VASCULAR TOXICITY OF MICROBEAM IRRADIATION DEPENDS ON THE STAGE OF CAPILLARY MATURATION

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Abstract

Purpose: Microbeam radiation therapy (MRT) is a promising preclinical form of radiosurgery first intended to treat effectively brain tumors. We hypothesize that blood vessels (especially microvessels) are mediating the tissue-sparing effect of MRT. The aim of this study was to describe the effects of MRT on chick chorio-allantoic membrane (CAM), as it represents an almost pure vascular system. In particular we intended to show that the effects of microbeam irradiation on CAM depend on the stage of vascular maturation. Thus we compared immature with mature CAM vessels, i.e. day 8 with day 12 of embryonic development.

Methods: CAMs were obtained by incubating chick embryos using the shell-free culture method. They were irradiated with multiplanar beams (width: 25 μm; interbeam spacing: 200 μm), at doses of 200 and 300 Gy and were evaluated morphologically and *in vivo* at different time points after irradiation. In addition, seamless (i.e. broad beam, like in currently used clinical protocols) radiation doses of 1, 2, 5 and 10 Gy were administered to growing CAMs.

Results: *In vivo* monitoring of day 8 CAM immature vasculature 6 hr after MRT revealed a near total destruction of the capillary plexus. In some samples the solitary capillaries in the path between the beams were partially preserved, due to the fact that the intact supplying vessels were running parallel between the beams. Surprisingly the supplying vessels, i.e. arteries and draining vessels (veins) were not affected by the irradiation. Conversely, at day 12, well defined lesions were observed in the microvasculature, with a typical, parallel distribution pattern along the beams. Light and electron microscopy revealed demarcated apoptotic capillary segments within the beams' width.
Time course of the changes at day 12 of development revealed that 15 minutes after a 300 Gy irradiation, the CAM thickness increased dramatically. The larger vessels as well as the capillary plexus conserved their normal morphology. TEM revealed only an enlargement of the interendothelial cell junctions, which could explain the oedema. Thirty minutes after a 300 Gy irradiation, CAM thickness was still increased, but less pronounced. The microvessels were completely congested in the beam path, demonstrating first signs of disruption as cytoplasmic vacuolisation of endothelium and persistence of intercellular gaps. Sixty minutes after a 300 Gy irradiation, the beam strips were clearly demarcated as a dead tissue and the affected, congested capillary segments as well as the surrounding tissue underwent apoptosis. The remaining vasculature recovered rapidly and CAM regained its normal thickness. Between 1h and 6h no additional morphological changes were observed. A seamless 10 Gy single dose ionizing radiation suppressed the embryonic angiogenesis in the CAM. The irradiated vasculature remained immature and corresponded to earlier developmental stages, most probably due to inhibition of cell proliferation. At the same time, the irradiation induced a very strong over-compensation in the contralateral, unirradiated part of the CAM, thus indicating systemic effects.

Conclusions: In the present study we demonstrate that the effects of MRT are most likely mediated by capillary damage, tissue injury occurring because of insufficient blood supply. Moreover, the vascular toxicity of MRT depends on the stage of vascular maturation: the uncovered, naked capillaries are much more vulnerable in comparison to the mature ones. Mature microvessels (covered by pericytes), supplying arteries and draining vessels (veins) are much more resistant and overcome MRT in a range of 200-300 Gy. The physiological effects of MRT appear in a short time, the most important structural alterations being present during the first 15-60 minutes after irradiation. On the other hand, the effects of seamless ionizing irradiation are present in long term (days in our system) and most likely are mediated by inhibition of the endothelial cell division.