Brain damage from $^{125}$I brachytherapy evaluated by MR imaging, a blood-brain barrier tracer, and light and electron microscopy in a rat model

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Changes in normal rat brain were studied acutely, and at 3, 6, 9, and 12 months following interstitial brachytherapy with high-activity $^{125}$I seeds. An 80-Gy radiation dose was administered to an area with a 5.5-mm radius. Effects were measured with magnetic resonance (MR) imaging (with and without gadolinium enhancement), leakage of horseradish peroxidase (HRP), electron microscopy, and light microscopy. Significant histological damage was seen at radiation doses above 295 Gy, and breakdown of the blood-brain barrier was observed only in tissue receiving a dose of 165 Gy or greater. Blood-brain barrier breakdown increased up to the 6-month time point, and thereafter appeared to stabilize or decrease. The area of blood-brain barrier disruption indicated by gadolinium-enhanced MR imaging was greater than that indicated by leakage of HRP.

Key Words • interstitial brachytherapy • brain damage • horseradish peroxidase • magnetic resonance imaging • blood-brain barrier • rat

The clinical applicability of interstitial brachytherapy is currently being examined in clinical studies of patients with cerebral neoplasms. This therapy appears to have unequivocal value in selected patients with recurrent malignant astrocytoma and perhaps a small group with recurrent solitary brain metastasis. The role for this modality in patients with refractory skull-base tumors is more difficult to evaluate, and the indications for brachytherapy as part of the initial treatment for patients with malignant astrocytoma is currently the subject of ongoing randomized studies.

Radiation-induced brain damage presenting early as cerebral edema and later as so-called “radiation necrosis” is a significant complication of interstitial brachytherapy, and has been studied in a small number of animal models. An improved understanding of the time course and specific pathophysiology of interstitial brachytherapy brain damage might allow neuro-oncologists to manipulate radiation parameters or perhaps add pharmacological agents to brachytherapy regimens to decrease morbidity. The present study was undertaken to produce a consistent small-animal model of interstitial radiation-induced brain damage quantitatively assessable by modern imaging techniques (magnetic resonance (MR) imaging) as well as standard light and electron microscopic examination and horseradish peroxidase (HRP) leakage.

Materials and Methods

Implantation Technique

Male F-344 rats, weighing 200 ± 20 gm (mean ± standard deviation), were used in all experiments. For seed implantation, animals were anesthetized with intraperitoneal pentobarbital and placed in a commercially available stereotactic frame.* The scalp was opened in the midline and a bone trough parallel to the midline, 8 mm long and 1 mm wide, was drilled 2.5 mm to the right of midline and centered on a point 4 mm anterior to the interaural line, such that the dura was not transgressed. An iodine-125 ($^{125}$I) seed (see next section) was placed in the trough and secured with a small piece of sterile tape. The scalp was closed with sutures and the animal placed in an individual cage within a large lead-lined fume hood for the duration of the irradiation period. The entire process was duplicated.

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* Stereotactic frame manufactured by Trent Wells, Southgate, California.
for all control animals except that a dummy seed was used instead of an active one. At the designated time for seed removal, the rats were anesthetized in an ether jar, the scalp was opened, and the seed was removed. Animals were then returned to the Animal Care Facility of the Toronto Western Hospital for the requisite observation period (0, 3, 6, 9, or 12 months) with food and water ad libitum. A total of 60 irradiated and 10 control animals were used in the present study.

Radioactive Isotope and Dosimetry

Iodine-125 seeds of mid to high activity (approximately 20 mCi) were used in all animals except control animals in which dummy seeds were used. The seed strength at the time of implantation was recorded and each rat was anesthetized with an intraperitoneal injection of pentobarbital. The rat was then placed in a Styrofoam holder and a 3-in, circular surface coil was positioned over the head. A protocol to obtain T₁- and T₂-weighted images was followed. The T₁-weighted imaging sequence outlined above was repeated after the rat had received an intravenous injection of Gd-DTPA (Magnevist, 1 mmol/kg) via femoral vein cutdown. The Gd-DTPA was allowed to circulate for 20 minutes before imaging was commenced. Time course measurements of signal intensity versus time showed that enhancement peaked at 20 ± 5 minutes and then decayed slowly with a time constant of 3.5 hours. Total scan time was less than 1 hour per animal. Control rats were imaged in the same way as treated rats.

The MR images were examined for evidence of enhancement with Gd-DTPA; the area of visible enhancement was traced onto parchment tracing paper and measured using a digitizing tablet as previously described. Drawings were calibrated using measurements of the fixed dehydrated brain sections of the same animal to correct for shrinkage during dehydration. Computer-assisted image analysis was used to measure the relative area of enhancement versus total area of the hemisphere. This ratio was multiplied by the total area of the hemisphere measured from vibratome sections taken from the same anatomical level to obtain an actual area (in square millimeters) of the gadolinium-enhanced lesion.

Horseradish Peroxidase

The integrity of the blood-brain barrier (BBB) after irradiation was evaluated using HRP in a 0.9% NaCl solution as a vascular tracer. Rats were divided into two groups: a short-term HRP circulation group to identify the sites of vascular leakage, and a long-term HRP circulation group to examine the extent of spread of blood-borne tracer and compare it with the spread of Gd-DTPA in the enhanced MR images.

Short-Term Circulation. After the final MR image, the rats were given an overdose of Somnotol intraperitoneally. The thoracic cavity was rapidly opened and HRP (100 mg/kg) was injected into the left cardiac ventricle. The injection was done slowly over 5 to 10 seconds to avoid a sudden increase in blood pressure. Thirty seconds after the start of tracer infusion the rats were decapitated and the brains were removed from the crania. The area of brain underlying the radioactive seed could be identified by its slightly discolored appearance. In the sham-operated rats the appropriate area was identified with reference to the groove in the overlying bone. The brain was bisected coronally

Fig. 1. Schematic diagram of a coronal section through the rat brain at the midpoint of a 125I seed (arrow). Isodose lines in this plane are circles centered on the seed.
through the midpoint of the seed's position. One-half was used to evaluate the histological changes in the brain parenchyma (see below). The other half was fixed by immersion in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for examination of BBB integrity.

After overnight fixation the brain fragments were washed twice in 0.1 M phosphate buffer and mounted in a vibratome. Seventy-micron coronal sections were cut and reacted for peroxidase activity using the cobalt-glucose oxidase method of Itoh, et al. The tissues were washed in 0.1 M Tris buffer (pH 7.6) and incubated in a 0.5% solution of cobaltous chloride in Tris for 10 minutes at room temperature followed by three washes in Tris. The tissues were then washed in phosphate buffer and incubated in a solution of 37°C temperature containing the following: 0.05% 3,3'-diaminobenzidine, 0.04% ammonium chloride, 0.2% D-glucose, and 0.003% glucose oxidase in phosphate buffer. The result of this incubation procedure is the deposition of a dark-brown reaction product at the site of the HRP enzyme. Sections were reacted in this mixture for 1 to 1.5 hours until the reaction product was of even intensity throughout the thickness of the tissues. Some of the sections were mounted on slides, dehydrated using a graded series of ethanol, and mounted on glass slides. These were examined under the light microscope to determine the intravascular and extravascular distribution of the reaction product. The remaining sections were used for electron microscopy as detailed below.

**Light Microscopy**

The other half of the removed rat brain was placed in formalin for 48 hours and then mounted in paraffin. Several adjacent sections through the midpoint of the 125I seed site were obtained and mounted on glass slides. Sections were stained with hematoxylin, phloxine, and saffron (HPS) for routine histology, glial fibrillary acidic protein to examine changes to astrocytes, Martius scarlet blue which stains fibrin and highlights fibrinoid degeneration of blood vessels, and Luxol fast blue which stains myelin.

All histology slides were examined and qualitative descriptions of blood vessel changes and cellular effects (reactive astrocytes) were recorded. Areas of radiation damage were measured by one blinded neuropathologist using a Zeiss microscope with an optical planimeter at a magnification of x 250. Radiation damage was considered to be a combination of one or more of the following: necrosis, inflammation, edema, calcification, fibrinoid damage of vessels, and/or fibrin deposition in brain parenchyma.

**Results**

**Magnetic Resonance Imaging**

The T₁- and T₂-weighted images showed little or no visible lesion at all time points after interstitial radiation. Gadolinium-enhanced T₁-weighted images did show relatively discrete increased signal intensity lesions in all irradiated animals. This was interpreted as reflecting the breakdown of the BBB (Fig. 2). Areas of BBB disruption appeared to increase up to the 6-month time point and thereafter decrease (Fig. 3). All visible BBB damage was seen in specimens with radiation doses above 165 Gy.

**Horseradish Peroxidase Leakage**

After 30-minute circulation times, HRP reaction product could be seen in the brain located immediately under the radioactive seed and extending into the tissue for a distance of 1 to 4 mm (Fig. 4). A comparison of the area of HRP deposition and Gd-DTPA enhancement after the same circulation time (30 minutes) revealed that the area of HRP reaction product was less...
FIG. 2. Magnetic resonance images of an animal that had received irradiation 6 months before (same animal as depicted in Figs. 4, 6, and 7). Left: T1-weighted coronal image through the midpoint of the 125I seed position. No obvious lesion is seen. Center: T2-weighted coronal image showing a slight increase in signal intensity at the radiation site (arrow). Right: T1-weighted image following administration of Gd-DTPA. Note the relatively discrete area of enhancement (arrow).

than half the Gd-DTPA area at 3- and 6-month time points. At 9 months after treatment, HRP extravasation was observed in only one animal and at 12 months no HRP extravasation could be detected in any animal (Fig. 3). In a small number of animals (about 10%) leakage of HRP along the corpus callosum across midline was observed. In acutely irradiated animals the area of HRP extravasation after 30 seconds of circulation, reflective of the area in which there is robust vascular leakage, was significantly less than the areas of HRP and Gd-DTPA enhancement after prolonged circulation times (Fig. 5).

Electron Microscopy

The most superficial millimeter of brain tissue immediately under the source of radiation consisted of necrotic or degenerative tissue. Vessels were either frankly disrupted or, if intact, had HRP-infiltrated cytoplasm indicating protein leakage through the endothelial membranes14 (Fig. 6A). Other vessel profiles showed swollen distorted endothelial cells (Fig. 6B). The interendothelial junctions in intact vessels appeared to be normal. The next millimeter layer included the corpus callosum and the superior aspect of the hippocampus posteriorly or striatum anteriorly and almost always contained leaking vascular segments (Fig. 6C). Here too occasional vessel profiles could be seen with HRP-infiltrated cytoplasm. Junctional clefts that were not continuous with either the lumen and ablumen did not contain tracer, although HRP could be found extending between adjacent endothelial cells as far as the first row of occluding junctional strands (Fig. 6D). Endothelial vesicles laden with HRP were often seen associated with HRP-infiltrated basement mem-

FIG. 3. Graph showing area of Gd-DTPA enhancement on magnetic resonance imaging (MRI), horseradish peroxidase leakage (HRP), and light microscopic damage at 0, 3, 6, 9, and 12 months following irradiation. Each point represents data from five to 12 animals. Bars represent standard errors of the mean.

FIG. 4. Photomicrograph showing horseradish peroxidase reaction product (hrp) in the same animal as depicted in Figs. 2, 6, and 7. n = necrotic tissue; cx = cerebral cortex; cc = corpus callosum; h = hippocampus; and lv = lateral ventricle containing hrp-laden choroid plexus. Arrowhead indicates the interhemispheric fissure.
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Fig. 5. Bar graph showing the area of apparent blood-brain barrier breakdown acutely after irradiation visualized on Gd-DTPA enhanced magnetic resonance images (MRI), horseradish peroxidase (HRP) circulated for 30 minutes comparable to Gd-DTPA circulation time, and HRP circulated for 30 seconds. Each bar represents data from six to eight animals. Error bars represent standard errors of the mean.

![Graph showing area of enhancement](image)

bran (Fig. 6E). Occasional vessel profiles with masses of vesicles were found, but most profiles contained the low density that is typical of the intact BBB. Vessels at 3- and 4-mm depth usually appeared normal at the electron microscopy level. These observations suggest that leakage of tracer from the vessels into the tissue occurred in a narrow zone close to the necrotic region. The mechanism of BBB leakage does not seem to include either vesicular transport or junctional abnormalities, but probably occurs directly through damaged endothelial cells.

Light Microscopy

On light microscopy a variety of pathological changes were observed. Some of these were characteristic of irradiation damage such as fibrinoid necrosis of vessels, fibrin “leaking” into the neuropil, large bizarre astrocytes, and calcification. Other changes were less specific, including necrosis, astrogliosis, and edema. Although the various pathological changes were generally found at the same site, noncontiguous foci of necrosis were occasionally present. Foci of calcification, when observed, were frequently distant from the primary focus of damage and were often not accompanied by other pathological alterations (Fig. 7). No histological damage was obvious in any tissue receiving less than 295 Gy.

Frank tissue loss was variable within each group and from group to group. However, the area of radiation damage did roughly parallel the area of abnormality on Gd-DTPA-enhanced MR images; the damage seemed to increase up to the 6-month time point, and thereafter stabilized (Fig. 3).

Discussion

Neurotoxicity following interstitial irradiation of brain tumors occurs at one or both of two time points following treatment. Early immediate deterioration due to cerebral edema and subsequent mass effect can complicate up to 10% of high-activity implants; obvious risk factors for this occurrence appear to be large tumor size, significant preexisting edema, and location of tumor in deep white or gray matter. Months to years later, mass effect due to a combination of recurrent tumor and radiation necrosis necessitates debulking craniotomy in up to 40% of patients treated with brachytherapy. Whether or not the causative lesion is predominantly recurrent tumor or radiation necrosis is a moot point and cannot be resolved by neuropathological examination of the tissue. Assessment of metabolic activity of the lesion as measured by positron emission tomography scanning may help us to better understand the nature and prognostic significance of these lesions. In any case, craniotomy and aggressive resection appear to be beneficial in decreasing mass effect, by both removing tissue and lessening edema.

It has been presumed that increased mass effect following interstitial irradiation is due to breakdown of the BBB due to the high dose of radiation delivered. This may be due to over-irradiation of “normal brain” surrounding the targeted tumor volume, and/or release of agents causing vasogenic edema from frankly necrotic tumor nearer the radiation seeds which receives extremely high radiation dose (for example, > 300 Gy).

Previous Animal Models

A number of animal models have been utilized to study interstitial irradiation brain damage. A common weakness of these animal studies (including the one described here) is that interstitial irradiation is being delivered in the artificial situation of normal brain without tumor, which is significantly different from the human clinical situation. However, useful information has been learned from animal experimentation.

Fike, et al., used quantitative computerized tomography (CT) and histological analysis following interstitial irradiation of canine brain and found frank coagulation necrosis at doses above 190 Gy (compared with 295 Gy in our study) and increased contrast enhancement (that is, BBB disruption) at doses above 60 Gy (compared with 165 Gy in our study). In Fike’s study high-activity removable 125I sources were used to deliver the radiation; this is similar to our study and to the clinical experience for patients with malignant brain tumors in the majority of institutions offering this modality of therapy.

Turowski, et al., also studied high-activity removable 125I brachytherapy in canine brain using quantitative CT scanning with specific attention to the effects of total dose and irradiated volume on normal brain damage. They concluded that: 1) tissue necrosis is related to total dose delivered, with a minimum effective dose averaging 180 to 200 Gy (compared with 295 Gy in our study); 2) vascular damage is less dependent on irradiated volume or total dose than on dose rate; and
FIG. 6. Electron micrographs of vessel profiles from viable and degenerating tissue within 2 mm of the $^{125}$I seed in the same animal as depicted in Figs. 2, 4, and 7. A: Degenerating endothelial cell (EC) infiltrated with horseradish peroxidase (HRP). The dark HRP reaction product can also be seen filling the lumen (L). Pericyte processes (P) forming part of the vessel wall are intact. × 10,700. B: Vessel profile showing two swollen abnormal endothelial cells (EC). This profile was found near the vessel shown in A. L = lumen. × 11,500. C: Normal-appearing vessel profile from the 2-mm block. The vessel profile is associated with extravasated HRP (asterisks). Traces of HRP remain in the lumen (L) near the cell membrane. P = pericyte. No obvious disruption of the vessel wall can be seen to account for the extravasated HRP. It is likely that HRP has spread from a distant site through the extravascular space. The square on the upper left aspect of the vessel wall indicates the area depicted at higher power in D. × 4675. D: Junctional cleft (JC) of the vessel shown in C, filled with HRP reaction product from the abluminal surface. Other clefts (arrowheads) within the junctional complex appear to be empty of HRP. L = lumen. × 132,350. E: Endothelial vesicles (arrowheads) filled with HRP associated with HRP-infiltrated basement membrane (BM). L = lumen of the vessel; EC = endothelial cells. × 26,350.
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3) edema is quantitatively related to volume of necrotic tissue.

Another group of studies using normal canine brain as a model present qualitative descriptions of brain damage following permanent implantation of low-activity radiation sources ($^{125}$I, iridium-192, and yttrium-90). In these papers, the histological zones identifiable around the seeds have been eloquently described and the area of central necrosis is noted to occur at doses above 180 Gy. Furthermore, this necrotic area tended to increase over a 1-year period and become progressively mineralized. In a few instances similar changes were observed in brain surrounding avian sarcoma virus-induced brain tumors treated with $^{125}$I brachytherapy. Extensive ipsilateral hemispherical cerebral edema was observed consistently and was attributed to a vasogenic edema-causing agent originating from the zone of necrosis. In a more recent study by Groothuis, et al., the BBB effects of permanent $^{125}$I seed implants were studied by quantitative autoradiography using carbon-14-labeled a-aminoisobutyric acid. The zones of radionecrosis and of BBB disruption increased up to approximately the 150-day time point and stabilized thereafter, which is quite similar to our findings. Groothuis, et al., observed the minimum radionecrosis dose to be about 270 Gy and the minimum BBB disruption dose to be about 135 Gy, which again compares closely with our findings in the present study.

Magnetic Resonance Imaging

Magnetic resonance imaging without gadolinium enhancement has been employed to assess radiation injury to the brain and has been found to be sensitive but rather nonspecific. Regarding the usefulness of MR imaging as an indicator of BBB disruption, there is emerging data in patients with brain tumors that Gd-DTPA has a role as an enhancing agent and as a semiquantitative BBB indicator. Gadolinium-DTPA is distributed mainly in the extracellular space and enhancement persists for approximately 1 hour following administration. The short half-life after intravenous administration (about 20 minutes) and the high binding constant (10$^{22}$ to 10$^{23}$) account for its stability and favorable kinetic properties. Experimental studies in dogs with osmotic BBB disruption have confirmed the usefulness of Gd-DTPA enhancement as an indicator of BBB breakdown. Furthermore, demonstration of BBB breakdown in an external radiation injury model in cats relied on gadolinium enhancement of T$_1$-weighted images. Similarly, Gd-DTPA-enhanced T$_1$-weighted images reliably demonstrated BBB disruption in our model that was not appreciated on plain T$_1$- or T$_2$-weighted images.

Correlation of MR Imaging, HRP, and Light Microscopic Lesions

The correlation of lesions indicated by Gd-DTPA-enhanced MR imaging, HRP leakage, and histopathological change provides some interesting insights into the relationship of BBB breakdown and frank histological damage. In the present model, the brain slice areas characterized by visible damage matched the area of HRP reaction product fairly closely, but were significantly smaller than areas of damage indicated by Gd-DTPA enhancement on MR images.

We interpret these results to mean that both gadolinium and HRP extravasate from the vessels at the narrow zone just under the necrotic tissue (the zone marked by HRP after 30 seconds) and then spread through the tissue with time down a concentration gradient.
lateral spread of both tracers along the corpus callosum that we observed in about 10% of animals supports this mechanism, since extracellular resistance is much lower in white than in gray matter and this would facilitate a more rapid spread of tracer within white matter tracts. Furthermore, Groothuis, et al., in a similar study used a vascular tracer that is trapped at the site of extravasation and so cannot spread within the brain. Their study also showed that BBB leakage was confined to a narrow zone immediately deep to necrotic tissue. On the basis of these results we suggest that the areas of apparent barrier leakage seen on contrast-enhanced MR images are overestimated due to spread of the tracer away from the leakage site.

It is not certain why gadolinium enhanced a larger area than HRP after the same circulation times. Flow of molecules within the brain extracellular space is due to bulk flow rather than diffusion, so the different sizes of these molecules (MW) do not explain the difference in tracer spread (Gd-DTPA, MW 550 vs. HRP, MW 40,000). Two possibilities could explain these results: either HRP is present in the same area as gadolinium but its concentration is below the limits of detectability (in this case, gadolinium could be considered to be a more sensitive tracer than HRP) or HRP may be binding to glycoproteins in the extracellular space, which limits its spread.

Time Course of Brachytherapy Brain Damage

The time course of 125I brain damage in this model was similar to that observed by some previous studies; the area of BBB disruption appeared to progress from time 0 to 6 months and thereafter stabilize or decrease. This indicates that the pathophysiological substrate of BBB disruption must be capable of some repair. Frank necrosis cannot be repaired in the cerebrum but apparently some subnecrotic damage can be partially repaired. The time course of brain damage in the present model also closely approximates the situation in human brain-tumor brachytherapy. In most clinical studies, the median time to maximum mass effect requiring surgical debulking following brachytherapy is approximately 6 months. However, this may be a coincidence since the peak time to tumor recurrence following any therapeutic intervention for de novo or recurrent malignant astrocytoma is also approximately 6 months.

Limitations of Study

The limitations of this study are numerous and worthy of discussion. The rat brain is about three orders of magnitude smaller than the human brain and perhaps also different in its response to radiation injury. However, previous work suggests that rat brain may actually be a reasonable model of the human situation in terms of radiation effects; a recent paper by Remler, et al., concludes that endothelial damage could be the principal mechanism mediating late radiation effects in rat brain. They observed that maximum BBB disruption after 60 Gy to a 0.5 cc hemispheric volume occurred around 100 days following irradiation. They also noted that the greater the radiation dose, the shorter the latency to BBB breakdown. Another obvious weakness is that normal brain is the model system in this study as opposed to the human clinical situation, in which tumor and brain around tumor constitute the targeted volume for interstitial irradiation. Certainly the edema and compression of brain around tumor are likely important ingredients in radiation damage that are not reproduced in our present model. The technique of radiation delivery (that is, laying the seed on the cerebrum as opposed to implanting it in the hemisphere) is also different from the human situation and was motivated by the concern that, in such a small volume as the rat cerebrum, the surgical trauma of seed implantation would produce too great an artifact and cloud our findings relating to BBB disruption. Finally, and perhaps most importantly, it is not clear exactly what the area of Gd-DTPA enhancement on MR imaging represents on the spectrum embracing normal brain, disrupted BBB, and frank tissue damage.

Future applications of the present model are planned to study the role of adding protectors of vascular damage (such as acetylsalicylic acid) in modifying the extent of brachytherapy-induced brain toxicity.

Acknowledgments

We thank Dr. Phil Leung for help with dosimetric calculations; Inge Frohn, Cindy Stewart, and Kay Hayakawa for technical support; and Denise Best for preparing the manuscript.

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Manuscript received January 11, 1990. Accepted in final form April 9, 1990. This work was supported by the Medical Research Council of Canada and the National Cancer Institute of Canada. Address reprint requests to: Mark Bernstein, M.D., F.R.C.S.(C), 25 Leonard Avenue, Suite 211, Toronto, Ontario M5T 2R2, Canada.