Effect of Low-Dose Total-Body Irradiation on Transplantability of Tumor Cells in Syngeneic Mice

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Low-dose radiation/Total-body irradiation/Tumor transplantability.

The effect of pretreatment with various low doses of total-body irradiation (TBI) on tumor cell transplantability in syngeneic mice was investigated. Two cell lines, EMT6 and SCCVII, and two strains of mice, were used. First, Balb/c mice were sham-irradiated or irradiated at 200 mGy, and 6–48 h later, 1000 EMT6 cells were inoculated in the hind legs. Based on the results, 0–1500 mGy of TBI was given 6 h before inoculation of 100 or 1000 cells in the subsequent experiments. All mice were observed for 50 days after transplantation. Tumors were judged as grown when the volume of palpable nodules exceeded 200 mm³. Tumor transplantability rate was significantly higher in the groups irradiated at 1500 mGy than in the sham-irradiated groups in both Balb/c and C3H/He mice. There were no differences in transplantability rates between the control group and the groups irradiated at various doses of 50–500 mGy. However, the mean time to tumor appearance was significantly elongated in Balb/c mice receiving TBI at 200 mGy and inoculated with 100 or 1000 EMT6 cells 6 h later. This phenomenon was also observed in Balb/c mice receiving 100 mGy TBI and inoculated with 1000 EMT6 cells. The present study might suggest that low-dose TBI to mice may delay tumor growth under certain conditions.

INTRODUCTION

With the development of diagnostic imaging and interventional radiology, there is an increasing interest in the effects of low-dose (< 200 mGy) radiation. Until recently, the risks of detrimental effects from exposure to low-dose radiation have been estimated by extrapolation from data obtained at high-dose radiation, using the linear no-threshold (LNT) model. However, much criticism has grown against the LNT theory since the introduction of "radiation hormesis" hypothesis indicating various beneficial effects of low-dose radiation on living organisms.1-3 Indeed, considerable amounts of evidence have accumulated, suggesting that living organisms, including humans, might respond differently to low-dose radiation than they do to high-dose radiation.

One of the beneficial effects of low-dose radiation may be stimulation of immune responses. Increase of immunological responses has been reported following low-dose irradiation.4-15 Also, a few studies have indicated suppression of tumorigenesis or lung metastases by adequate low doses of total-body irradiation (TBI).6-9,13 Low-dose radiation is also known to induce radioprotective responses and increase the level of radioprotective substances and DNA-repair enzymes.7,9,16-21 However, such beneficial effects of low-dose radiation do not appear to be universally accepted, and at any dose levels, radiation is still believed to be hazardous in most clinics. In order to properly evaluate the effect of low-dose radiation and the difference between high and low doses of radiation, more investigations seem necessary. In this study, therefore, we investigated the effect of low-dose TBI on transplantability of two tumor cell lines cultured in vitro in syngeneic mice. To our knowledge, this experimental design has never been used by other investigators.

MATERIALS AND METHODS

Cell Line and mice

EMT6 mammary sarcoma cells of Balb/c mice and SCCVII squamous cell carcinoma cells of C3H/He mice were used. Both cell lines were cultured in Eagle’s minimum essential medium containing 12.5% fetal bovine serum. Characteristics of these cell lines have been described previously.22,23 Both cells were subcultured on the day before tumor cell inoculation. Female Balb/c and C3H/HeN mice.

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Short Communication


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were purchased from Nihon SLC Co. Ltd. (Hamamatsu, Japan) at the age of 7–8 weeks. Before giving irradiation, all of the mice were accommodated for 5–7 days in sterile polypropylene cages (usually 8 mice per cage) containing a sterile paddy as bedding. They were maintained under specific pathogen-free conditions at 24°C ± 2°C and 60% ± 10% relative humidity, and were provided with nutritional chow (CRF-1, Charles River Laboratories Japan Inc., Yokohama, Japan) and reverse osmosis (RO) water ad libitum. The RO water rejects approximately 95% to 99% of all ionic materials including bacteria, viruses and pyrogens. Forty or 50 mice were used per group. The use of experimental animals for this study was approved by an ethics committee for animal experiments at Nagoya City University.

Irradiation

Irradiation was performed using a 210-kVp X-ray machine (0.5 or 10 mA with a 2-mm Al filter; Chubu Medical Co., Yokkaichi, Japan). Mice received TBI without anesthesia and physical restraint in an acrylic box at doses of 50–1500 mGy at a dose rate of 40 mGy/min for lower-dose irradiation (<500 mGy) or 1 Gy/min for a dose of 1500 mGy. The dose was calibrated using a RAMTEC 1000 dosimeter (Toyo Medic, Tokyo, Japan). This machine has been used in our previous biological studies and radiation procedure has been described in more details. All irradiation was given to 8-week-old mice.

Tumor transplantability studies

Exponentially-growing EMT6 and SCCVII cells cultured in vitro were trypsinized and suspended in phosphate-buffered saline (PBS). The cell density in the PBS was counted with a Coulter counter and diluted with PBS to a density of 10^6 or 10^7 cells/ml. Then 10 μL of the suspensions of EMT6 and SCCVII (i.e., containing approximately 100 or 1000 cells) were injected subcutaneously into both hind legs of Balb/c and C3H/HeN mice, respectively, at 6–48 h after TBI or sham irradiation to the mice. Thereafter, development of tumor masses was checked every day until day 50. Tumor transplantability was judged separately in the right and left legs. The three dimensions of each tumor were measured using a caliper and tumor volume was estimated using the formula π/6 × product of the three dimensions. Tumors were judged as grown when the volume of palpable nodules exceeded 200 mm^3. In the previous study, EMT6 and SCCVII cells from in vitro culture were injected into Balb/c mice and C3H/HeN mice, respectively, as was done in this study, and the grown tumors were pathologically examined; the two tumors were considered to derive from original EMT6 (mammary sarcoma) and SCCVII (poorly-differentiated squamous cell carcinoma) tumors, respectively. Curves for tumor transplantability were generated by the Kaplan-Meier method and differences between pairs of survival curves were examined by the logrank test using a computer program StatView version 5 (SAS institute Inc., Cary, NC, USA). The mean time to tumor development was also estimated using the StatView; this mean time represented the average period from tumor cell injection to tumor appearance in mice developing the tumors, but data from mice with no tumor development were also taken into account as censored data in a non-parametric analysis. Differences in the mean time to tumor development were examined by t-test. Using a computer program PRISM version 4.0c (GraphPad Software, Inc., San Diego, CA, USA), the statistical analyses were confirmed and figures were generated.

RESULTS

In some mice, tumor growth was observed only in one of the legs, but there was no difference in the tumor transplantability rate between the right and left legs. In the first experiment, 200 mGy of TBI was given to Balb/c mice and at 6–48 h thereafter, 1000 EMT6 cells were inoculated. The tumor transplantability rate was between 74% and 88% at day 30, but there were no differences in transplantability rates between sham-irradiated mice and mice irradiated at 200 mGy 6–48 h beforehand. Also, there were no differences among mice irradiated at various intervals before tumor cell inoculation (data not shown). From the results of this experiment, it was considered difficult to determine the optimal interval between TBI and tumor cell inoculation. However, based on the results of previous studies showing stimulation of immune responses by low-dose TBI, all subsequent irradiation was given 6 h before tumor cell inoculation.

Figures 1 and 2 show tumor transplantability curves for EMT6 tumors following TBI at 50–1500 mGy and inoculation of 1000 and 100 EMT6 cells, respectively, performed at 6-h intervals. Tumor transplantability significantly increased in mice receiving 1500 mGy of TBI in both experiments. Other doses of TBI did not significantly influence tumor transplantability in both experiments, but when the mice were irradiated at 200 mGy and 100 EMT6 cells were inoculated, tumor transplantability tended to be lower than that of control groups (53% vs 65%, p = 0.19; Fig. 2). Although tumor transplantability did not significantly decrease by giving low-dose TBI, the time for the tumor to become apparent was significantly prolonged in some groups. Figure 3 shows mean time to tumor growth in Balb/c mice inoculated with 1000 or 100 EMT6 cells as a function of the TBI dose. In the group inoculated with 1000 EMT6 cells, the mean time to tumor appearance was significantly longer in mice receiving 100 or 200 mGy than in mice receiving sham-irradiation (p = 0.0026 and 0.0012, respectively). Also in the group inoculated with 100 EMT6 cells, the mean time to tumor appearance was significantly longer in mice receiving 200 mGy than in mice receiving sham-irradiation (p = 0.0056).
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Fig. 1. Tumor transplantability curves for EMT6 tumors in Balb/c mice receiving 0 to 1500 mGy of total-body irradiation given at 6 h before inoculation of 1000 EMT6 cells. Each group consisted of 40 inoculation sites. There was a difference between the group irradiated at 1500 mGy and the sham-irradiated group (*p < 0.0001).

Fig. 2. Tumor transplantability curves for EMT6 tumors in Balb/c mice receiving 0 to 1500 mGy of total-body irradiation given at 6 h before inoculation of 100 EMT6 cells. Each group consisted of 40 inoculation sites. There was a difference between the group irradiated at 1500 mGy and the sham-irradiated group (*p = 0.0027).

On the other hand, the mean time to tumor appearance was significantly shorter in mice receiving 1500 mGy than in mice receiving sham-irradiation.

In SCCVII tumors, TBI and tumor cell inoculation were performed at 6-h intervals. After inoculation of 1000 SCCVII cells, tumor transplantability rate at day 50 was 40% for the sham-irradiated group and 70% for the group receiving 1500 mGy TBI (p = 0.0007), and after inoculation of 100 cells, it was 7.5% for the sham-irradiated group and 48% for the group receiving 1500 mGy TBI (p < 0.0001). However, there were no differences in tumor transplantability rates between the sham-irradiated group and other groups irradiated at 50–500 mGy (data not shown). Also, there was no elongation of the mean time to tumor appearance by 50–500 mGy of TBI (data not shown).

DISCUSSION

Low-dose radiation has been shown to stimulate the immune system of various living organisms. Various immunological parameters including natural killer activity, concanavalin A-induced proliferative response of the splenocytes, antibody-dependent cellular cytotoxicity, CD8+ lymphocytes and some cytokines, have been shown to increase following low-dose TBI.3-10 Sakamoto et al.6)
found that TBI at 100 mGy potentiated the effect of local irradiation given 12 h later to squamous cell carcinomas in WHT/Ht mice. They also observed that 100 and 150 mGy of TBI had suppressive effect on distant metastases of the tumor. Hashimoto et al.\(^9\) reported suppression of lung metastases and increase of CD8+ lymphocytes in a rat tumor model after TBI with 200 mGy. As reviewed by Hosoi\(^8\) recently, there exists still other data suggesting anti-tumor effects of low-dose irradiation, but only a limited proportion of clinicians, including radiologists, believe in such hormetic effects. Therefore, it was considered necessary to further investigate the effects of low-dose irradiation in various experimental systems.

We used two tumor lines because EMT6 tumors were supposed to be somewhat immunogenic to Balb/c mice, whereas SCCVII tumors were not so to C3H/He mice in the 1980's (Professor K. Ono, personal communication). From the results of this study, this appears to be no longer true, since tumor transplantability rates after inoculation of 100 and 1000 cells were lower in SCCVII than in EMT6 tumors. Anyhow, since the effect of low-dose irradiation is not yet universally believed, investigation with as many tumor lines as possible was considered meaningful. To our knowledge, our experimental design has never been used by other investigators.

In this type of experiments, optimal timing between low-dose radiation and tumor cell inoculation may be difficult to determine. We did not find any difference among various timings. Several studies indicate that enhancement of immune response occurs at 6–24 h after low-dose irradiation.\(^6\)–\(^9\) In this experiment, however, it is unclear which interval between low-dose TBI and tumor cell inoculation is most efficient in rejecting tumor cells, because it is unknown at which time after tumor cell inoculation the immune response plays the most important role. The timing should be a topic of further study as well as the effect of low-dose irradiation on tumor transplantability. There may be a possibility that repeated stimulation with low doses of radiation works better, since many studies have indicated that immune response by low-dose TBI is temporary.\(^6\)–\(^9\)\(^,\)\(^11\)

We could not find statistically significant differences in overall tumor transplantability curves, using the logrank test in both tumor lines. However, the mean time to tumor appearance was significantly elongated in the Balb/c mice receiving 200 mGy of TBI 6 h beforehand, after inoculation of both 100 and 1000 EMT6 cells. This phenomenon was also observed in the mice receiving 100 mGy TBI and inoculated with 1000 EMT6 cells. We consider that the phenomenon of delay in tumor appearance should be a manifestation of stimulation of immune responses, and under more optimal conditions inducing stronger stimulation, tumor transplantability rates may be lowered by low-dose TBI. In EMT6 tumors, repeated administration of low-dose TBI may be a next step to be investigated. In SCCVII tumors, tumor transplantability rates were relatively low after 100 and 1000 cells, so inoculation of larger numbers of tumor cells may be investigated together with the optimal method of low-dose irradiation. We think that anti-tumor effects and immune responses by low-dose irradiation may be proven experimentally under optimal conditions for each living organism.

In summary, we observed elongation of the mean time to tumor growth in Balb/c mice transplanted with EMT6 tumors by low-dose TBI. Although this observation alone may not be a strong evidence for anti-tumor effects of low-dose irradiation, the present study would warrant further investigations under various conditions, and we will continue to investigate the effect of low-dose radiation towards the final goal of utilizing it in clinical cancer therapy.

**REFERENCES**

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