Int. J. Radiation Oncology Biol. Phys., Vol. 80, No. 5, pp. 1522–1532, 2011

0360-3016/$ - see front matter

ELSEVIER

doi:10.1016/j.ijrobp.2011.03.018

BIOLOGY CONTRIBUTION

MICROBEAM RADIATION-INDUCED TISSUE DAMAGE DEPENDS ON THE STAGE OF VASCULAR MATURATION

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Purpose: **To explore the effects of microbeam radiation (MR) on vascular biology, we used the chick chorioallantoic membrane (CAM) model of an almost pure vascular system with immature vessels (lacking periendothelial coverage) at Day 8 and mature vessels (with coverage) at Day 12 of development.**

Methods and Materials: **CAMs were irradiated with microplanar beams (width, ~25 μm; interbeam spacing, ~200 μm) at entrance doses of 200 or 300 Gy and, for comparison, with a broad beam (seamless radiation [SLR]), with entrance doses of 5 to 40 Gy.**

Results: ***In vivo*** **monitoring of Day-8 CAM vasculature 6 h after 200 Gy MR revealed a near total destruction of the immature capillary plexus. Conversely, 200 Gy MR barely affected Day-12 CAM mature microvasculature. Morphological evaluation of Day-12 CAMs after the dose was increased to 300 Gy revealed opened interendothelial junctions, which could explain the transient mesenchymal edema immediately after irradiation. Electron micrographs revealed cytoplasmic vacuolization of endothelial cells in the beam path, with disrupted luminal surfaces; often the lumen was engorged with erythrocytes and leukocytes. After 30 min, the capillary plexus adopted a striated metronomic pattern, with alternating destroyed and intact zones, corresponding to the beam and the interbeam paths within the array. SLR at a dose of 10 Gy caused growth retardation, resulting in a remarkable reduction in the vascular endpoint density 24 h postirradiation. A dose of 40 Gy damaged the entire CAM vasculature.**

Conclusions: **The effects of MR are mediated by capillary damage, with tissue injury caused by insufficient blood supply. Vascular toxicity and physiological effects of MR depend on the stage of capillary maturation and appear in the first 15 to 60 min after irradiation. Conversely, the effects of SLR, due to the arrest of cell proliferation, persist for a longer time.** **© 2011 Elsevier Inc.**

Chick chorioallantoic membrane, Microbeam radiation therapy, Seamless irradiation, Synchrotron-generated X-rays, Vascular maturation.

INTRODUCTION

Microbeam radiation therapy (MRT), a preclinical form of radiosurgery first intended to treat brain tumors, uses arrays of multiple, parallel, very thin (~25–75-μm-wide), millimeter-high microplanar beams (MPB) of synchrotron X-rays (energy range, 50–350 keV), spaced 100 to 400 μm apart, that deliver high doses (~150–4,000 Gy), typically in <1 sec (1); energy spectrum, dose rate and other physical parameters depend on the characteristics of the synchrotron. The microbeam irradiation is based on a spatial fractionation of the dose, instead of the temporal fractionation currently used in clinical protocols (1). MRT generates two main regions of dose deposition: the peak and the valley. The first corresponds to the path of the MPB; the second to the tissue swaths sited between the peaks,

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The study was supported by grant no. 31003A-116243 from the Swiss National Research Foundation.

Conflict of interest: none.

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Acknowledgment—We thank Barbara Krieger for help with figures; the European Synchrotron Radiation Facility for access to the ID 17 beamline; and Pierrick Regnard, Géraldine Le Duc, and Dominique Dalléry for highly qualified support in the preparation of the experiment.

We also thank Walter Burkard for the excellent technical assistance.

Received Aug 19, 2010, and in revised form Feb 21, 2011. Accepted for publication March 20, 2011.

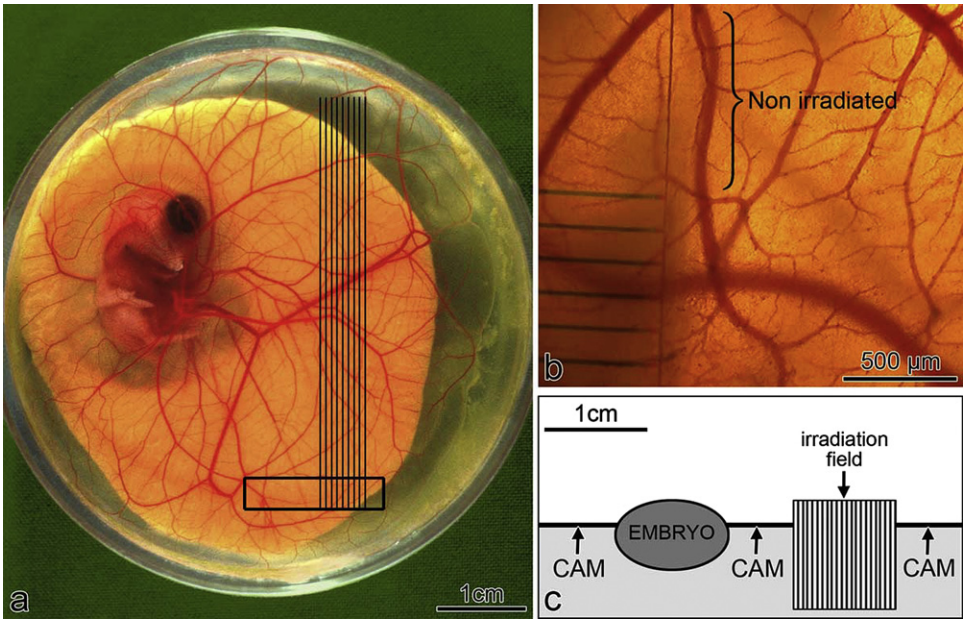

Fig. 1. CAM is shown as the thin well-vascularized membrane on the top of the shell-free cultured chick embryo. (a) Black parallel lines illustrate the MR beam conduit. (b) Black stripes on the radiochromic film indicate the entering path of the vertical microplanar beam in the irradiated region: the on-center spacing of these stripes is ∼20.0 μm. (c) Schematic representation of the position of the embryo, the CAM, and the irradiation field.

exposed to a lower dose, resulting from the scatter of secondary electrons and photons from adjacent peaks (1). MR allows dose deliveries with typical exposition times of less than 1 sec, versus SLR, which is usually applied for minutes (1).

The technique of synchrotron X-ray-generated microbeams, initiated in the 1990s at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory (2), has since then been developed in a few centers worldwide, mostly at the European Synchrotron Radiation Facility and the National Synchrotron Light Source (3–5). Prospectively, unidirectional MRT might be used in very young children, *e.g.*, for hindbrain tumors or lesions in or near the spinal canal, with intent to palliate their condition without serious neurological sequelae using peak entrance doses that generate valley doses clearly below the tolerance dose of normal tissue (3). For deeper lesions, irradiation protocols with several ports might be considered.

The first study of the biological effects of 22-MeV cylindrical deuteron microbeams was conducted with mouse brain (6, 7); after exposure to 140 Gy, mainly neurons were missing in the otherwise intact 25-μm-wide beam path, without any major vascular damage, even after exposure to 4,000 Gy. Conversely, 140 Gy, administered through a beam of 1-mm diameter, caused the complete destruction, necrosis, of the cortex in the beam path (8).

To explain the extreme resistance of the brain to MR, an identical basic hypothesis emerged from most studies: primary radiobiological effects in the brain were probably due to injury to the capillaries, with consequent ischemia-mediated damage (8). Because blood capillaries in the mouse brain are spaced ∼65 μm apart, a 25-μm-wide beam may miss enough capillaries to maintain tissue perfusion. Furthermore, tissue necrosis within the path of the microbeam was avoided by regeneration of blood vessels from contiguous, minimally irradiated vasculature outside the path (8, 9). Slatkin *et al.* (2) postulated that "this exceptional resistance to subacute brain damage by X-rays may be attributed to hyperplasia and immigration of minimally irradiated endotheliocytes and oligodendrogliocytes (or their precursors) between the slices". Similarly, duck embryonic brain irradiated unidirectionally *in ovo*, tolerated doses at least three times higher than those delivered by a broad beam (10). The normal brain microvasculature was observed *in vivo* in irradiated mice. From 2 h to 3 months after irradiation, no damage was present following 312 Gy MR (5). Thus, rapid microvessel repair by undamaged neighboring endothelia must have taken place. Furthermore, the capillary plexus of chick chorioallantoic membrane (CAM) was shown to be severely damaged in the peak zones after a dose of 300 Gy but only minimally affected in the valley zones, where it was repaired within 24 h (11).

Capillaries, arterioles, and venules obviously play an important role in mediating tissue-sparing effects of MR. In the present study, we studied (*1*) to what extent the effects of MR are mediated by vascular damage versus the vascular maturation stage and (*2*)the temporal pathophysiological changes induced by MR (*3*) compared to those caused by SLR. CAM, our model, represents an almost pure vascular system in which maturation of uncovered, naked capillaries at Day 8 of development to mature capillaries covered with pericytes at Day 12 can be observed (12).

METHODS AND MATERIALS

CAM assay

Fertilized eggs from a commercial hatchery were transferred to petri dishes and incubated at 37°C under a humidified atmosphere