Patients with Lung Cancer Receiving Chemotherapy in Relation to Bone Marrow Toxicity

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The frequencies of sister chromatid exchanges (SCE) in the peripheral lymphocytes of patients with lung cancer before and after initial chemotherapy were serially measured and the correlation between SCE frequencies and bone marrow toxicity was evaluated. The addition of mitomycin C to vindesine plus cisplatin increased SCE frequencies significantly in patients with non-small cell lung cancer. A significant increase in frequencies of SCE was observed in patients treated with cyclophosphamide plus adriamycin plus vincristine as compared with those treated with cisplatin plus etoposide in small cell lung cancer. A significant inverse correlation was observed between SCE frequencies in the peripheral lymphocytes 7 days after treatment (x) and the nadir value/pre-treatment value of platelets (y) ($r = -0.685$, $P = 0.0007$, $y = 0.842 - 0.022x$). The relation between SCE frequency and leukopenia showed the same trend as thrombocytopenia, but the correlation was not statistically significant ($r = -0.444$, $P = 0.057$). SCE assay may have potential clinical use for the prediction of chemotherapy-induced thrombocytopenia.

Key words: Sister chromatid exchanges — Bone marrow toxicity — Chemotherapy — Lung cancer

Sister chromatid exchange (SCE) assay has been utilized for identification of genotoxic agents,¹⁻³ and for chemosensitivity testing.^{4,5} However, little is known about the relationship between frequencies of SCE and bone marrow toxicity induced by chemotherapy. In our previous study,⁶ frequencies of SCE in the peripheral lymphocytes during chemotherapy tended to fluctuate inversely with respect to platelet and leukocyte cell counts. Frequencies of SCE in the peripheral lymphocytes tended to be the highest within one week from the start of chemotherapy and to decrease to the pre-treatment level 3-5 weeks after chemotherapy. A study by Abe *et al.*⁷ showed that nimustine, which is well known to cause severe and persistent myelosuppression, induced an extremely profound and prolonged increase of SCE frequency. Therefore, we serially evaluated the frequencies of SCE in peripheral lymphocytes of patients with lung cancer during initial chemotherapy and compared them with myelosuppression.

MATERIALS AND METHODS

SCE analysis For the SCE analysis, heparinized blood samples were taken serially, before and after initial chemotherapy, from twelve patients with primary lung cancer

who had received no prior chemotherapy or radiotherapy. Samples were cultured in RPMI-1640 medium supplemented with 17% fetal calf serum, penicillin (100 IU/ml), streptomycin (100 μ g/ml) and 0.2 ml phytohemagglutinin M (Difco). After a 24-h culture, 10 μ M 5-bromodeoxyuridine was added to the culture bottles. After an additional 48-h incubation in the dark, in a highly humidified 5% CO₂ incubator at 37°C, colcemid (0.1 μ g/ml) was added to the culture 4 h prior to harvest. Harvested cells were then treated with a hypotonic solution (0.075 M potassium chloride) and fixed with methanol/acetic acid (3:1). Slides were prepared by forceful blowing of cell suspensions and were air-dried. The sister chromatids were differentially stained according to the method described by Perry and Wolff,⁸ with some modification. In each sample, more than 20 metaphases showing differences in chromatid staining were photographed and then scored for SCE frequency analysis. The statistical significance of differences was evaluated using Student's *t* test and correlation coefficient. Differences yielding observed significance levels $\geq 5\%$ were not considered statistically significant.

Treatment Patients with non-small cell lung cancer (NSCLC) received vindesine plus cisplatin (VP: vindesine 3 mg/m² on days 1, 8 and 15, cisplatin 80 mg/m² on day 1) or mitomycin C plus vindesine plus cisplatin (MVP: mitomycin 8 mg/m² on day 1, vindesine 3 mg/m²

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on days 1 and 8, cisplatin 80 mg/m² on day 1). Patients with small cell lung cancer (SCLC) received cyclophosphamide plus adriamycin plus vincristine (CAV: cyclophosphamide 800 mg/m² on day 1, adriamycin 50 mg/m² on day 1, vincristine 1.4 mg/m² on day 1) or cisplatin plus etoposide (PE: cisplatin 80 mg/m² on day 1, etoposide 100 mg/m² on days 1, 3 and 5). All drugs were administered intravenously and all regimens were repeated every 3–4 weeks.

The absolute counts of platelets, white blood cells and hemoglobin were measured before chemotherapy and one or two times weekly for four weeks after it.

RESULTS

Serial measurement of SCE frequency before and after chemotherapy Table I shows frequencies of SCE/metaphase in the peripheral lymphocytes before and after initial chemotherapy. A significant increase in SCE frequency was observed in all patients within one week from the start of chemotherapy and the frequencies of SCE tended to decrease to the pretreatment level 3 weeks after chemotherapy. To determine the difference of SCE frequency for each treatment arm, 100 metaphase cells obtained from four patients were scored for MVP vs. VP regimen and 50 metaphase cells obtained from two patients for CAV vs. PE regimen. The addition of mitomycin to VP regimen increased SCE frequency significantly in patients with NSCLC. A significant increase in SCE frequency was observed in SCLC patients treated with CAV regimen compared to those with PE regimen.

Correlation between SCE frequency and bone marrow toxicity induced by chemotherapy We analyzed fre-

quencies of SCE/metaphase in the peripheral lymphocytes 7 days after treatment and platelet count in 20 patients (12 patients in Table I and another 8 patients who were examined for SCE frequency twice, before and 7 days after chemotherapy). The correlation between SCE frequency and thrombocytopenia is shown in Fig. 1. A significant inverse correlation was observed between frequency of SCE (x) and platelet nadir value/pretreatment value (y). As demonstrated, the correlation coefficient

Fig. 1. Correlation of nadir value/pretreatment value of platelets (y) with SCE frequencies/metaphase (x). $y = 0.842 - 0.022x$, $r = 0.685$, $P = 0.0007$. Twenty patients were studied.

Table I. Serial Measurement of SCE Frequency/Metaphase in the Peripheral Lymphocytes before and after Chemotherapy

Chemotherapy ^{a)}	No. of metaphases counted	SCE frequency/metaphase (mean $\pm$ SD) ^{b)}					
		Before	Days after treatment				
			2–3	4–5	7	14	21
MVP	100	7.1 $\pm$ 3.4	16.8 $\pm$ 4.4 ^{c)}	16.8 $\pm$ 5.7 ^{d)}	18.0 $\pm$ 5.7 ^{e)}	11.4 $\pm$ 3.8	11.4 $\pm$ 4.3 ^{e)}
VP	100	8.7 $\pm$ 3.1	13.6 $\pm$ 4.6	14.4 $\pm$ 3.2	13.3 $\pm$ 4.5	10.9 $\pm$ 2.8 ^{e)}	8.3 $\pm$ 3.3
CAV	50	7.4 $\pm$ 2.9	21.6 $\pm$ 7.9 ^{e)}	NT	19.5 $\pm$ 4.8 ^{e)}	11.6 $\pm$ 4.0	9.5 $\pm$ 3.8
PE	50	8.9 $\pm$ 3.4	10.3 $\pm$ 3.3	11.9 $\pm$ 4.5	12.6 $\pm$ 3.5	12.3 $\pm$ 4.5	10.0 $\pm$ 3.2

a) MVP, mitomycin + vindesine + cisplatin; VP, vindesine + cisplatin; CAV, cyclophosphamide + adriamycin + vincristine; PE, cisplatin + etoposide. Four patients received the MVP or VP regimen and two patients received the CAV or PE regimen.

b) SCE values represent mean $\pm$ SD of 100 metaphase cells for the MVP or VP regimen and 50 metaphase cells for the CAV or PE regimen.

c) $P < 0.001$. d) $P < 0.05$: MVP vs. VP, CAV vs. PE.

e) Only 50 metaphase cells could be analyzed.

cient (r) was -0.685 ($P=0.0007$, $y=0.842-0.022x$). The relationship between nadir value/pre-treatment value of white blood cells or nadir value/pre-treatment value of hemoglobin and SCE frequency/metaphase in the peripheral lymphocytes was evaluated. No correlation was found between SCE frequency/metaphase and either leukopenia ($r=-0.444$, $P=0.057$) or anemia ($r=0.063$) (data not shown).

DISCUSSION

The VP regimen has been one of the standard treatments for advanced NSCLC.^{9, 10} Recently, the additional effect of mitomycin C (MMC) in the VP regimen has been investigated.^{11, 12} The present study showed that the MVP regimen induced a significant increase in SCE frequency in the peripheral lymphocytes compared to the VP regimen. Although the mechanism of SCE is not well understood, one should take into consideration the possibility of an increase of the frequency of secondary malignancies by adding MMC in the combination of V and P.

In recent studies of secondary tumors in patients with SCLC, the cumulative risk of secondary acute non-lymphocytic leukemia and preleukemia reached 14%–25%, especially when alkylating agents were used, 3 or 4 years after the start of chemotherapy.^{13, 14} CAV has been shown to be an effective first-line regimen for SCLC.¹⁵ More recently, the EP regimen has demonstrated an equal or stronger anti-tumor effect for SCLC.^{16, 17} In the present study, the CAV regimen induced a significant increase in SCE frequency in the peripheral lymphocytes as compared with the EP regimen despite the small number of patients investigated. From these points of view, it appears that the EP regimen is superior to the CAV regimen.

In the clinical study of chemotherapy, it is important to predict leukopenia and thrombocytopenia, which sometimes induce fatal infection and bleeding, respectively. A study by Kao-Shan *et al.*¹⁸ demonstrated that the frequency of SCE was significantly increased in smokers in both bone marrow cells and peripheral blood lympho-

cytes, and they indicated that peripheral lymphocytes were more sensitive to possible damage by carcinogenic agents than bone marrow cells. Therefore, we examined the correlation between SCE frequency in the peripheral lymphocytes and bone marrow toxicity induced by chemotherapy. We found a significant inverse correlation between SCE frequency/metaphase and platelet nadir value/pre-treatment value, and therefore measurement of SCE values in peripheral lymphocytes appears to have a potential application in the clinical prediction of chemotherapy-induced thrombocytopenia. The relation between SCE frequency and leukopenia showed the same trend as thrombocytopenia, but the correlation was not statistically significant. This may be because of withdrawal or delay of administration of the second or third dose of vindesine owing to leukopenia in some patients treated with the VP or MVP regimen. We evaluated SCE frequency 7 days from the start of chemotherapy because the PE regimen consisted of a five-day schedule and a significant increase in SCE frequency at 7 days from the start of chemotherapy was observed in all patients in Table I compared to the pretreatment value ($P<0.01$, $t=3.29\sim14.09$). At present, we are involved in studies to try to identify the correlation between SCE frequencies in the peripheral lymphocytes 24 h from the start of chemotherapy and bone marrow toxicity, because maximum elevation of SCE frequency was observed 24 h from the start of chemotherapy^{6, 7, 19} and it might be more useful to predict bone marrow toxicity as early as possible in the clinical setting. The relation between tobacco smoking and SCE frequencies could not be analyzed because only 2 of 20 patients were non-smokers in this study.

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