

Direct Granular Cell Death in the Rat Cerebellum Observed in the Paths of Very High Dose X-ray Microbeams

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Introduction: The extraordinary sparing effects of x-ray microbeams (MBs) on the normal central nervous system has been studied at the NSLS where the research was initiated in early 1990s, and at the European Synchrotron Radiation Facility (ESRF), Grenoble, France. The studies indicate that single exposure to arrays of parallel, thin (<100 μm -wide), planar slices of 50-150 keV synchrotron-generated x-ray MBs are tolerated by normal tissues at 10-20-fold higher in-beam doses than conventional beams [1-7]; these arrays also preferentially destroy tumors [2, 6, 7]. Studies are underway at the NSLS to understand the biological mechanisms underlying the MB effects. The current hypothesis is that the effects are mediated, at least in part, by the microvasculature. We believe recovery to the vasculature occurs when lethally injured endothelial cells in normal tissue are replaced by the neighboring, undamaged endothelial cells between the MBs [1]. An important step in understanding these effects is to be able to demarcate the paths of MBs on the histology tissue stained with hematoxylin and eosin. This can be done by using sparse MBs of very high doses, i.e., several times higher than MB doses expected to be relevant to radiotherapy in patients. The effect was reported by Slatkin et al. [1] and Laissue et al. [2, 4] at time points of 30 d or larger post-irradiation.

Materials and Methods: Mature Fischer 344 rats bearing intracranial 9LGS tumors were irradiated anteroposteriorly using a unidirectional MB array, 10 mm wide and 10 mm high, with 27 μm beam width, 200 μm beam-spacing, 800 Gy in-beam entrance dose, and 120 keV median energy. No acute dose effects were observed. The rats were euthanized, using a $\text{CO}_2\text{-O}_2$ mixture, at 1, 3, and 8 hr, and at 2, 4, 16, and 30 days after irradiation, with 2 rats in each group. The brains were recovered, fixed in formalin, and axial cuts were embedded in paraffin. Serial sections (5 μm) were stained with H&E.

Results: At 3 hr post-irradiation, the cerebellum of the rats clearly showed the MB paths as bands of granular cells with darkened nuclei (Fig. 1). The cells had a pyknotic appearance by 8 hr (Fig. 2). By 2 d the cells started to disappear (Fig. 3), and by 16 d they totally disappeared, leaving lucent empty bands in the cerebellum (Fig. 4). The banding effect in the cerebrum also was visible, although at a later time (3 wk) and with less strength, probably due to the much smaller density of neurons in the gray matter of the cerebrum. The banding in tumor was transiently visible, apparent only at 1 to 8 hr time points, probably due to the tumor's rapid growth. We note that MB banding in the cerebellum was observed earlier in rat [1] and in piglets [4] at longer times.

Conclusions: We conclude that a) MBs at extremely high doses deplete neurons from the cerebellum, and b) the effect can be used to show the MB paths in the brain tissue sections at time points as short as 3 hr.

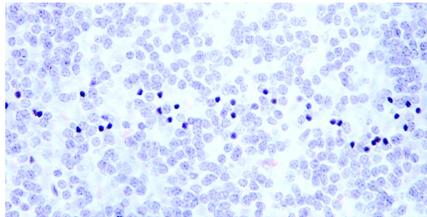


Figure 1. A MB path in the cerebellum 3 hr post-irradiation as darkened nuclei of neurons (400X).

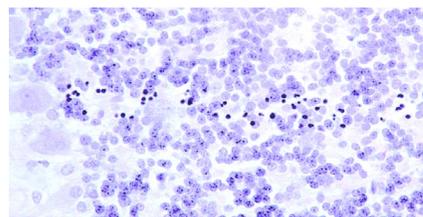


Figure 2. An MB path seen in the cerebellum 2 d post-irradiation (400X).

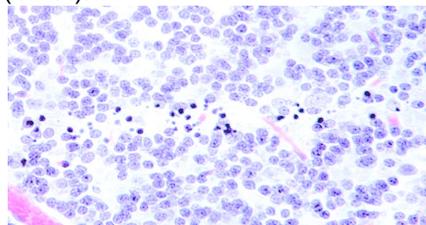


Figure 3. The MB path in the cerebellum 4 d post-irradiation. Some of the neurons have disappeared.

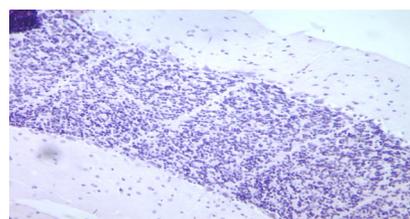


Figure 4. The evenly-spaced MB paths at 16 d post-irradiation (200X) are demonstrated by fairly complete disappearance of neurons in the cerebellum.

References:

1. D.N. Slatkin, et al. *Proc. Nat. Acad. Sci.*, 92: 8783-8787, 1995.
2. J.A. Laissue, et al., *Int. J. Cancer*, 78: 654-660, 1998.
3. J.A. Laissue, et al., SPIE Conference Proceedings Vol. 3770, pp. 38-45, 1999.
4. J.A. Laissue, et al, SPIE Conference Proceedings Vol. 4508, pp. 65-73, 2001.
5. F.A. Dilmanian, et al., *Cell. Molec. Biol.*, 47: 485-494, 2001.
6. F.A. Dilmanian, et al., *Neuro-Oncology*, 4: 26-32, 2002.
7. F.A. Dilmanian, et al., *Rad. Res.*, 2003 (in press)

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