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Biological Mechanisms Underlying the Preferential Destruction of Gliomas by X-ray Microbeam Radiation

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Beamline: X17B1

Introduction: Studies at the NSLS and at the European Synchrotron Radiation Facility (ESRF), Grenoble, France, showed that a single exposure to arrays of parallel, thin (<100 μm-wide), planar slices of 50-150 keV synchrotron x-ray beams (microbeams, MBs) spares normal tissues at 10-20-fold higher in-beam doses than does conventional beams [1-7], and preferentially destroys tumors [2, 6, 7]. These effects may be mediated, at least in part, by the tissues' microvasculature. In particular, it was suggested that surviving endothelial cells lying between the paths of the MBs replace their lethally injured neighbors in the direct paths of MBs (1). Understanding the mechanisms underlying these effects is of utmost importance in evaluating the clinical potential of microbeam radiation therapy (MRT).

Methods and Materials: Mature Fischer 344 rats bearing intracranial 9LGS tumors were inoculated with 10,00 9LGS. The rats were irradiated at day 14 of inoculation, when the tumor was ~4 mm. The irradiation was anteroposteriorly using a unidirectional 10 mm x 10 mm MB array with 27 μm beam width, 200 μm beam spacing, 800 Gy in-beam entrance dose, and 120 keV median energy. No acute effects were observed. Two rats each were euthanized at 1, 3 , 8 hrs, and at 2, 4, 16, and 30 days after irradiation. The rats were either euthanized using a CO_2-O_2 mixture, or first were deeply anesthetized, injected intracardially with FITC-albumin (a fluorescing dye that does not cross the intact blood-brain barrier), and euthanized 2 minutes later using intracardial tissue perfusion with saline and formalin. The brain was embedded in paraffin, sectioned in 5 μm slices, and stained with hematoxylin and eosin (H&E).

Results: A potentially significant finding was our observation of the disruption of tumor vessels and associated necrosis starting at 8 hr post-irradiation (Figs. 1-3). Although it is known that when rapidly proliferating tumors, such as intracranial 9LGS, become large they often outstrip their blood supply and develop central tumor necrosis, the effect is not expected to be significant in small tumors (~4 mm in Figs. 1-3). Despite this, the damage was quite prevalent in these MB-irradiated tumors, starting 8 hour post-irradiation. It included regions of pyknotic/apoptotic cells with red blood-cell extravasation into the tumor tissue (Figs. 1, 3). In many cases, including that shown in Fig. 1, the region of the damage coincided with the MB paths. Perfusion with FITC-albumin assessed vascular leakage. Fig. 2 is the fluorescence image of the same tissue section as Fig. 1; it vividly displays large FITC-albumin leakage from the destroyed vessels into tumor. No vascular damage or leakage was observed in the normal tissues of the cerebrum and cerebellum. For example, no fluorescent leakage is seen in the left part of Fig. 1 that shows the normal cerebrum at the edge of the tumor. Fig. 4, the control brain, shows an unirradiated tumor 19 days after inoculation (~6 tumor diameter) in which there is no necrosis or vascular damage. This tumor is expected to be larger than the irradiated tumor of Figs. 1-3 because the latter was irradiated at day 14 after inoculation and examined 8 hours after irradiation. These findings support our assumption that the necrosis and red blood-cell extravasation of Figs. 1-3 are caused by MB irradiation.

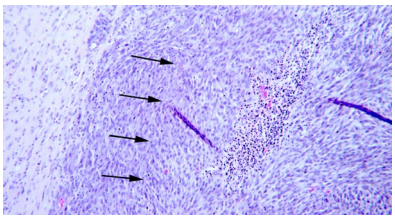

Figure 1. Vessel disruption and tumor necrosis 8 hr post-irradiation (100X). Four MB paths are faintly visible.

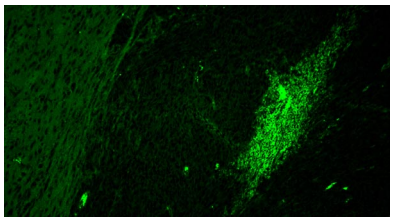

Figure 2. Fluorescence image of the necrotic region of Fig. 1 with FITC-albumin, showing vascular disruption and blood leakage.

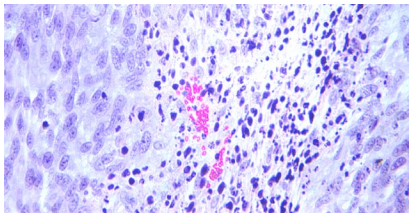

Figure 3. The vascular extravasation of Figs. 1 and 2 at 400 X, revealing details of the vascular effect and necrosis.

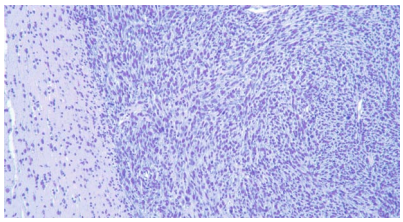

Figure 4. Edge of a non-irradiated 19-day tumor at ~6 mm diameter. Vascular damage or necrosis are not visible.

Conclusions: If this effect is confirmed by more detailed experiments it will a) largely explain the tumor-specific toxicity of MBs, and b) have significant implications for further enhancing MRT by improving drug delivery specifically to the tumor.

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