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LEL/BrdU Double Staining: a Quantitative Study of Endothelial Cell Proliferation in the Rat Cerebellum Irradiated with X-ray Microbeams

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Beamline(s): X17B1

Introduction: It has been reported that normal tissue, including the brain of the rat and the duck embryo, tolerated appreciably higher doses of microbeams compared to broad beam irradiation [1]. Our current hypothesis for the normal tissue sparing effects of MRT is that the microvasculature endothelial cells between microbeams survive and later proliferate or migrate/spread, therefore replacing the lethally injured endothelial cells in the direct path of microbeams. The present study was designed to examine the role of endothelium proliferation in the recovery of the rat brain tissue after irradiation with arrays of parallel x-ray microbeams. Rat cerebellum was used as a model.

Methods and Materials: Sixteen Fischer 344 rats were irradiated in their cerebellum using an array of microbeams, each 80 μm in width and 6 mm in height, spaced 400 μm apart. The entrance dose in each beam was 200 Gy. After the irradiations the animals were injected intraperitoneally (ip) with bromodeoxyuridine (BrdU) at certain time interval (described below) before the animal was euthanized using the tissue perfusion method and the brain tissue was dissected for histology. The euthanasia time points, post-irradiation, were 1, 1.5, 2, 2.5, 3, 3.5, and 4 days. The BrdU was administered in 4 injected doses of 15 mg BrdU per dose at 12 hours, 9 hours, 6 hours, and 3 hours before euthanasia. Two rats were irradiated in each group. Cerebellum samples were processed and sectioned using a regular procedure. The sections were stained with BrdU secondary antibodies conjugated to fluorescein. The process was followed by staining with Lycopersicon Esculentum Lectin (LEL) conjugated to rhodamine to label endothelial cells.

Results: The increase in the number of proliferating endothelial cells per cerebellum section from day 1 to days 4 is significant [Fig 1]. The results may indicate that the microbeam irradiations initiated a process of cell proliferation that accelerated during the period of the measurements, but probably did not reach its peak. This effect is in accordance with our hypothesis that endothelial cells which resided between the microbeams at the time of irradiation proliferate after the irradiation. Besides the endothelial cells, other cell types such as glial cells, also regenerated significantly after microbeam irradiation, as it was expected.

References: 1. F.A. Dilmanian, G.M. Morris, G. Le Duc, X. Huang, B. Ren, T. Bacarian, J. Kalef-Ezra, I. Orion, T. Sandhu, X. Y. Wu, Z. Zhong and H.L. Shivaprasad, "Response of avian embryonic brain to spacially segmented x-ray microbeams," *Cell. Molec. Biol.*, 47, 485-494, 2001

Fig 1: Detection of endothelial cell proliferation after microbeam irradiation. A, The number of double stained cells, represented by the purple curve, shows a significant increase over a period of 4 days. B, Proliferating endothelial cells are identified by changing the wavelength of fluorescence, and localizing BrdU positive cells (middle panel) in the contour of endothelial cells labeled by LEL-Rhodamine (upper panel); a composite picture was made to confirm the double stained endothelial cell, as pointed by the arrow (lower panel).

