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A New Experimental System for Irradiating Tumors in Mice Using a Linear Accelerator under Specific Pathogen-Free Conditions

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We developed a reliable system for the irradiation of xenografted tumors in mice which allows for accurate local irradiation under specific pathogen-free conditions. The system presented here consists of acrylic supports for mice and an acrylic box connected to a pump through 0.22 μm pore-sized filters. Mice with xenotransplanted tumors growing on their right hind legs were set on the supports and put into the box in a laminar flow hood. The tumors of 7 mice were irradiated simultaneously with X-rays of 6 and 10 MV generated by a linear accelerator at a dose rate of 3.1-4.7 Gy/min. The air was ventilated through filters during irradiation in the closed box. Microorganism tests confirmed that no bacteria entered or left the box. One of the significant characteristics of this setup is that it allows for irradiation under conditions of acute hypoxia, which is obtained using an integrated tourniquet. The dose variation among 7 tumors was less than 1 %. The rest of the mouse's body was shielded effectively by a half-field technique and a lead block. As a result, the whole body dose for the mice was 0-4% of the total dose absorbed by the tumor. Due to the high dose rate and the ability to irradiate 7 mice simultaneously under specific pathogen-free conditions, this new system can be considered a time-saving and valuable tool for radiation oncology research.

Key words: animal experiment, mouse, radiotherapy, linear accelerator, specific pathogen-free

Accurate local irradiation of mouse tumors is indispensable in many radiation oncology experiments. However, actual irradiation is often far from ideal due to the following limitations. First, as devices for irradiation are often set in a room under conventional conditions, it is sometimes difficult to maintain mice under specific pathogen-free (SPF) conditions during irradiation. Second, the relative biological effectiveness (RBE) of the radiation beams used for radiobiological experiments often differs from that of the beams used for clinical radiotherapy (1). Third, experimental irradiation is often time-consuming due to the need to reproduce clinical fractionation schedules. Therefore, it would be effective and time-saving if many mouse tumors could be irradiated simultaneously with uniform doses at high dose rates. Finally, scattered radiation on the mouse bodies should be strictly avoided. Sophisticated dosimetry is the basis of radiation experiments when these devices are used for local irradiation, but many reports have not provided details of the dosimetry used with their devices. Herein, we present a new system for accurate and time-saving local irradiation of mouse tumors under SPF conditions.

Materials and Methods

Phantom experiments. We used X-rays of 6 and 10 MV from a linear accelerator (Mevatron 77, Toshiba Medical Co., Tokyo, Japan) for irradiation, because these are the same as those used for clinical radiotherapy. First, we measured the dose distributions of these beams in order to determine the most suitable positions in which to set the mouse tumors. We defined

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X- and Y-axes as shown in Fig. 1. We measured dose distributions according to depth, as shown in Fig. 2A. The water bath was irradiated in a 40 × 40 cm field at a 100 cm source-surface distance (SSD). The distributions were measured along the X-axis from 0 to + 30 cm at each depth from 14 to 300 mm with a dosimetry system (DynaScan, Computerized Medical Systems Inc., MO, USA). Scattered radiation outside the irradiation fields was measured, as shown in Fig. 3A. We used a half-field technique (2) to avoid scattered radiation, and examined whether or not a lead block was effective in preventing scattered radiation. The lead block was set in a 100-mm-thick acrylic plate. The X-ray film (Kodak X-Omat TL, Eastman Kodak Co., NY, USA) was put on the acrylic plate. The film was irradiated with X-rays of 0.07 Gy through the acrylic plate at a dose rate of 3.1–4.7 Gy/min in a 36 × 36 cm field at an 80 cm SSD. Scattered radiation was measured parallel to the X-axis for the -X area half-field (X-HF), and parallel to Y-axis for the -Y area half-field (Y-HF), as shown in Fig. 3B. Other film was irradiated with X-rays of 0.5 Gy at a dose rate of 4.7 Gy/min with and without a lead block for the Y-HF. The dose distributions inside the irradiation fields were examined at distances of 5–25 mm from the axis, as shown in Fig. 3C. The doses were scanned parallel to the Y-axis in the X-HF, and parallel to the X-axis in the Y-HF. The film was irradiated with X-rays of 0.07 Gy using the half-field technique and the lead block. Irradiation in acute hypoxia was achieved with an integrated tourniquet. The

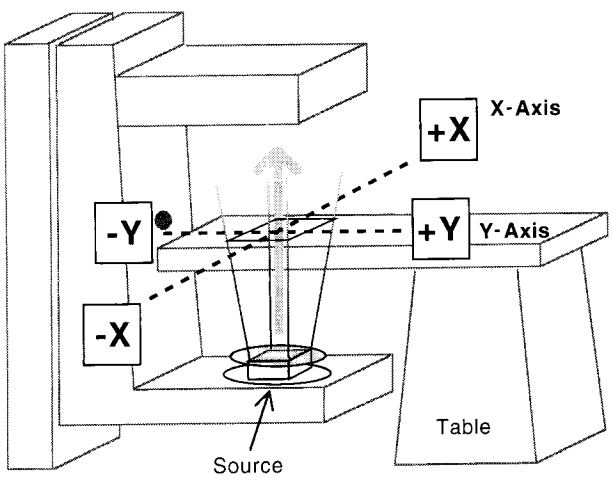

Fig. 1 The X- and Y-axis scheme. Irradiation with megavoltage X-rays is performed across these axes.

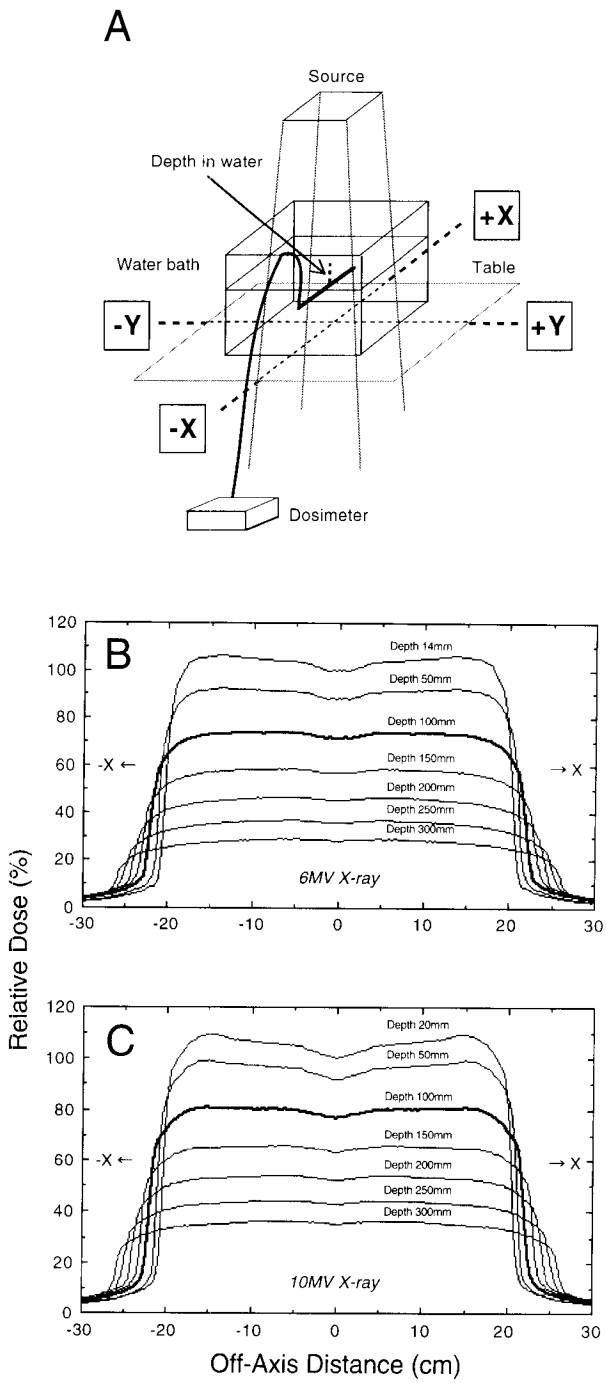

Fig. 2 Dose distributions of megavoltage X-rays. (**A**) The measurement scheme using a dosimetry system. The dose distributions of 6 MV (**B**) and 10 MV (**C**) X-rays. The dose distributions, which were measured from 0 to + 30 cm along the X-axis, were symmetrical on the graphs to indicate distributions from 0 to − 30 cm along the X-axis. The relative doses (%) are the rates of measured doses for the doses at 0 cm at 14 mm (**B**) and 20 mm (**C**) depth.

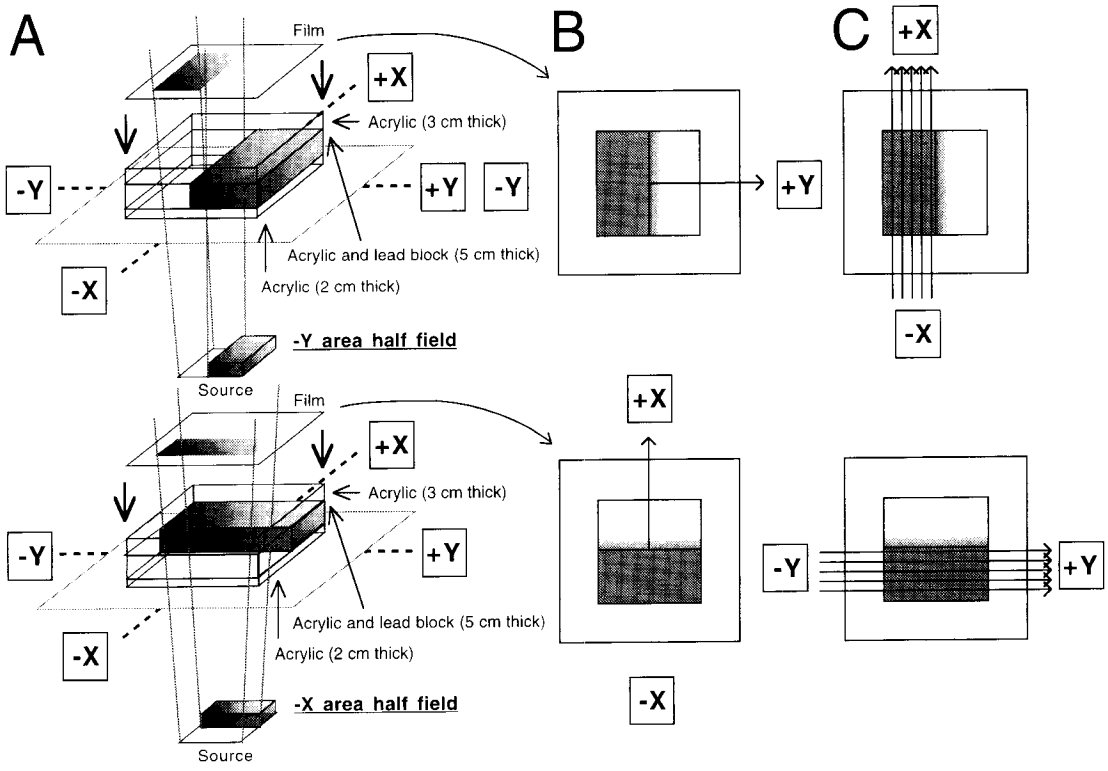

Fig. 3 The dose measurement scheme using X-ray films. (**A**) The scheme of experiments. The scan direction on X-ray films for the detection of the scattered radiation doses outside the irradiation field (**B**) and the doses inside the irradiation field (**C**).

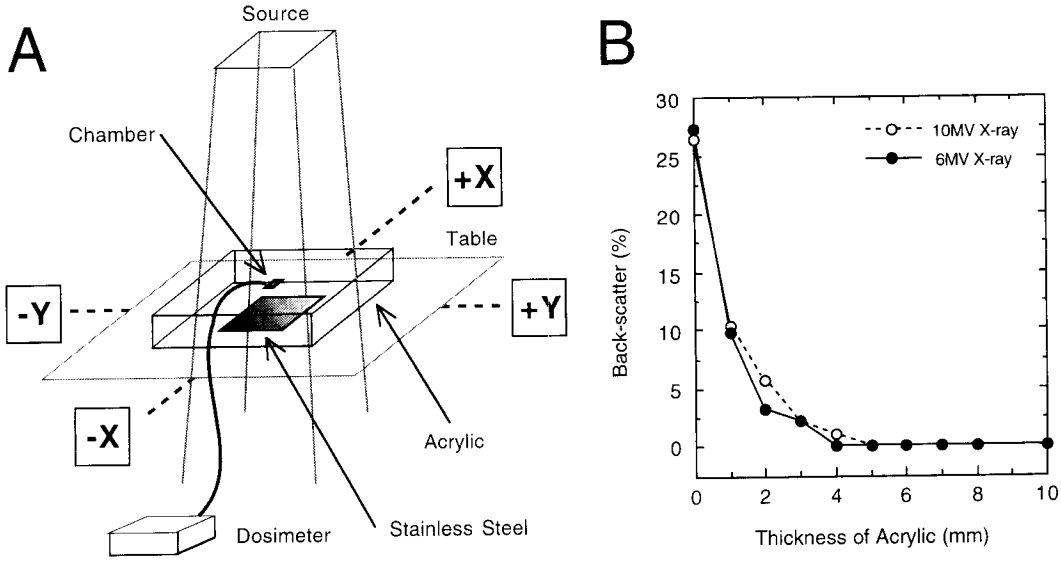

Fig. 4 Back-scattered radiation from a stainless steel plate. (**A**) The measurement scheme using a dosimetry system. (**B**) Back-scattered radiation from the stainless steel plate. The horizontal axis indicates the thickness of the acrylic spacer. The vertical axis indicates the increase in back-scattered radiation (%) with and without the stainless steel plate.

blood supply to the tumors was stopped before and during irradiation by clamping the thighs of the mice with 3-mm-thick stainless steel clamps. We measured the back-scattered radiation from the stainless steel plate, as shown in Fig. 4A. A 0.03 cm^3 shallow chamber (IONEX2532/3, Nuclear Enterprises Ltd., Reading, England) was put on 0–10 mm thick acrylic spacers, which were set on a 3-mm-thick stainless steel plate. The stainless steel plate was irradiated with X-rays through the chamber and an acrylic spacer in a 10×10 cm field and at a 100 cm source-chamber distance (SCD). The dose on the surface of the acrylic spacer was measured with a dosimeter (IONEX2500/3, Nuclear Enterprises Ltd.) with and without the stainless steel plate to determine which thickness of acrylic spacer was effective in preventing back-scattered radiation from the stainless steel clamps.

Dose measurement in the device made for local irradiation of mouse tumors. We measured the doses of 9 mouse tumors with the new device. The 0.03 cm^3 shallow chamber was put on the same positions where the mouse tumors were set 2 cm inside the irradiation field at 3 cm intervals within a 30 cm width. The chamber was irradiated with X-rays at a dose rate of 3.1–4.7 Gy/min in a 4.8×32 cm field at an 80 cm SCD. The X-HF and Y-HF were irradiated with 6 and 10 MV X-rays, respectively, and the doses were measured with a dosimeter.

Microorganism tests in the system. We examined the presence of bacteria in the irradiation system using stamp methods (3). The supports, which were sterilized with ethylene oxide gas, were put into a box which was closed with a lid, as shown in Fig. 5B. A pump (Fig. 5k) sent air into the box through 0.22 μm pore-sized HEPA filters (HEPA capsule filter, Gelman Sciences, MI, USA, Fig. 5j) for 6 h. The control support, which was sterilized with ethylene oxide gas, was put on a linear accelerator table for 6 h. The air leaving the box through the filter was also examined. The box was set on the table without a lid. The air from the tube, which was connected to a filter, was trapped on culture dishes in the laminar flow hood for 6 h. Ten positions on each support and culture dish were stamped with agar sausages (Stamp Media BHI Set, Eiken Kizai Co., Tokyo, Japan). These agars were incubated in dishes 6

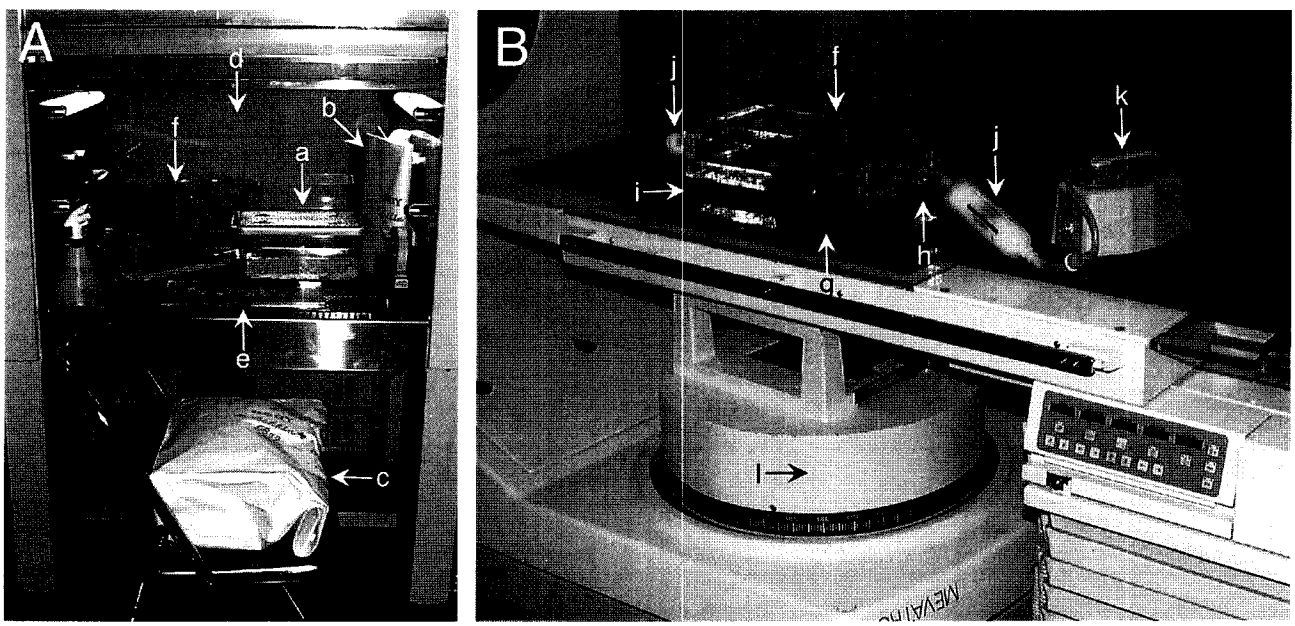

Fig. 5 System made for local irradiation of mouse tumors under specific pathogen-free conditions. The mice are put into an autoclaved cage (a) and then into an autoclaved sterilization bag (b) in the animal facility. Then the bag (c) is put into a laminar flow hood (A and d). The mice are anesthetized, taped onto supports (e), and put into the box (f). A lid is put on the box, and the box is connected to HEPA filters (j) via tubes. Then the box is put on a linear acceralator table. The box is put on 2-cm-thick (g) and 5-cm-thick (h) acrylic plates and a 5-cm-thick lead block (i). The positions of the mouse bodies are set on the lead block. A pump (k) is connected to the HEPA filters (j), so that air flows into the box during irradiation.

cm in diameter for 24 h at 37 °C, after which the number of colonies was counted.

Results

Phantom experiments. The dose distribution of megavoltage X-rays changed depending on the depth of target due to build-up and flattening filter (4) of the linear accelerator. Figs. 2B and 2C indicate that although the dose distributions became flat, the doses decreased as the depth increased. We decided to irradiate the mouse tumors at a 10 cm depth through a 10-cm-thick acrylic plate in the following experiments. As far as possible, those areas of the mouse body which are outside the area to be irradiated should be protected from scattered radiation. Scattered radiation outside the irradiation fields decreased when a lead block was used, as shown in Fig. 6A. Figs. 6B and 6C indicate that the combined use of the half-field technique and a lead block is effective to prevent scattered radiation. Scattered radiation doses in the mouse bodies at 1 cm outside the irradiation fields were 4 % and 3 % of the maximum tumor doses in X-HF and Y-HF with 10 MV X-rays, respectively, and 3 % of the maximum tumor doses with 6 MV X-rays. The doses inside the irradiation fields increased as the distance from the edge of the irradiation fields increased, as shown in Fig. 7. The doses became almost uniform at more than 10 mm from the edge of the irradiation fields. We decided to set the mouse tumors 20 mm from the edge of the irradiation fields. The 6 MV X-rays in X-HF and 10 MV in Y-HF showed more uniform dose distributions than the 6 MV X-rays in Y-HF and 10 MV in X-HF. The acrylic spacers decreased back-scattered radiation from a 3-mm-thick stainless steel plate, as shown in Fig. 4B. The back-scattered radiation on the surface of the steel plate was 27 % and 26 % with 6 MV and 10 MV X-rays, respectively. This back-scattering of 6 MV and 10 MV X-rays decreased to 0 % when acrylic spacers of 4 mm and 5 mm thickness, respectively, were used.

The device made for local irradiation of mouse tumors. Based on the results of the phantom experiments, we constructed a device for the local irradiation of mouse tumors, as shown in Fig. 8. This device consists of 2-cm-thick (Fig. 8a) and 5-cm-thick (Fig. 8b) acrylic plates, a 5-cm-thick lead block (Fig. 8c), a 2-cm-thick acrylic box (Fig. 8d), a lid for the box (Fig. 8e) and supports for mice made of 1-cm-thick acrylic plates (Figs. 8f and 8g). The box has connections on both sides for ventilation with filtered air. We made two kinds of supports for the mice: Fig. 8f shows a support which can be used for aerobic experiments and Fig. 8g shows a support with 3-mm-thick stainless steel clamps which cut off blood

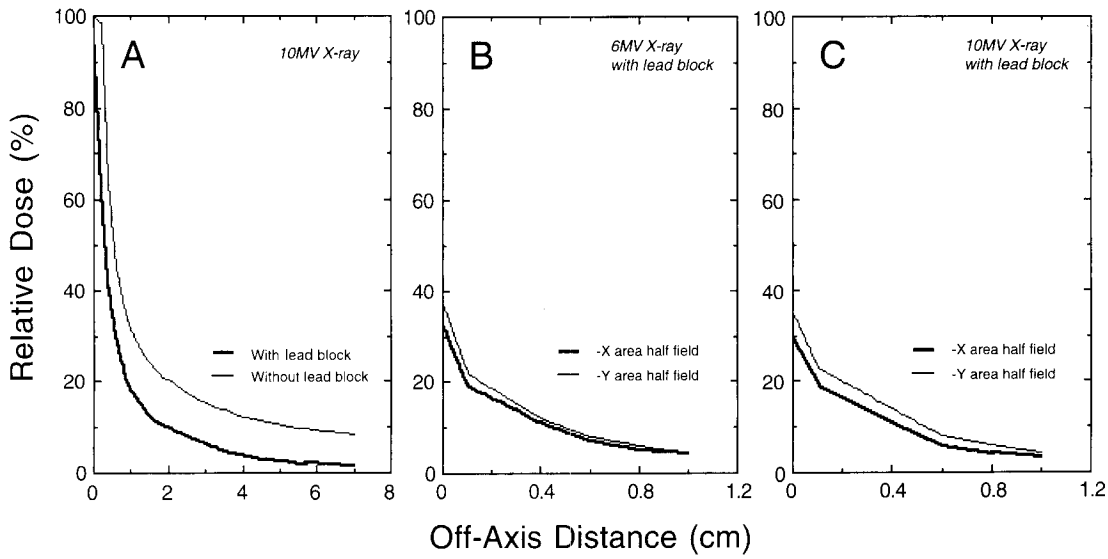

Fig. 6 Scattered radiation doses outside the irradiation fields from 10 MV (**A** and **C**) and 6 MV (**B**) X-rays. The horizontal axis indicates the distance from the edge of the irradiation field. The vertical axis indicates the actual radiation received as a percentage of the maximum dose on the axis (**A**) and that of the maximum doses inside the irradiation fields (**B** and **C**).

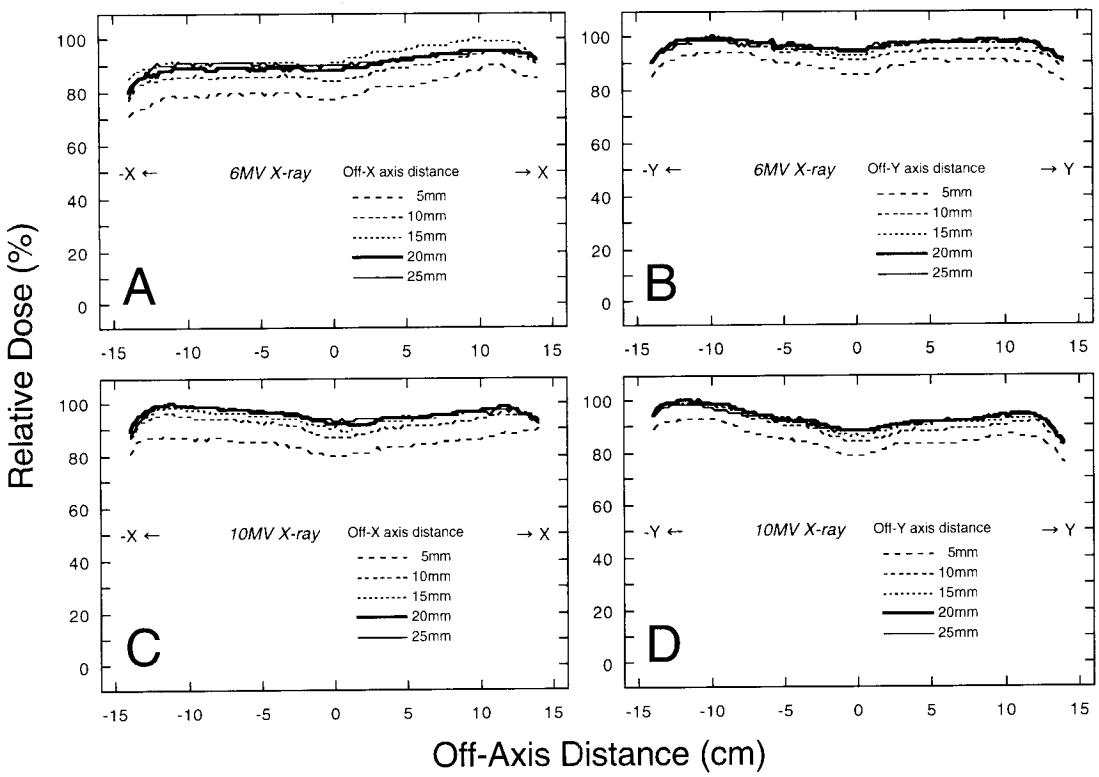

Fig. 7 Doses inside the irradiation fields. The horizontal axis indicates the off-axis distance. The vertical axis indicates the actual radiation received as a percentage of the maximum possible dose inside the irradiated fields. The -X area half-field (**B** and **D**) and -Y area half-field (**A** and **C**) were irradiated with X-rays of 6 MV (**A** and **B**) and 10 MV (**C** and **D**).

flow to the mouse thighs for use in experiments involving hypoxia in the tumors. The 5-mm-thick acrylic spacers (Fig. 8h) are inserted under the clamps to prevent back-scattered radiation. Nine mice can be taped on either support at 3 cm intervals within a 30 cm width.

The system for local irradiation under SPF conditions. The mice used for radiation experiments are put into an autoclaved cage (Fig. 5a) and then into an autoclaved sterilization bag (HM-62, Hogy Medical Co., Ltd., Tokyo, Japan, Fig. 5b) in the animal facility. The mice in the bag (Fig. 5c) are brought into a laminar flow hood (Figs. 5A and d), and are anesthetized and taped onto the supports (Fig. 5e) in the laminar flow hood. The mouse tumors and bodies are set 20 mm inside and 10 mm outside the irradiation fields, respectively. The mice on supports are put into the box (Fig. 5f). The lid is put on the box, and the box is connected to 0.22 μm pore-sized HEPA filters (Fig. 5j) via tubes, after which it is put on the linear accelerator table. The box is put on 2-cm-thick (Fig. 5g) and 5-cm-thick (Fig. 5h) acrylic plates and a 5-cm-thick lead block (Fig. 5i), as shown in Fig. 5B. The positions of the mouse bodies are set on the lead block. The pump (Fig. 5k) is connected to the HEPA filters and air flows into the box during irradiation. The tumors are irradiated with 6 MV X-rays in the X-HF and with 10 MV X-rays in the Y-HF. After irradiation, the mice are returned to cages in laminar flow hood. The cages are put into autoclaved sterilization bags and returned to the breeding rooms of the animal facility. Thus, the mice are treated in a closed system, which maintains SPF conditions throughout the experiments.

Doses in the device made for local irradiation. Fig. 9 shows that the doses at 7 tumor positions were uniform except at each end of the device. The doses ranged from 98 to 102 % when the off-axis distances were changed from -11 to $+11$ cm. The average $\pm$ standard deviation (SD) of these 7 doses was 100 ± 1 %, when the dose at the central position was set at 98 %.

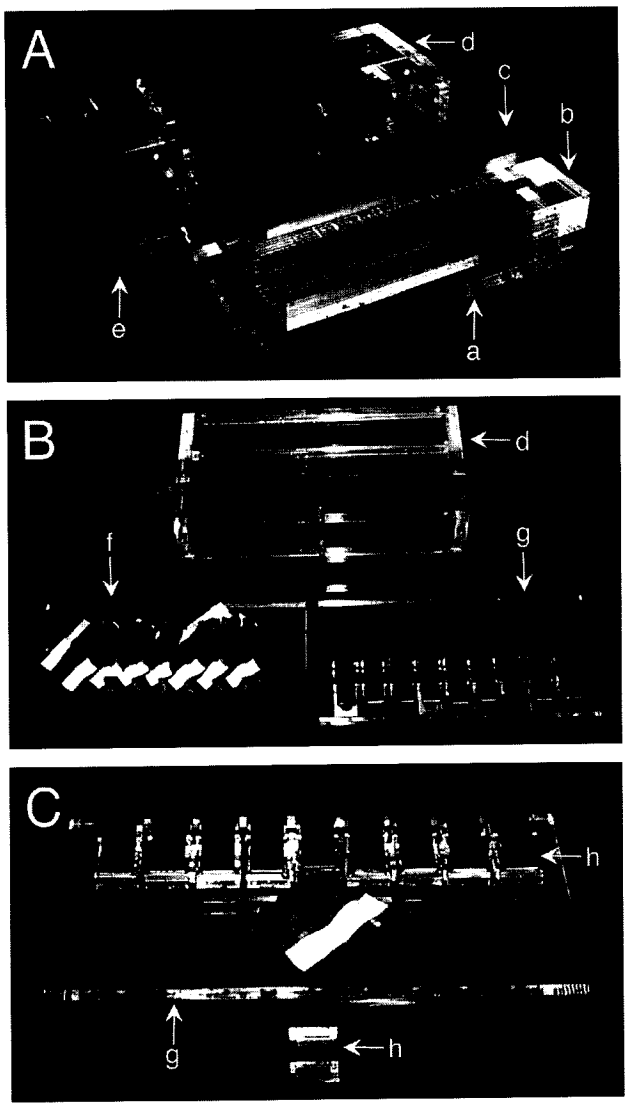

Fig. 8 Device made based on phantom experiments. This device consists of 2-cm-thick (a) and 5-cm-thick (b) acrylic plates, a 5-cm-thick lead block (c), a 2-cm-thick acrylic box (d), a lid (e), supports for the mice made of 1-cm-thick acrylic plates (f and g), and 5-mm-thick acrylic spacers (h).

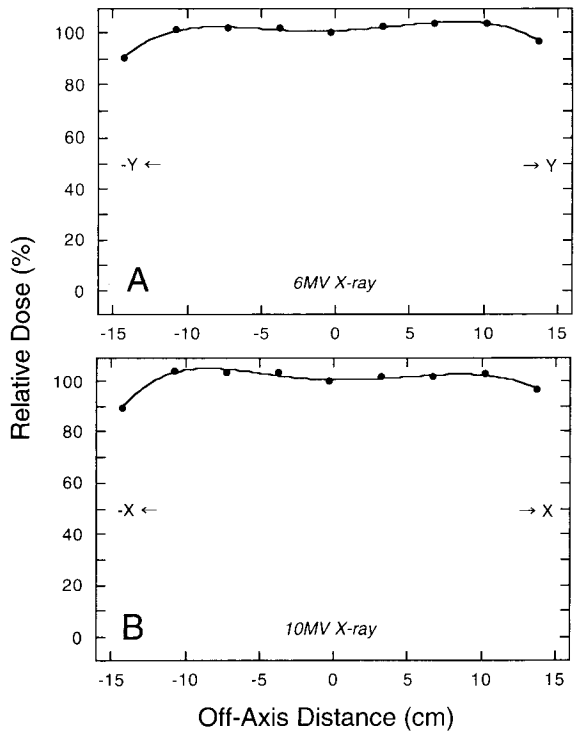

Fig. 9 Doses in the device made for local irradiation of mouse tumors. The doses at 9 tumor positions, which were 2 cm inside the irradiation field at 3 cm intervals, were measured. The horizontal axis indicates off-axis distance, while the vertical axis indicates the doses received as a percentage of the dose received in the central position. The -X area and -Y area half-fields were irradiated with X-rays of 6 MV (**A**) and 10 MV (**B**), respectively.

Microorganisms in the system. The average number of colonies ± SD was 0 ± 0 per dish using this system and those in control were 2 ± 1 per dish, indicating that the air filters in the system intercept bacterial contamination from outside. Moreover, the average number of colonies ± SD was 0 ± 0 per dish when the outflow of air from the box was examined, indicating that filters on both sides of the box also prevented bacterial contamination of the irradiation room from inside of the box.

Discussion

Accurate local irradiation of mouse tumors is indispensable for many experiments in radiation oncology. Various devices are used to achieve this goal and to produce various types of radiation such as low-voltage X-rays, megavoltage X-rays and γ-rays. Low-voltage X-rays such as ones of 100–250 kV are most frequently used, but the RBE of these beams is higher than the RBE of beams used in clinical radiotherapy. The RBE of megavoltage X-rays and ^{60}Co γ-rays is in the range 0.8–0.9 compared to low-voltage X-rays (1). Although the devices which produce megavoltage X-rays and γ-rays are favorable from the viewpoint of RBE, irradiation of

mouse tumors under SPF conditions is sometimes difficult because these devices are usually set under conventional conditions. The tumor control dose (TCD)-50 method (5–7) is a reliable and frequently used method for analysis of the *in vivo* effects of radiotherapy. The goal of the TCD-50 method is tumor cure, so mice must be treated under SPF conditions throughout their lives. Only a few institutes have devices using radioisotopes, such as ^{137}Cs, which are suitable for local irradiation of mouse tumors under SPF conditions (5–7).

Stüben *et al.* reported the creation of an excellent and time-saving experimental irradiation system using a linear accelerator (8), although the system is designed to be used under conventional conditions. Their system makes possible simultaneous irradiation of 10 mice with dose variation of less than 4 % at a dose rate of 2.5 Gy/min. Our new system allows simultaneous irradiation of 7 mice with dose variation of 1 % SD at a dose rate of 3.1–4.7 Gy/min. The improvements in dose variation and dose rate may be due to the performance of our linear accelerator which has an excellent flattening filter (4) and a short SSD of 80 cm. The scattered radiation in mouse bodies was 8 % with their system and 4 % with our system. The reduction in scattered radiation was brought about by the combined use of a half-field technique and a lead block.

One of the significant characteristics of the setup is the ability to irradiate under conditions of acute hypoxia, which is achieved with an integrated tourniquet. Acute hypoxia is often necessary for *in vivo* studies on repopulation and reoxygenation after irradiation (6, 9). If the mouse thighs are constricted with clamps, starting 5 min before irradiation and continuing throughout irradiation, complete hypoxia can be achieved. This procedure is performed under general anesthesia, and is well tolerated by the mice.

This new system is a closed system. 2 HEPA filters on both sides of the box completely intercept bacterial contamination from outside and inside. This makes possible irradiations of human tumors transplanted onto immunodeficient mice such as nude and SCID mice (5).

In conclusion, with this new system accurate and time-saving local irradiation of mouse tumors under SPF conditions can be performed. Therefore, this system appears to be a suitable tool for radiation oncology research.

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