Sister Chromatid Exchanges in Lymphocytes of Cancer Patients Receiving Mitomycin C Treatment[1]

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ABSTRACT

Peripheral lymphocytes from cancer patients receiving mitomycin C treatment were examined for cytogenetic effects. The treatment consisted of i.v. injections of mitomycin C at a dose of 4 mg given twice a week for 2 weeks.

The lymphocytes were cultured in vitro for 72 hr with phytohemagglutinin and 5-bromodeoxyuridine, and then sister chromatid exchanges were scored. Before treatment with mitomycin C, the frequencies of sister chromatid exchanges in lymphocytes of cancer patients were similar to those of healthy controls. After the first and second treatments in vivo with mitomycin C, the frequencies of sister chromatid exchanges increased with time, reached a peak in about 24 hr, and then returned to the pretreatment level in about 48 hr, in contrast to the case of in vitro exposure to mitomycin C. After the third and fourth injections, however, the frequency increased further and did not return to the original level. The significance of the specific kinetics of change in the sister chromatid exchange frequency after in vivo treatments is discussed in relation to cancer chemotherapy.

INTRODUCTION

Several anticancer chemotherapeutic agents have cytogenetic effects and induce not only mutation in bacteria and cultured mammalian cells but also cancers in laboratory animals (16). Recently, it has been reported that chromosome aberrations, the majority of which are balanced rearrangements, persist for many years in children who have survived for extended periods after chemotherapy of cancer (15). If we could routinely monitor some of the complicated biological effects of anticancer agents in patients receiving chemotherapy, we should be in a better position to assess the clinical value of treatment. Recently, techniques for scoring SCE's[3] in mammalian cells have been much improved (2, 8, 10, 11, 13, 19, 23), and SCE's are now detectable at levels 1 or 2 orders of magnitude lower than chromosome aberrations (17, 21). Increased frequencies of SCE have been reported in peripheral lymphocytes of cancer patients receiving chemotherapy (12, 17, 20). We have made detailed measurements of SCE in peripheral lymphocytes taken from cancer patients at various times during MMC treatment, using a dose and injection schedule chosen on the basis of therapeutic effects. Since MMC is a potent inducer of SCE (1, 5, 9, 14, 18), we were able to

obtain reproducible data on MMC damage in vivo as shown in the present paper. To our surprise, the pattern of changes in the frequencies of SCE in lymphocytes during MMC treatment in vivo was markedly different from that expected from previous findings in vitro (7).

MATERIALS AND METHODS

MMC Treatment. Cancer patients (gastric, thyroid, and breast cancer) were treated with MMC (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) i.v. at a dose of 4 mg twice a week.

Cell Culture. On the basis of previous results (6), human peripheral lymphocytes were cultured as follows. Samples of 0.9 ml of whole heparinized blood were added to 9.5 ml of Eagle's minimal essential medium (The Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan) containing 20% fetal bovine serum (Flow Laboratories, Stanmore, N. S. W., Australia) and 0.25 ml of phytohemagglutinin (Grand Island Biological Company, Grand Island, N. Y.). For detection of SCE, 5-bromodeoxyuridine (Sigma Chemical Company, St. Louis, Mo.; 25 μM final concentration in the medium) was added, and the cultures were kept in the dark. All cultures were incubated at 37° under 5% CO_2 in air for 72 to 96 hr, and 2 hr before harvesting the cells 0.15 ml of Colcemid (Grand Island Biological Company) was added to each culture.

Slide Preparation. Cells were suspended in 0.075 M KCl solution for 12 min, fixed in methanol:acetic acid (3:1) for 30 min, washed twice with fresh fixative, dropped onto the surface of a wet glass slide, and dried in air. For scoring SCE, slides were processed essentially as described by Goto et al. (4). The slides were stained with 10^{-4} M Hoechst 33258 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for 10 min, rinsed with tap water, and mounted in 0.174 M Na_2PO_4:0.013 M sodium citrate (pH 7.0). They were then illuminated with two 15-watt black light lamps (National Electric Co., Ltd., Osaka, Japan) for about 40 min and then restained with 2% Giemsa (Merck, Darmstadt, West Germany) in 0.05 M phosphate buffer, pH 6.8, for 20 min. The slides were then rinsed twice with acetone, once with acetone:toluene (1:1), and twice with toluene and air dried. Coverslips were mounted with Permount (Fisher Scientific Company, Fair Lawn, N. J.).

Scoring. Average SCE's for all the chromosomes of at least 20 intact metaphase spreads are shown.

RESULTS

As shown in Table 1, there was no significant difference in the numbers of SCE's per lymphocyte of healthy controls and cancer patients without MMC treatment. Furthermore, no statistically significant influences of age or sex on the SCE frequencies were detected.

Table 1

SCE frequency in peripheral lymphocytes of controls and cancer patients before treatment

Group	Sex	Age (yr)	Diagnosis	No. of cells	No. of SCE/cell
Control					
1	M	34		50	9.38 ± 3.56[a]
2	F	36		50	7.88 ± 3.87
3	M	24		50	9.74 ± 2.74
4	M	30		50	7.86 ± 3.51
5	F	25		50	10.86 ± 4.36
6	M	29		50	7.84 ± 3.37
Patient					
1	M	51	Thyroid cancer	50	7.98 ± 3.24
2	M	49	Gastric cancer	50	11.18 ± 6.19
3	F	60	Gastric cancer	50	9.64 ± 4.09
4	M	61	Gastric cancer	20	7.70 ± 3.08
5	M	50	Gastric cancer	20	8.87 ± 3.56
6	F	70	Gastric cancer	20	7.10 ± 2.72
7	F	34	Gastric cancer	20	7.40 ± 3.06
8	F	43	Breast cancer	20	8.20 ± 4.27
9	F	13	Thyroid cancer	20	7.60 ± 2.48
10	M	33	Thyroid cancer	20	9.50 ± 4.20
11	M	50	Gastric cancer	20	7.10 ± 3.26

[a] Average ± S.D.

In one patient with gastric cancer, the SCE frequencies were determined immediately before surgery, 1 and 5 days after surgery, and after MMC treatment. This patient was given MMC by i.v. injection at a dose of 4 mg twice a week for 2 weeks. As shown in Chart 1, surgical operation had no marked influence on the frequency of SCE's, but after MMC treatment the frequency of SCE's increased to greatly above the control level. To detect the possible influence of residual MMC remaining in the patient's plasma, which would increase SCE-inducing lesions during culture *in vitro*, before incubation some of the blood cells obtained from the patient were washed by centrifugation to separate them from plasma. As shown in Chart 1, *D* and *E*, there was no difference between the frequencies in unwashed and washed leukocytes.

In 3 patients with gastric cancer, the frequencies of SCE's were determined serially during the course of MMC treatment. As shown in Chart 2, the SCE frequency gradually increased after a single i.v. injection of 4 mg of MMC, and after 24 hr it reached a peak of about 10 SCE's higher than the level before treatment. Then it decreased, returning to the pretreatment level 48 hr after MMC treatment. As shown in Chart 3, a second injection of MMC resulted in a similar pattern of change in SCE frequency with time. However, after the third injection of MMC, the increased SCE frequency did not return to the original level but remained high throughout the 3-day period before the fourth injection. The fourth injection then resulted in a further increase of 10 SCE's in the already elevated level. However, the SCE values of lymphocytes of the 2 patients were at the pretreatment level when we examined them about 3 months later.

DISCUSSION

MMC, which is widely used as an anticancer chemotherapeutic agent, is a very strong inducer of SCE *in vitro* (18). When human peripheral lymphocytes cultured *in vitro* are exposed to MMC, some of the MMC-induced lesions are repaired during culture (7), but some lesions remain unrepaired and persist, causing SCE through at least 3 cell cycles (6). These
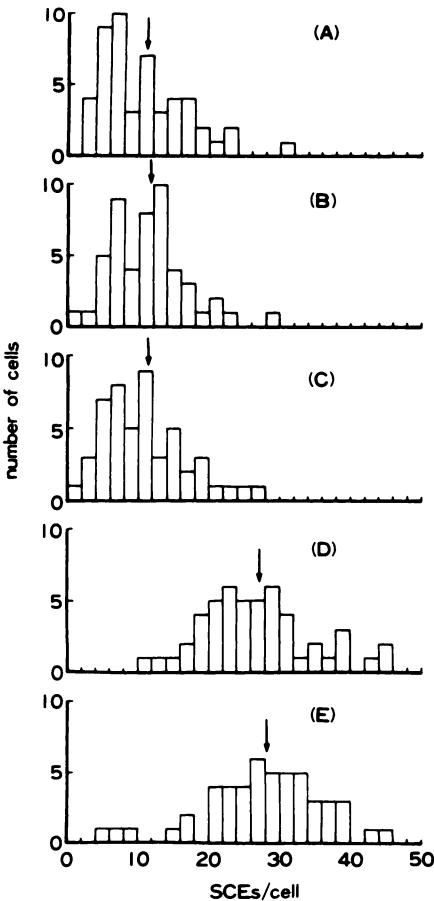

Chart 1. SCE frequencies in a 49-year-old man with gastric cancer before surgery (A), 1 (B) and 5 (C) days after surgery, and after MMC treatments (i.v. injections of 4 mg, twice a week for 2 weeks) (D, E). The average number of SCE before treatment was not significantly different from that of healthy subjects, although the distribution was somewhat wider. The frequency was significantly higher after MMC treatment (p < 0.01). There was no significant difference between the frequencies in cells cultured with (E) and without (D) washing, indicating that the increase after MMC treatment was not induced by MMC during incubation. Arrows, average SCE frequencies per cell: A, 11.18 ± 6.19 (S.D.); B, 11.86 ± 5.42; C, 11.56 ± 5.88; D, 27.36 ± 7.90; E, 28.30 ± 8.55.

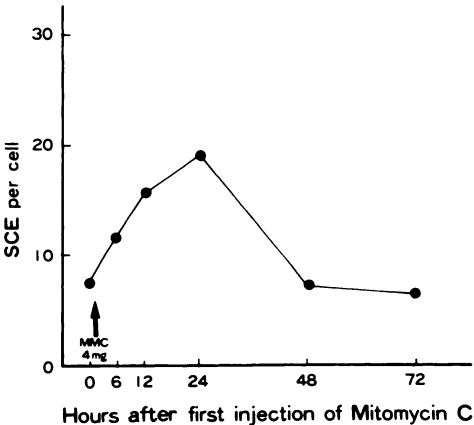Hours after first injection of Mitomycin C

Chart 2. Serial determinations of the SCE frequency in a 61-year-old man with gastric cancer after a single injection of MMC. After i.v. injection of 4 mg of MMC, the SCE frequency increased to a peak after 24 hr and then returned to the original value after 48 hr.

findings led us to expect that MMC lesions in cells of MMC-treated patients should be detectable by scoring SCE in peripheral lymphocytes after culture *in vitro*.
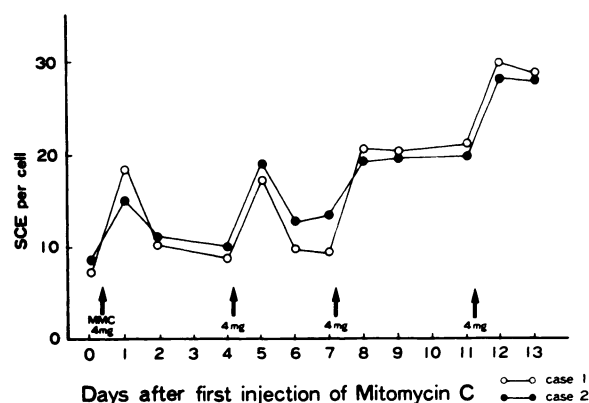
Chart 3. Frequencies of SCE in 2 patients with cancer determined during MMC treatment for 2 weeks. MMC (4 mg) was injected i.v. twice a week for 2 weeks. Case 1, 70-year-old woman with gastric cancer; Case 2, 50-year-old man with gastric cancer.

Accordingly, we cultured blood samples taken from cancer patients treated with MMC and then measured the frequencies of SCE's in lymphocytes after stimulation with phytohemagglutinin. We found no statistically significant difference between the SCE frequencies in peripheral lymphocytes of healthy controls and cancer patients receiving no chemotherapy (Table 1). Moreover, our results showed that the SCE frequency was not influenced by age, sex, or surgical operation (Table 1; Chart 1). However, a preliminary test clearly demonstrated that MMC treatment in vivo increased the frequency of SCE (Chart 1). This increase was not due to MMC remaining in the blood sample during in vitro incubation, because washed and unwashed blood cells had similar SCE frequencies (Chart 1). Therefore, we next made detailed observations on SCE in lymphocytes from patients receiving MMC therapy.

Patients with cancer were given i.v. injections of 4 mg of MMC twice a week. The first injection of the drug caused nearly linear increase in SCE frequency with a peak after about 24 hr (Chart 2). This finding is consistent with the report of Ishii and Bender (6) after in vitro treatments that SCE values increase linearly for about 30 hr after addition of MMC to cultures of human peripheral lymphocytes. It is very puzzling that, 2 days after the first MMC administration, the SCE values of lymphocytes exposed to MMC in vivo had decreased to nearly the control level (Charts 2 and 3), since the SCE values of lymphocytes exposed to MMC in vitro remain virtually constant for at least 48 hr after treatment with MMC (150 ng/ml for 4 hr) (7). After the third and fourth i.v. injections of MMC, however, the SCE values of lymphocytes treated in vivo remained constant from Day 1 until at least Day 2 or 3 after MMC injection (Chart 3), as in the in vitro-treated lymphocytes (7).

The pattern of changes in SCE frequencies shown in Chart 3 is similar to that observed by Stetka et al. (22) in rabbits given 8 injections of MMC at weekly intervals. Thus, rapid decrease in SCE frequency between 24 and 48 hr after MMC in vivo treatment is observed only after the first and probably the second treatments, either in humans or rabbits.

Ishii and Bender (6) clearly demonstrated after in vitro treatments that a large proportion of the MMC-induced DNA lesions persists for at least 72 hr and can cause SCE in the third cell cycle of cultured lymphocytes with almost the same efficiency as in the first and second cell cycles. The decrease in SCE's

after the first and second in vivo treatments with MMC that we observed may be due to some unknown damage-eliminating system(s) other than DNA repair detected in cultures exposed to MMC in vitro.

A more likely explanation of this decrease is that it is caused by dilution of MMC-damaged lymphocytes with "fresh" lymphocytes having negligible SCE-inducing damage and/or by selective death of MMC-damaged lymphocytes in the peripheral blood. It has been reported that, after i.v. injection of MMC, the level of MMC and rates of its disappearance vary in different tissues and organs of mice and guinea pigs (3). In some organs, the level of MMC may be so low that the lymphocytes are not appreciably damaged. Thus, it is quite conceivable that lymphocytes in a reservoir pool may be almost free from MMC damage owing to a very low level of MMC in the pool and that MMC administration induces their release into the peripheral blood in about 1 day. Alternatively, lymphocytes damaged by MMC may be killed selectively. Further studies are required on the decrease in the MMC-induced SCE frequency observed after in vivo treatments.

The kinetics of the increase in the SCE frequency after MMC treatment in vivo is only superficially compatible with that observed after in vitro treatment because the level of MMC in the blood reaches a peak within a few min after i.v. injection of MMC and then decreases at a high rate of about 90%/hr, reaching a negligible value in about 3 hr (3), whereas the concentration of MMC does not change appreciably during incubation in vitro. Therefore, the continuous rise in the SCE frequency after in vivo treatment for 24 hr is not readily explainable from the results of in vitro exposure. Whatever the mechanisms of increase and decrease in the SCE frequency after MMC treatment in vivo, it seems certain that MMC-induced damage of peripheral lymphocytes of patients is mostly eliminated within 2 days after the first and second treatments. We do not know whether the disappearance is due to repair in individual cells, to differential lethality and/or the substitution of an undamaged cell population, or to some other mechanism. However, the damage, i.e., SCE-inducing lesions detectable after culture in vitro, persists for a fairly long time after further in vivo treatments with MMC. Thus, if the intervals between MMC treatments in vivo were longer, the MMC-induced damage of the peripheral lymphocytes might diminish rapidly even after third and fourth treatments. There may also be other ways of enhancing the recovery of peripheral lymphocytes from MMC damage. Measurement of SCE values in peripheral lymphocytes should be a useful probe in attempts to improve the therapy of cancer not only with MMC but also with other anticancer agents.

ACKNOWLEDGMENTS

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M. Ohtsuru et al.

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