Dose-related Effects of Single Focal Irradiation in the Medial Temporal Lobe Structures in Rats
—Magnetic Resonance Imaging and Histological Study—

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Abstract

The dose-related effects of single focal irradiation on the medial temporal lobe in rats were investigated by sequential magnetic resonance imaging and histological examination. Irradiation of 200 Gy as a maximum dose using 4 mm collimators with a gamma unit created an area of necrosis consistently at the target site within 2 weeks after irradiation. Irradiation of 100 Gy caused necrosis within 10 weeks, and 75 Gy caused necrosis within one year. Irradiation of less than 50 Gy did not induce necrosis consistently, although a restricted area of necrosis was created in the medial temporal structures including the intraparenchymal portion of the optic tract. 75 Gy may be the optimum dose for creating necrosis consistently in the medial temporal lobe structures. However, careful dose planning considering both dose-time and dose-volume relationships in necrosis development is necessary to avoid injury to vulnerable neural structures such as the optic tract when applying radiosurgical techniques to treat functional brain disorders in medial temporal lobe structures such as temporal lobe epilepsy.

Key words: radiosurgery, temporal lobe, epilepsy, radiation necrosis

Introduction

Stereotactic radiosurgery allows the application of single high-dose focal irradiation to a focal region without affecting the surrounding brain structures. This less invasive technique has been applied to the treatment of organic brain diseases such as arteriovenous malformation and brain tumors as well as functional brain disorders such as thalamic pain, parkinsonian tremor, and temporal lobe epilepsy. However, the therapeutic results in patients with functional disorders have not always been consistent, in contrast to the well-established efficacy in organic brain lesions.

Such inconsistent outcomes for functional radiosurgery may be partly due to uncertainty about the optimum radiation dose for this radiosurgical approach. Various clinical and experimental studies have been reported, but results may differ with radiation method, period of observation, and irradiated neural structures.

This study investigated the dose-related effects of single focal irradiation in the medial temporal lobe structures of the rat, to clarify the optimum dose for application of radiosurgical techniques to functional brain disorders located in this area such as temporal lobe epilepsy.

Methods

Male Sprague-Dawley rats weighing 270 to 300 g underwent single focal irradiation using a Gamma Unit (Elekta, Stockholm, Sweden) at the right amygdala centromedian nucleus based on Paxinos & Watson’s rat brain atlas (anteroposterior +6.2 mm, left 4.5 mm, height +2.0 mm) (Fig. 1). Rats were placed in a stereotactic frame adapted to the Gamma Unit under pentobarbital anesthesia (40 mg/kg intraperitoneally) and target coordinates were calculated using biplane x-ray films.
Fig. 1  Diagram showing dosimetry for single focal irradiation in the medial temporal lobe of the rat. The target center is the centromedian nucleus of the amygdala (Amy). CC: corpus callosum, CPu: caudate putamen, EC: external capsule, Hip: hippocampus, IC: internal capsule, Opt: optic tract, Th: thalamus. Bar = 1 mm.

collimators were used for irradiation. The target area covered by the 80% isodose line was calculated as a sphere of 4 mm in diameter. The maximum irradiation dose at the center of the target was 200, 100, 75, 50, or 25 Gy.

T1-weighted (repetition time [TR] 25 msec, echo time [TE] 30 msec) and T2-weighted (TR 350 msec, TE 100 msec) magnetic resonance (MR) images of the coronal section of the rat brain were obtained using a surface coil under pentobarbital anesthesia at 2-week intervals for 16 weeks to observe the early changes after irradiation, except for rats which received 200-Gy irradiation, which were sacrificed at 2 weeks after irradiation because of severe brain edema. The late changes were observed in another series of rats irradiated with 25 to 100 Gy by MR imaging obtained one year after irradiation.

Rats were sacrificed after MR imaging by an overdose of pentobarbital, then perfused with heparinized saline and 4% paraformaldehyde. The brains were fixed by 4% paraformaldehyde and embedded in paraffin. Histological examination used HE, Klüver-Barrera, myelin basic protein, and glial fibrillary acid protein (GFAP) staining.

Results

I. Early changes by MR imaging

200 Gy: All three rats had an extensive area of low T1 and high T2 signal intensity 2 weeks after irradiation (Fig. 2A). The high T2 signal extended along the white matter to the ipsilateral thalamus, hypothalamus, striatum, and to the contralateral external capsule.

100 Gy: All four rats had an area of high T2 signal intensity 8 to 10 weeks after irradiation (Fig. 2B), whereas the area was T1 isointense in three rats and hypointense in one rat. The high T2 signal intensity tended to extend widely to various ipsilateral brain structures as observed in the 200 Gy group.

75 Gy: One of five rats had an area of low T1 and high T2 signal intensity at the target site 8 weeks after irradiation, which increased in size afterwards (Fig. 2C). No significant MR imaging changes were
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Fig. 3  Magnetic resonance images (left column: $T_1$-weighted images, right column: $T_2$-weighted images), demonstrating late changes caused by single focal irradiation at the medial temporal lobe 1 year after irradiation. A: 100 Gy, B: 75 Gy, C: 50 Gy, D: 25 Gy.

observed in the other four rats during the observation period of 16 weeks.

50 Gy: One of five rats had an area of low $T_1$ and high $T_2$ signal intensity 16 weeks after irradiation (Fig. 2D). No significant MR imaging changes were observed in the other four rats during the observation period.

25 Gy: None of the five rats showed significant MR imaging changes for 16 weeks.

II. Late changes by MR imaging

100 Gy: One rat had an area of low $T_1$ and high $T_2$ signal intensity with a sharp margin at the target site corresponding to the 50% isodose line (Fig. 3A).

75 Gy: All three rats had an area of low $T_1$ and high $T_2$ signal intensity at the target site corresponding to the 80% isodose line (Fig. 3B).

50 Gy: Three of four rats had spherical areas of low $T_1$ and high $T_2$ signal intensity localized at the medial temporal lobe structures including the amygdala and the intraparenchymal portion of the optic tract (Fig. 3C).

25 Gy: One of three rats had a spherical area of low $T_1$ and high $T_2$ signal intensity of 2 mm in diameter localized at the amygdala and the intraparenchymal portion of the optic tract (Fig. 3D).

III. Early histological changes

200 Gy: Rats were sacrificed 2 weeks after irradiation because of severe brain edema. Necrosis was observed in all three rats at the target site corresponding to the 50% isodose line. The area of necrosis contained histolysis, karyolysis, microhemorrhage, and hyaline degeneration of the arteriole walls. The necrosis was surrounded by an area of edema, demyelination, and GFAP-positive astrocytosis.

100 Gy: Necrosis was observed in all four rats corresponding to isodose lines from 50% to 80% at the target site 16 weeks after irradiation. The area of necrosis contained cavity formation with calcification, infarction, capillary thickening, and thrombosed vessels. The area surrounding the necrosis demonstrated edema, demyelination, astrocytosis, and dilatation of vessels. An extensive GFAP-positive area was observed throughout the ipsilateral hemisphere in all rats (Fig. 4 upper).

75 Gy: Necrosis with cavity formation was observed in one of five rats at the target site corresponding to the 80% isodose line 16 weeks after irradiation. Other animals showed an area of GFAP-positive astrocytosis, widening of the perivascular space, pallor staining of Nissl’s granules, and pyknosis in the target area.

50 Gy: An oval-shaped area of necrosis was observed in one of four rats at the target area including the intraparenchymal portion of the optic tract. Demyelination and GFAP-positive astrocytosis were observed in the target area in the other rats.

25 Gy: Necrosis was not observed in any of the five rats. However, edema, GFAP-positive astrocytosis around vessels, and pallor staining of Nissl’s granules were identified in the target area in two rats.

IV. Late histological changes

100 Gy: Histological changes at 1 year after irradiation in one rat demonstrated an area of liquefied necrosis with a sharp margin corresponding to the 50% isodose line in the target area. Demyelination and GFAP-positive astrocytosis were localized...
Fig. 4 Photomicrographs showing glial fibrillary acid protein (GFAP)-positive astrocytosis. upper: 100 Gy, 16 weeks after irradiation. An extensive GFAP-positive area is present throughout the hemisphere. ×10. lower: 75 Gy, 1 year after irradiation. The GFAP-positive area is localized at the sharp, thin margin of the necrosis. ×20. R: GFAP-positive reactive astrocytosis, C: cavity created by necrosis.

only at the margin of the necrosis.

75 Gy: An area of liquefied necrosis with a sharp margin corresponding to the 80% isodose line was observed 1 year after irradiation in all three rats. Demyelination and GFAP-positive astrocytosis were localized only at the margin of the cavity created by necrosis (Fig. 4 lower).

50 Gy: An area of liquefied necrosis with a sharp margin was demonstrated in three of four rats. The cavity was oval with the largest diameter of 0.5 to 3.5 mm. The center was located at the optic tract. Demyelination and GFAP-positive astrocytosis were localized at the margin of the cavity.

25 Gy: An area of liquefied necrosis of 2 mm was observed at the site of the optic tract in one of three rats (Fig. 5). Demyelination and GFAP-positive astrocytosis were localized at the margin, where macrophages with hemosiderin, thickening of the arteriolar walls, and arterial thrombosis were observed.

Fig. 5 Photomicrographs showing a spherical area of liquefied necrosis at the optic tract 1 year after 25 Gy irradiation. Demyelination and astrocytosis are localized at the sharp, thin margin of necrosis (N). left: HE stain, ×10; right: Klüver-Barrera stain, ×10.

Discussion

Previous studies of single focal high-dose irradiation in normal brain tissue have suggested a dose-related correlation between irradiation dose and time to development of necrosis (Table 1). An autopsy series in patients with malignant pain found that over 160 Gy of single focal irradiation was necessary to consistently create an area of necrosis at the thalamus. Focal irradiation of 150 Gy by 8 mm collimators in baboons created an area of necrosis in the thalamus and pons 24 weeks after irradiation, whereas 100 Gy by 4 mm collimators resulted in the appearance of an area of necrosis 90 days after irradiation in rats, and irradiation with 75 Gy by 10 mm collimators created an area of necrosis after 6 months in cats. Although these results suggest the presence of a direct relationship between dose and time to appearance of necrosis, few quantitative studies have been performed.

The present study demonstrated a clear relationship between irradiation dose and time to development of necrosis following single focal irradiation. Irradiation with the maximum dose of 200 Gy caused necrosis at the target site within 2 weeks after irradiation, 100 Gy within 10 weeks, and 75 Gy within 1 year after irradiation. Irradiation of less than 50 Gy did not consistently induce necrosis.
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within 1 year after irradiation, although a restricted area of necrosis was created at the medial temporal structures including the amygdala and the intraparenchymal portion of the optic tract.

The volume of irradiation is another important factor in estimating the probability of radiation necrosis, since necrosis is associated with larger volumes of irradiation. The threshold dose for the development of necrosis in mice at 24 days following irradiation was 140 Gy for a 1 mm beam, 360 Gy for a 0.25 mm beam, and 500 Gy for a 0.075 mm beam. A non-linear isoeffect relationship has been found between the volume and the dose of irradiation. However, insufficient experimental data are available to clarify this dose-volume relationship, although larger irradiation volumes tend to result in necrosis even with a lower dose, as necrosis developed 12 months after irradiation with 30 Gy using a 20 × 10 mm collimator in rabbits.

The present study compared MR imaging findings to histological findings. An extensive high T2 signal intensity area observed within 16 weeks of irradiation over 100 Gy corresponded to necrosis and an area of edema, demyelination, and GFAP-positive astrocytosis. The extensive GFAP-positive area in the whole ipsilateral hemisphere in the early phase was considered to be the reaction of astrocytes to brain edema caused by radiation injury of the vascular endothelium. In contrast, one year after irradiation, GFAP-positive astrocytosis was confined to a sharp, thin margin of necrosis with no obvious histological changes in the surrounding tissue.

This study suggests that 75 Gy is the optimum dose for creating necrosis consistently within 1 year in the medial temporal lobe structures corresponding to the 80% isodose line using 4 mm collimators. However, much lower dose induces necrosis in vulnerable neural structures such as the intraparenchymal portion of the optic tract. Careful dose planning considering both the dose-time and dose-volume relationship for the development of radiation necrosis is necessary to apply radiosurgical techniques to functional disorders in medial temporal lobe structures. Further study of the functional

Table 1 Literature review of reports on necrosis formation following single focal irradiation in the brain; radiation size, dose, and observation period

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Subject</th>
<th>Apparatus</th>
<th>Radiation size (mm)</th>
<th>Dose (Gy)</th>
<th>Observation period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larsson (1958)</td>
<td>rabbit</td>
<td>185-MeV proton beam</td>
<td>1.5</td>
<td>200</td>
<td>14 days</td>
</tr>
<tr>
<td></td>
<td>goat</td>
<td>185-MeV proton beam</td>
<td>2 × 7</td>
<td>200</td>
<td>4 wks, 7 wks</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2 × 10</td>
<td></td>
<td></td>
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<tr>
<td>Rexed et al. (1960)</td>
<td>rabbit</td>
<td>185-MeV proton beam</td>
<td>1.5</td>
<td>200</td>
<td>10 wks</td>
</tr>
<tr>
<td>Zeman et al. (1961)</td>
<td>mouse</td>
<td>n.d.</td>
<td>1</td>
<td>140</td>
<td>24 days</td>
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<td></td>
<td></td>
<td></td>
<td>0.25</td>
<td>360</td>
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<td>0.075</td>
<td>500</td>
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<tr>
<td>Larsson et al. (1963)</td>
<td>human</td>
<td>proton beam</td>
<td>n.d.</td>
<td>200</td>
<td>62 days</td>
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<tr>
<td>Kjellberg et al. (1964)</td>
<td>n.d.</td>
<td>Bragg peak of proton beam</td>
<td>n.d.</td>
<td>240</td>
<td>20 days</td>
</tr>
<tr>
<td>Andersson et al. (1970)</td>
<td>goat</td>
<td>185-MeV proton beam</td>
<td>7 × 2</td>
<td>200</td>
<td>18 mos</td>
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<tr>
<td>Steiner et al. (1980)</td>
<td>human</td>
<td>gamma knife</td>
<td>3 × 5</td>
<td>160</td>
<td>n.d.</td>
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<td>3 × 7</td>
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<tr>
<td>Lunsford et al. (1990)</td>
<td>baboon</td>
<td>gamma knife</td>
<td>8 (collimators)</td>
<td>150</td>
<td>24 wks</td>
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<tr>
<td>Lo et al. (1992)</td>
<td>rabbit</td>
<td>heavy charged</td>
<td>20 × 10</td>
<td>30</td>
<td>12 mos</td>
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<td></td>
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<td>particle beam</td>
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<tr>
<td>Kondziolka et al. (1992)</td>
<td>rat</td>
<td>gamma knife</td>
<td>4 (collimators)</td>
<td>200</td>
<td>21 days, 90 days</td>
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<td></td>
<td></td>
<td>100</td>
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<td>Spiegelmann et al. (1993)</td>
<td>cat</td>
<td>Linac</td>
<td>10 (collimators)</td>
<td>150</td>
<td>6 mos</td>
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<td>100</td>
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<td>75</td>
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<tr>
<td>Blatt et al. (1994)</td>
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<td>Linac</td>
<td>10 (collimators)</td>
<td>125</td>
<td>3.5 wks</td>
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<tr>
<td>Kamiryo et al. (1996)</td>
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<td>gamma knife</td>
<td>4 (collimators)</td>
<td>120</td>
<td>1 mo, 4 mos</td>
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<tr>
<td>Present study</td>
<td>rat</td>
<td>gamma knife</td>
<td>4 (collimators)</td>
<td>200</td>
<td>16 wks, 1 yr</td>
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n.d.: not described.
changes in epileptic neural structures following irradiation of sub-necrotic doses\textsuperscript{6,8,10,18,21,27,28} will be necessary before clinical trials in patients with epilepsy can be undertaken.

\textbf{Acknowledgments}

The authors wish to express their appreciation to Prof. Yuzo Iwasaki (National Miyagi Hospital, Miyagi) for his helpful comments on histological findings.

\textbf{References}


Neurol Med Chir (Tokyo) 39, January, 1999
Commentary

This article confirms the dose-related effect on single focal irradiation by gamma unit on the medial temporal lobe in rats. Recently, surgical treatment for temporal lobe epilepsy has been tried in many patients due to the progress in neuroradiological neurophysiological diagnosis. Trials of radiosurgery for epileptic focus have also increased due to the progress in gamma knife and stereotactic radiosurgical devices. Warnke et al. reported that seizures are decreased after radiosurgery of low grade glioma. Regis et al. and Arita et al. reported decreased frequency of seizures in patients with hamartoma and temporal lobe epilepsy after radiosurgery. However, there are few studies concerning the optimal dose based on fundamental research like this experiment. This research is valuable in this area as well. Pathological changes after high dose irradiation of brain are necrosis, astrocytosis and demyelination. If irradiation was 75 Gy in one fraction, necrosis of the medial temporal lobe occurred within one year. The authors concluded that this dose is the optimal dose for application to functional brain disorders. However, the further experimental and clinical studies are necessary to decide the optimal dose for treatment of functional brain disorders.

References


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Ishikawa et al. carefully studied the dose-related effects of single focal irradiation using a Gamma Unit on the normal rat brain. The medial temporal lobe was irradiated with 25, 50, 75, 100, or 200 Gy as a central dose using 4 mm collimators and the brain was studied sequentially by magnetic resonance imaging and histological examination up to one year after irradiation. They found that 75 Gy was the optimum dose for creating brain necrosis consistently in the target site. This study provides important information for the application of stereotactic radiosurgery to the treatment of brain disorders such as temporal lobe epilepsy.

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