Radiotoxicity After Strontium-89 Therapy for Bone Metastases Using the Micronucleus Assay

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The purpose of this study was to evaluate the degree of cytologic radiation damage to lymphocytes after ^{89}Sr therapy using the cytokinesis-blocked micronucleus assay. The chromosomal damage to lymphocytes exposed to ^{89}Sr in vivo should result in augmentation of the number of cells with micronucleus. **Methods:** We studied eight patients with painful bone metastases, who were treated with 111 MBq ^{89}Sr. Isolated lymphocytes collected from the patients 1 wk after therapy were harvested and treated according to the cytokinesis-blocked method of Fenech and Morley. The number of micronuclei per 500 binucleated cells was scored by visual inspection. As controls, lymphocytes from the same patients before therapy were also studied. For three patients, serial blood samples were examined for a maximum of 2 mo after therapy. In an in vitro study, lymphocytes from five normal volunteers were exposed to doses varying from 0.25 to 1.0 Gy and studied with the same method. **Results:** The mean number (±s.d.) of micronuclei per 500 binucleated cells after treatment was significantly increased ($p < 0.05$) as compared to control subjects (17.1 ± 3.0 compared to 6.0 ± 1.7). Thereafter, the number of micronuclei recovered gradually by 6 wk following therapy and, in one case, nearly to the baseline range in 2 mo. The number of micronuclei after 0.53 ± 0.13 Gy of external irradiation was nearly equivalent to that after ^{89}Sr therapy. **Conclusion:** The relatively low frequency of lymphocyte micronuclei exposed to ^{89}Sr in vivo supported the contention that short-term nonstochastic damage with 111 MBq ^{89}Sr in patients with painful bone metastases is minimal.

Key Words: micronucleus assay; painful bone metastases; strontium-89 therapy; radiotoxicity

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Pain is one of the most feared consequences of cancer. Adequate control of such pain should be possible with a combination of approaches including narcotic analgesics and nonnarcotic modalities (*1*). Bone metastases cause pain and compromise the quality of life for a large number of patients with cancer. Bone metastases often present as the first evidence of disseminated disease and are the most common sites of primary bone-metastasizing primary tumors. Treatment is primarily palliative when it is designed to relieve pain, prevent fractures, maintain activity and mobility and, if possible, prolong survival (*2*).

Bone-seeking radiopharmaceuticals play a significant role in the treatment of the pain of osteoblastic metastatic disease. Strontium-89, an analog of calcium injected in the chloride form, was first suggested for the palliation of bone pain from metastatic disease in 1942 (*3*). It has been widely available for the reduction or relief of pain of osteoblastic metastatic disease (*4–6*), with blood and bone marrow toxicity the dose-limiting factors in ^{89}Sr radiotherapy. Although dose estimation studies have been performed (*7,8*), to our knowledge, cytologic examination of radiation exposure to lymphocytes in vivo with ^{89}Sr therapy has not been extensively performed.

The purpose of our study was to evaluate the degree of cytologic radiation-induced damage to lymphocytes in vivo after therapy with 111 MBq ^{89}Sr using the CBMN assay. Because of likely differences between low-dose rate internal ^{89}Sr irradiation and high-dose rate external x-ray irradiation in relative biological effectiveness and/or dose rate factor, a comparison of the frequency of micronuclei between lymphocytes irradiated in vivo by ^{89}Sr and in vitro by x-ray can only yield relative dose equivalents, not relative absorbed doses.

MATERIALS AND METHODS

Patients

Eight patients were admitted to clinical trials of ^{89}Sr therapy at the Department of Nuclear Medicine, Kanazawa University Hospital (Kanazawa, Japan). All patients were diagnosed with prostatic cancer, breast cancer or colon cancer and underwent surgery and bone scintigraphy. After informed consent was obtained, these eight patients were administered 111 MBq ^{89}Sr to control the severe pain from multiple bone metastases.

Lymphocyte Isolation and Culture

Venous blood was collected from each of the eight patients before therapy as a control and 1 wk thereafter. Serial blood samples were obtained periodically from three patients up to a maximum of 2 mo after therapy.

Peripheral blood lymphocytes were separated from whole blood on lymphocyte separation medium (ICN Biomedicals, Inc., Aurora, OH) after centrifugation (400 *g* for 30 min, 18°C), washed twice in Hank's balanced salt solution and resuspended in McCoy's modified medium 5A containing 15% heat-inactivated fetal calf serum. The lymphocytes were cultured according to the method of Fenech and Morley (*9*) in a 25-ml tissue culture flask at a concentration of 0.5×10^6 cells/ml. An optimum concentration of phytohemagglutinin (5 μg/ml, Difco Laboratories, Detroit, MI) was used to stimulate the lymphocytes to transform and divide in the culture. The cells were cultured at 37°C in a humidified atmosphere containing 10% CO_2. A solution of Cytochalasin B (Aldrich Chemical Co., Milwaukee, WI) was added to the culture 44 hr after PHA stimulation to give a final concentration of 3.0 μg/ml. The cultures were terminated 72 hr after initiation.

In Vitro External Irradiation

In an in vitro study, lymphocytes were collected from five normal volunteers. Irradiation of the lymphocytes was performed

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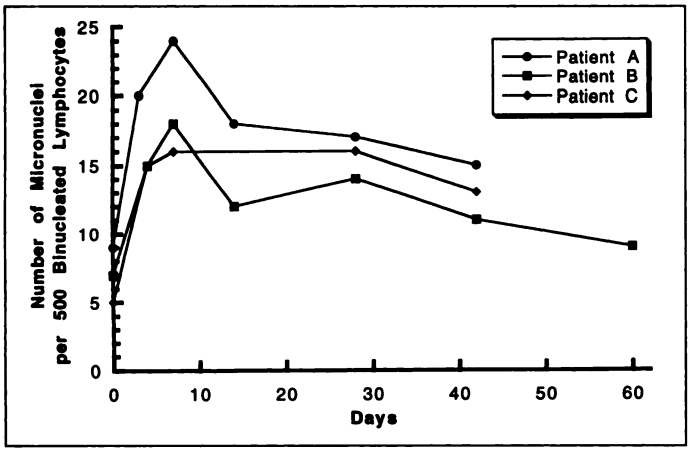

FIGURE 1. Serial change of number of micronuclei per 500 binucleated cells in three patients up to a maximum of 2 mo.

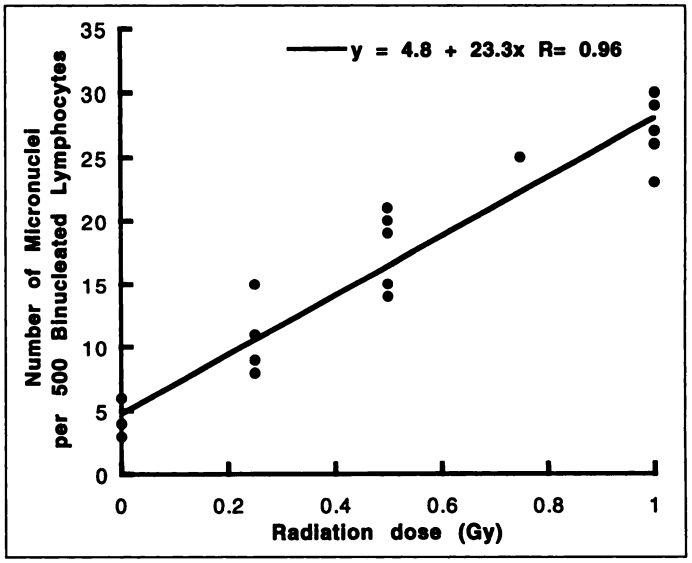

FIGURE 2. Number of micronuclei per 500 binucleated cells after external irradiation in vitro (n = 5).

using MBR-1505R2 x-ray equipment (Hitachi, Tokyo, Japan) with the following conditions: 150 kV, 2 mA, 1.0-mm Al filter, 2.14 Gy/min dose rate, at room temperature, in a 25-ml tissue culture flask. The applied doses were 0.25, 0.5, 0.75 and 1 Gy. After irradiation, the lymphocytes were cultured according to the same method described above.

Micronucleus Assay

The acridine orange fluorescent staining procedure was applied to the CBMN assay in this study, according to the method of Hayashi et al. (*10*). The prepared lymphocytes were stained with 0.1% aqueous solution of acridine orange. These lymphocytes were smeared on clean glass slides. An Olympus BH-RFL fluorescent microscope with BG-12 excitation filter and 0-515 barrier filter was used for observation. Micronuclei stained yellowish green with green fluorescence. The number of micronuclei per 500 binucleated cells was scored by visual inspection.

Statistical Analysis

Data are expressed as means ± s.d. A paired t-test was used to test the statistical significance of the difference in the frequencies of micronuclei before and after ^{89}Sr therapy. Probability (p) values of <0.05 were considered significant.

RESULTS

Evaluation of the Cytologic Radiation Damage to Lymphocytes After Strontium-89 Therapy

The number (mean ± s.d.) of micronuclei per 500 binucleated lymphocytes was 6.0 ± 1.7 before and 17.1 ± 3.0 after ^{89}Sr therapy, a statistically significant increase ($p < 0.05$).

In Patient A, the frequency of micronuclei peaked at 7 days after therapy of ^{89}Sr and, thereafter, gradually decreased by 6 wk. In Patient B, it peaked at 7 days and slightly increased at 28 days. Thereafter, it decreased gradually and recovered nearly to baseline (i.e., pretherapy) levels by 2 mo. In Patient C, the peak was considered to be present between 7 days and 4 wk after therapy. Thereafter, it gradually decreased by 6 wk (Fig. 1).

Evaluation of the Cytologic Radiation Damage to Lymphocytes After External Irradiation

In the external irradiation study, lymphocytes from five normal volunteers were collected. The number of micronuclei per 500 binucleated lymphocytes was observed after x-ray irradiation doses of 0.25, 0.5, 0.75 and 1 Gy. These data fit a nonthreshold, linear dose–response function ($y = 23.3x + 4.8$, $r = 0.96$) (Fig. 2). The number of micronuclei per 500 binucleated lymphocytes after 0.53 ± 0.13 Gy of external irradiation in vitro was, thus, nearly equivalent to that after ^{89}Sr therapy in vivo.

DISCUSSION

Fenech and Morley (*9*) developed the CBMN assay as a method for measurement of chromosomal damage in mitogen-stimulated human lymphocytes. This assay has been extensively used in routine mutagen/carcinogen screening programs to detect agents that cause chromosomal breakage and spindle dysfunction (*11,12*). Using this assay, we recently evaluated the cytologic radiation damage to lymphocytes induced by ^{131}I therapy (*13*) and could estimate the biological damage of ^{131}I therapy. Using the same assay, this study evaluates the adverse biological effect of ^{89}Sr therapy. Dose estimation after ^{89}Sr therapy has already been described (*7,8*), whereas biological dosimetry has not been reported previously for patients treated with ^{89}Sr.

With this assay system, we evaluated the degree of cytologic radiation-induced damage to lymphocytes in vivo after therapy with 111 MBq ^{89}Sr. Our in vivo study demonstrated that the frequencies of lymphocyte micronucleus in cells exposed to ^{89}Sr were significantly higher than those before therapy, used as controls. The frequency of micronucleus after therapy decreased gradually after the peak value, probably owing to the long half-life of lymphocytes, which has been considered to be variable (from ~100 to 500 days) (*14,15*). Two months after therapy, the frequency of micronucleus returned to nearly the baseline range in the one case examined. We could not collect blood samples after 6 wk from two patients. Our results suggested that cytologic damage to lymphocytes exposed to ^{89}Sr may be minimal, with gradual recovery seen a few months after therapy. However, M'Kacher et al. (*16*) showed that the biological damage persisted for up to 2 yr after ^{131}I therapy using conventional cytogenetic and chromosome 4 painting methods. The repair of internal radiation damage in lymphocytes in vivo is still not well understood. Further studies using a variety of techniques are needed to clarify this issue.

We compared the micronucleus incidence in in vivo and in vitro irradiated lymphocytes to estimate the effective absorbed radiation dose to lymphocytes in vivo. We determined that the cytologic damage to the lymphocytes exposed to ^{89}Sr in vivo may be considered to be equivalent to the damage observed after an external irradiation of 0.53 ± 0.13 Gy in vitro. The dose

rate from ^{89}Sr is lower than that from x-rays, and exposure time is protracted and consistent with known dose rate reduction factors (*2–10*), with which the biological effect of a given dose is generally reduced (*17*). Therefore, when compared to an equally effective high dose-rate acute external radiation exposure in vitro, the in vivo dose accumulated over 1 wk of low dose-rate chronic radiation exposure may be significantly higher.

Blood and bone marrow are the dose-limiting organs in ^{89}Sr therapy for bone metastases. Blake et al. (*8*) reported that the radiation doses in 14 subjects receiving 1.48 MBq/kg ^{89}Sr were 1 cGy/MBq to the red marrow. These results were remarkably close to the estimation by Silberstein and Williams (*18*) of 1.0 cGy/MBq to the red marrow. They used the ICRP model of strontium dosimetry to estimate the absorbed dose to red marrow. The mean internal radiation exposure estimated for our patients of 0.53 Gy (mean 2.07 MBq/Kg) is considered to be relatively low compared to these calculated results (1.1 Gy to red marrow at our doses). This is because our biological estimates may be too low, as mentioned above. However, an accurate estimate of marrow dose by their methods was imprecise due to its widespread distribution in the body and the difficulty in defining a suitable region of interest.

The long-term stochastic hazards of palliative treatment with ^{89}Sr have not been clearly established. We believe that our results suggest a minimal short-term nonstochastic hazard, although, theoretically, a long-term stochastic hazard may be present. However, at present, the long-term hazards of ^{89}Sr therapy are of less concern because of the limited life expectancy of such patients.

The procedures described here are the only simple, currently available procedures for routine evaluation of radiation-induced chromosomal damage (*19,20*). Although radiation damage to lymphocytes exposed to ^{89}Sr therapy in vivo may be minimal, based on the results of comparative cytologic studies, additional comparative studies monitoring other radionuclide therapies in patients given larger bone marrow doses such as ^{131}I radioimmunotherapy may be useful in defining optimal therapeutic strategies in such patients.

CONCLUSION

The relatively low frequency of lymphocyte micronuclei induced by ^{89}Sr in vivo supported the contention that short-term nonstochastic damage induced by this therapy in patients with painful bone metastases is minimal.

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