Acute Immunohistochemical Changes in Murine Brain after Stereotactic Radiosurgery May Suggest Novel Intervention Targets

Lisa Feldman  MD, PhD Dept of Neurosurgery, VCU, Virginia USA
William Broaddus  MD, PhD Dept of Neurosurgery, VCU, Virginia USA
Karen Liu  MSc Dept of Pharmacology, U of Auckland, Auckland NZ
Colin Green PhD, DSc Dept of Ophthalmology, U of Auckland, Auckland NZ
Simon O’Carroll  MSc, PhD Dept of Anatomy and Med Imaging, U of Auckland, NZ
Barbara Fackelmeier  BSc, PhD Dept of Anatomy and Med Imaging, U of Auckland, NZ
Tony Anene-Maidoh  MS, MD Dept of Neurosurgery, VCU, Virginia USA
Joel Garbow  PhD, Dept of Radiology, Washington University in St. Louis, Missouri USA
Jian Guan  MD, PhD Dept of Pharmacology, U of Auckland, Auckland NZ
Disclosure

• In compliance with the Accreditation Council for Continuing Medical Education’s (ACCME) Standards for Commercial Support of CME, VCU CME discloses all current relationships which program faculty report having with companies whose products and/or services they may discuss during their presentation. In addition, all presenters must disclose to the audience when any material they present recommends an off-label or unapproved use of a drug, procedure or piece of equipment.

• Lisa Feldman discloses having no relevant financial relationships to report.
Background

• Cerebral radiation necrosis (RN), a late-complication of radiation therapy and stereotactic radiosurgery (SRS), can result in debilitating symptoms, including seizures, increased intracranial pressure, neurological deficits, and death.

• Current therapies include medications, such as steroids and Bevacizumab, or in severe cases, surgery. All treatments are either non-curative, or are associated with significant risks.

• The pathophysiology of RN has not yet been conclusively elucidated, making it difficult to identify novel therapies to reduce or prevent this serious complication.

• Here, we use a murine model of RN to study the early immunohistochemical changes in irradiated mouse brains after Gamma Knife SRS.
Methods

• We utilized a mouse model of RN developed by Garbow, et al., in which healthy mice are irradiated with a single-fraction, single hemispheric 50-Gy radiation dose (targeting left hemisphere cortex ~3 mm posterior to bregma) using the Leksell Gamma Knife® (GK) Perfexion™ (Elekta; Stockholm, Sweden).

• Mice (n=5/cohort) were sacrificed 6, 24 hrs, 96 hrs, 1, 2 and 3 weeks after SRS, time points which precede the appearance of frank radiation necrosis, either radiographically, or by conventional (H&E) histology.

• Immunohistochemistry (IHC) was performed on formalin-fixed, 40 micron-thick cryosections of brain to assess for biological changes in brain tissue.

• IHC antibodies were optimized for mouse brain and then analyzed regionally (hippocampus, striatum, thalamus and cortex) using Image J (https://imagej.nih.gov/ij/). Two-way ANOVA were performed using GraphPad Prism 7.0, comparing irradiated vs control hemispheres.

• Primary antibodies against Glial Fibrillary Acidic Protein (GFAP), isolectin B-4, Fibrinogen, connexin 43 (Cx43), interleukin-1β (IL-1 β) and inflammasome gene nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NPLR-3) were used to mark reactive astrocytes, microglial cells, leakage of the BBB, gap junctions, and pro-inflammation cytokines respectively in both control and radiated hemispheres in 5 different time points. IHC staining was evaluated, both in density and area, using Image J in control and radiated hemispheres.
Increase in astrocyte density in the irradiated hemispheres (on left) was detected from 96 hrs, particularly in the CA4 and dentate gyrus area of the hippocampus and the cortex. GFAP increase further spread through the cortex and the rest of hippocampus by week 1. The changes in week 2 and week 3 are mainly the increase of density around the same area. Photo F shows the morphology of GFAP positive astrocyte in the hippocampus (x40).
Glial Fibrillary Acidic Protein (GFAP)

A) Anterior Cortex

- Control side
- Irradiated side

(p=0.045) *
(p=0.0004)

B) Striatum

- Control side
- Irradiated side

C) Posterior Cortex

- Control side
- Irradiated side

(p<0.0001) *
(p<0.0001)

D) Hippocampus

- Control side
- Irradiated side

(p<0.0001) *
(p<0.0001) *
(p<0.02) *
Isolectin B4 (IB4)

A. Anterior Cortex

B. Striatum

C. Posterior Cortex

D. Hippocampus

After Radiation

Average density of IB4

<table>
<thead>
<tr>
<th>Week</th>
<th>Control side</th>
<th>Irradiated side</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(p=0.02)

(p<0.0001)

(p=0.007)

(p=0.0006)

(p=0.006)

(p=0.0002)

(p<0.0001)
Fibrinogen

![Average density of Fibrinogen over time after radiation](chart)

**Connectin 43 (Cx43)**

![Average density of Cx43 over time after radiation](chart)

**Interleukin-1 Beta (IL-1β)**

![Average area of IL-1β over time after radiation](chart)

**NLRP-3**

![Average density of NLRP-3 over time after radiation](chart)

* (p<0.0001)  
* (p<0.0001)  
* (p<0.0001)  
* (p=0.004)  
* (p=0.03)  
* (p<0.01)
Discussion

- **GFAP** is differentially expressed across irradiated brain regions and time:
  - Significantly upregulated in the anterior cortex 3 weeks after irradiation.
  - Significantly upregulated in the posterior cortex 1, 2 and 3 weeks after irradiation.
  - Significantly upregulated in the hippocampus 96 (but not 24) hours; 1, 2 and 3 weeks after irradiation.

- **IB4** is differentially expressed across irradiated brain hemisphere and time:
  - Significantly upregulated in the anterior cortex after 3 weeks.
  - Significantly upregulated in the posterior cortex, striatum and hippocampus 1, 2 and 3 weeks after irradiation.

- **Fibrinogen** is significantly upregulated in irradiated hemispheres 1, 2, 3 week after irradiation.

- **IL-β** is significantly upregulated in irradiated hemispheres within 96 hours after irradiation.

- **Cx43** is significantly upregulated in irradiated hemispheres 2 weeks after irradiation.

- **NLRP-3** is not significantly differentially expressed in irradiated hemispheres 24 or 96 hours after irradiation.
Summary Points

- **Glial changes, as measured by GFAP expression**, occur rapidly (within < 1 week of irradiation) in the post cortex and hippocampus, and within 3 weeks in anterior cortex of irradiated brains.

- **Microglia changes, as assessed IB4**, occur rapidly and diffusely across all measured brain regions (ant and post cortex, striatum and hippocampus) as early as 1 week post radiation.

- **Irradiation affects blood brain barrier (BBB)**, as measured by gap junction Cx43, with significant increase in expression 2 weeks after radiation
  - BBB is affected by irradiation later than glial and vascular changes
  - This is consistent with the delayed BBB disruption seen clinically in RN.

- Radiation results in a robust **inflammatory response as measured by**:
  - An extremely rapid upregulation of pro-inflammatory cytokine **IL-1β**, within 96 hours of irradiation, likely leads to astrocyte and glial activation.
  - **Fibrinogen**, which is significantly upregulated 1,2 and 3 weeks following irradiation, likely infiltrates brain through leaky blood-brain barrier.

- All of these upregulations occur 3 weeks earlier than changes observed by MRI or H&E.

- SRS tends to affect hippocampus more quickly and robustly than other regions.