

High Dose Synchrotron Microbeam Radiation Therapy of Normal Mouse Skin: An Immunohistochemical Study of DNA Repair, Proliferation and Apoptosis

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The biological effects of MRT are not well understood. The aim of this study was to identify suitable histological and immunohistochemical markers of the acute damage to normal mouse skin from high dose MRT.

Experiments were performed at the SPring-8 synchrotron, Japan. The beam (median energy 110 keV) was segmented spatially into microplanar arrays of very narrow beams approximately 30 microns wide, with a peak-to-peak separation of 200 microns. The raised dorsal skin-flap of normal mice was irradiated with single fraction, unidirectional, synchrotron microbeam radiation or broadbeam radiation of increasing doses (200 Gy, 400 Gy and 800 Gy). Mice were culled at intervals of 6, 12, 24, 48 hours or 5 days post irradiation. Skin sections were harvested and fixed in formalin and returned to Australia. Histological and immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue sections at laboratories in Australia and the UK.

At 48 hrs post-irradiation with a broadbeam of radiation, there was a significant reduction in cellular proliferation for 400 Gy and 800 Gy. By 5 days post-irradiation with broad-beam radiation, severe tissue damage was observed, including; necrosis, epidermal denuding, significantly damaged hair follicles and sebaceous glands. In contrast, at 48 hrs post-irradiation with MRT there was an increase in proliferation of epidermal cells in the irradiated regions. The increase in proliferation was also observed at 5 days for the 400 Gy and 800 Gy MRT groups. Apoptotic and pyknotic cells appeared at 12 and 24 hours post irradiation and were more numerous for broadbeams compared to microbeams. Immunohistochemical staining for phosphorylation of histone H2AX (gamma-H2AX), a marker for DNA double-strand breaks, revealed intense 'stripes' or 'tracks' of positive staining at locations where microbeams had traversed the tissue. The tracks appeared to become wider and less distinctive with time (out to 48 hrs).

This work confirms and extends previously published observations by other MRT research groups in Europe and the USA showing that high dose synchrotron microbeam radiation therapy confers a sparing effect on normal tissue compared to broad beam radiation fields.

KEYWORDS: Microbeam Radiation Therapy, Normal tissue response, Immunohistochemistry