EFFECTS OF FRACTIONATED RADIATION ON THE BRAIN VASCULATURE IN A MURINE MODEL: BLOOD–BRAIN BARRIER PERMEABILITY, ASTROCYTE PROLIFERATION, AND ULTRASTRUCTURAL CHANGES

HONG YUAN, PH.D.,*‡ M. WALEED GABER, PH.D.,* KELLI BOYD, D.V.M., PH.D.,‡ CHRISTY M. WILSON, M.S.,* MOHAMMAD F. KIANI, PH.D.,§ AND THOMAS E. MERCHANT, D.O., PH.D.†*

*Department of BioImaging, College of Health Science Engineering, University of Tennessee Health Science Center, Memphis, TN; †Department of Radiological Sciences, St. Jude Children’s Research Hospital, Memphis, TN; ‡Animal Research Center, St. Jude Children’s Research Hospital, Memphis, TN; and §Departments of Mechanical Engineering and Radiation Oncology, Temple University, Philadelphia, PA

Purpose: Radiation therapy of CNS tumors damages the blood–brain barrier (BBB) and normal brain tissue. Our aims were to characterize the short- and long-term effects of fractionated radiotherapy (FRT) on cerebral microvasculature in mice and to investigate the mechanism of change in BBB permeability in mice.

Methods and Materials: Intravital microscopy and a cranial window technique were used to measure BBB permeability to fluorescein isothiocyanate (FITC)-dextran and leukocyte endothelial interactions before and after cranial irradiation. Daily doses of 2 Gy were delivered 5 days/week (total, 40 Gy). We immunostained the molecules to detect the expression of glial fibrillary acidic protein and to demonstrate astrocyte activity in brain parenchyma. To relate the permeability changes to endothelial ultrastructural changes, we used electron microscopy.

Results: Blood-brain barrier permeability did not increase significantly until 90 days after FRT, at which point it increased continuously until 180 days post-FRT. The number of adherent leukocytes did not increase during the study. The number of astrocytes in the cerebral cortex increased significantly; vesicular activity in endothelial cells increased beginning 90 days after irradiation, and most tight junctions stayed intact, although some were shorter and less dense at 120 and 180 days.

Conclusions: The cellular and microvascular response of the brain to FRT is mediated through astrogliosis and ultrastructural changes, accompanied by an increase in BBB permeability. The response to FRT is delayed as compared with single-dose irradiation treatment, and does not involve leukocyte adhesion. However, FRT induces an increase in the BBB permeability, as in the case of single-dose irradiation. © 2006 Elsevier Inc.


INTRODUCTION

Radiation therapy directed at brain tumors can damage the glial, neuronal, and vasculature compartments of the brain, limiting the doses of radiation that can be safely delivered to patients (1–4). The causes and interconnections of the side effects of radiation treatment are not yet fully understood (5). Our studies, as well as those of others, have shown that ionizing radiation can disrupt the blood–brain barrier (BBB). This disruption may exacerbate radiation-induced brain toxicity (5–9). Research into irradiation effects in normal brain tissue has been principally limited to studies of single-dose irradiation (5, 6, 9–12). However, large single doses of radiation are not commonly used in the clinic; exceptions include radiosurgery, pulsed high-dose-rate brachytherapy, and some hypofractionated palliative treatment regimens. Although fractionated radiotherapy (FRT) is the leading mode of radiation delivery, the effects of fractionated doses of ionizing radiation on the brain microvasculature have not been thoroughly investigated (13–15). In previous studies (16), we have observed a difference in cellular response to fractionated and single-dose brain treat-
ments; the single-dose—response was rapid, within hours of treatment, whereas the fractionated response was delayed until the end of treatment (30 days) (16).

In a previous study (9), we characterized the effects of large single doses of irradiation on the cerebral microvasculature, showing that ionizing radiation increases the BBB permeability to fluorescein isothiocyanate (FITC)-dextran molecules of various sizes. We also found that the increased BBB permeability is associated with an increase in cell adhesion (9), findings that are consistent with those of other researchers (13, 14, 17, 18).

In the current study, we characterized the acute effects (during and right after), as well as the long-term effects (up to 180 days postirradiation) of fractionated irradiation on BBB permeability in normal mouse brain pial vessels. Intravital microscopy and a closed cranial window technique were used. Using immunohistochemistry and electron microscopy (EM) techniques, we also investigated the relationship between changes in the ultrastructure of the brain and the irradiation-induced changes in BBB permeability. This study develops a fractionated RT animal model that was used to study the radiation-induced changes in BBB permeability, endothelial cell adhesion, structural changes caused by radiation, as well as the cellular response, especially astrocytic activity. The model can be used further to study interventional methods aimed at ameliorating the side effects of RT.

METHODS AND MATERIALS

Animals

Male C57BL/6J mice (6–7 weeks of age) were purchased from Harlan Laboratories (Frederick, MD) and used in the study. All protocols followed were approved by the Animal Care and Use Committees of St. Jude Children’s Research Hospital and the University of Tennessee Health Science Center (Memphis, TN). We also followed the policy guidelines of the National Institutes of Health for the humane care and use of laboratory animals.

Cranial window preparation

Cranial window surgery was performed according to our previously published protocol (19). Briefly, animals were anesthetized with an i.m. injection of a solution of ketamine (100 mg/kg) and xylazine (10 mg/kg). The animals were then placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). We removed the scalp, underlying soft tissue, skull bone, and dura. A 4 × 6 mm glass window that extended from the bregma to lambdoid sutures and was centered on the midsagittal suture was placed and fixed over the surgically exposed cerebral cortex using cyanoacrylate glue. After surgery, animals were allowed to recover for 1 week before data collection was initiated.

Experimental design

Mice were divided into two study groups. In the acute effects group (n = 6), cranial window surgery was conducted 1 week before the start of the fractionated irradiation, and BBB permeability was measured after 1, 2, 5, 10, 15, and 20 irradiation fractions (1 radiation fraction per day). Each animal served as its own control. Animals in the long-term effect group (n = 40) were subdivided into 4 groups (each with 10 animals) on the basis of time from the start of the fractionated radiation: 60 days, 90 days, 120 days, and 180 days. Each long-term effect group included irradiated mice (n = 6) and sham mice (n = 4); cranial window surgery was conducted on all animals in the long-term group 1 week before the measurements started. BBB permeability and cell adhesion were measured in all of the animals in the long-term groups at 60, 90, 120, and 180 days from the start of fractionated irradiation.

This schedule was designed to allow us to follow the acute and long-term effects of brain irradiation. As there was no precedence to go by, we chose 1 month as an adequate interval to follow the changes induced by FRT. Experiments were terminated at 180 days after the start of FRT (equivalent to one quarter of the life expectancy of the mice), which we considered to be sufficient to observe the long-term changes induced by radiation treatment.

Radiation treatment

All radiation treatments were administrated with a 6-MV X-ray linear accelerator (Siemens Primus, Concord, CA). Fractionated irradiation was delivered to the whole brains of the mice at a daily dose of 2 Gy, 5 days/week for 4 weeks, for a total of 40 Gy. Animals were placed in a customized chamber, which could hold 12 mice, connected to a gas anesthesia (2% isoflurane) machine. Animals were placed on a fluoroscopy unit (Phillips Medical Systems, New York, NY) radiation simulator (Siemens, Concord, CA) to determine the treatment area, and a customized lead shielding block (17 cm × 17 cm) was molded according to the measured area. During irradiation, the customized, lead shielding block covered the bodies of the animals to ensure that only the brains were irradiated. Tissue-equivalent material (1 cm thick) was placed above the head of each animal to establish electronic equilibrium. To ensure proper positioning of the animals, they were imaged during irradiation using the digital portal system EPID (Siemens, Concord, CA).

BBB permeability and cell adhesion measurement

Measurement of BBB permeability and cell adhesion using intravital microscopy has previously been detailed (9, 19). Briefly, to visualize cerebral microcirculation, animals were anesthetized with a solution of ketamine and xylazine, as described above, and placed in a stereotaxic frame under a fluorescence intravital microscope. As a permeability tracer, we used FITC-dextran molecules (Sigma, St. Louis, MO) with molecular sizes of 4.4 and 38.2 kD. Before dye injection, a microvascular region with 2 to 3 venules and clear parenchymal tissue was chosen, and a reference image (for localization of vessel) was first recorded under epifluorescence illumination. A bolus of FITC-dextran in saline (10 mg/kg body weight) was injected through the retro-orbital vein. To minimize fluorophore excitation, changes in brain tissue intensity levels were digitally recorded after injection every 1 s for the first 60 s, every 30 s for 5 min, and then every 1 min for 20 min. The images were later analyzed off-line by using MetaMorph software (Universal Imaging Co., West Chester, PA) to measure the extravasation rate of FITC-dextran from vessels to tissue, which is proportional to the slope of the change of tissue intensity vs. time (19).

Leukocyte adhesion was measured by injecting Rhodamine-6G (0.4 mg/kg body weight; Sigma, St. Louis, MO) through the retro-orbital vein to stain the leukocytes. A leukocyte in contact with the vessel wall was considered to be adherent if it did not move for 30 s; this measure is expressed as the number of adherent