



Spinal cord tolerance

Response of the rat spinal cord to X-ray microbeams

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ABSTRACT

Background and purpose: To quantify the late dose-related responses of the rat cervical spinal cord to X-ray irradiations by an array of microbeams or by a single millimeter beam.

Materials and methods: Necks of anesthetized rats were irradiated transversely by an 11 mm wide array of 52 parallel, 35 μ m wide, vertical X-ray microbeams, separated by 210 μ m intervals between centers. Comparison was made with rats irradiated with a 1.35 mm wide single beam of similar X-rays. Rats were killed when paresis developed, or up to 383 days post irradiation (dpi).

Results: Microbeam peak/valley doses of $\approx 357/12.7$ Gy to 715/25.4 Gy to an 11 mm long segment of the spinal cord, or single beam doses of ≈ 146 –454 Gy to a 1.35 mm long segment caused foreleg paresis and histopathologically verified spinal cord damage; rats exposed to peak/valley doses up to 253/9 Gy were paresis-free at 383 dpi.

Conclusions: Whereas microbeam radiation therapy [MRT] for malignant gliomas implanted in rat brains can be safe, palliative or curative, the high tolerance of normal rat spinal cords to similar microbeam exposures justifies testing MRT for autochthonous malignancies in the central nervous system of larger animals with a view to subsequent clinical applications.

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Microbeam radiation therapy (MRT) uses arrays of synchrotron-generated X-ray microbeams [MBs]. MRT is currently being considered for the palliative treatment of malignancies at radiosensitive neurological and ophthalmological sites, particularly in very young individuals [1], because experimental studies have shown that the central nervous system of rodents and piglets can tolerate much higher radiation doses delivered by spatially separated MBs [1] than those delivered by a single, uninterrupted, macroscopically wide beam (SB). High-dose, high precision radiotherapies with reduced probabilities of normal tissue complications offer prospects of improved therapeutic ratios [2]. Such improvements might be achieved by spatial inhomogeneity of dose to, and/or by reductions of uniformly irradiated volumes in targeted tissues.

In the present study, the total circumference of an 11 mm long segment of the cervical spinal cord (CSC, *intumescentia cervicalis*) of young adolescent rats was exposed to graded doses of spatially separated synchrotron X-rays, delivered by an array of 52 vertical

MBs. The valley dose, situated between any two adjacent microbeam peak doses in the array, is likely to be the most important limit for a normal tissue radiation response. In principle, if tissues in the ‘valleys’ are the reservoirs of reparative cells, valley doses should not exceed the highest dose from a single uniform beam that will be tolerated by normal tissues in the targeted zone. Knowing the valley dose is required for the prescription of a safe treatment plan involving unidirectional or stereotactic MB irradiation of a lesion in the CSC, whereas any tumor-palliative dose of SB X-rays delivered in a single fraction is unsafe. In this study, the MB dose in an 11 mm long segment of the CSC that produced paralysis in 50% of rats (i.e., the ED₅₀) was compared with the ED₅₀ delivered by an SB of synchrotron X-rays to a 1.35 mm wide segment of the *intumescentia cervicalis*; the segment irradiated by the SB was 26% shorter than the cumulative length of the 52 vertical MBs.

Materials and methods

Animals

Sixty-four specific pathogen-free rats (SPF Fischer, body weight 225–278 g, 8–12 weeks old) were used, 36 males for the MB and 28

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females for the SB arm. Irradiations were carried out under xylazine/ketamine (61.5/7.7 mg kg⁻¹ i.p.) anesthesia, after induction by 5% isoflurane in air. Animal care conformed to French licenses 380324 and A3818510002 and to Swiss licenses 104/01 and 29/00.

Microbeams (MBs)

Among the third generation of synchrotron sources, the European Synchrotron Radiation Facility (ESRF), Grenoble, France, is currently one of the most suitable for the production of highly collimated, quasi-parallel arrays of X-ray microbeams ranging in energy from 50–600 keV. A multi-slit collimator (MSC) inserted into the beam produces steep dose gradients which are delivered to the target volume within a fraction of a second. The sharp dose gradients between peaks and valleys are preserved even after penetration of the microbeams deep into tissue. The wiggler source, at a distance of 40 m from the storage ring, provides a fan beam of orthovoltage (mean energy $\approx$ 100 keV) synchrotron X-rays, about 40 mm in width and 1 mm in height [3]. The animals are moved vertically through the beam in combination with a very fast shutter system that defines the upper and lower limit of the irradiated target zone. In the ID17 hutch of ESRF, the rats, in a prone position on a remotely controlled stage, were irradiated so that the right side of their body would interrupt the horizontal fan beam. The midline of an approximately 11 mm wide array of 52 vertical, minimally divergent, approximately 35 μ m wide (SD: $\pm$ 2.9 μ m) and 0.5 mm high MBs, spaced at about 210 μ m center-to-center [ctc] intervals, was aligned to the interval between the 5th and 6th cervical vertebral bodies, i.e., about at the middle of the *intumescencia cervicalis*, the segment of the cervical spinal cord [CSC] within the six vertebrae C3 to T1 containing most of the neurons serving the forelimbs. The alignment was triangulated from bony landmarks displayed for that purpose in online radiographs from a fast read-out, low noise CCD X-ray camera. For irradiation, the stage was moved upward through the fixed MBs produced by the TECOMET^R multislit collimator [4], at a speed (mm/s) depending on the momentary ring current and the planned irradiation dose. The elevation of the rat for nearly 21 mm through the 0.5 mm high MBs ensured that the total circumference of the cord was irradiated and reduced the possibility that body movements might widen absorbed dose penumbras around the irradiated tissue slices. Incident peak doses at 3 mm depth in tissue were 334, 472, 669, and 945 Gy. Eight, 9 or 10 rats were irradiated per MB dose group; 4 male rats served as sham irradiated controls.

Single beam (SB)

Rats were also irradiated at ID17 hutch using a 1.35 mm wide, 0.5 mm high collimator. The SB was centered so that its anterior margin was about 20 mm posterior to the nadir of the *incisura intertragica*. Elevation of the stage with the rat by 25 mm resulted in SB irradiation of a total circumference of a spinal cord length of about 1.35 mm, directly in line with the SB. This length was about 25% less than was the cumulative spinal cord length of about 1.8 mm exposed, geometrically in line, to the peak dose of the 52 MBs. Filtration by 16 mm Al, upstream from the collimator, increased the median X-ray energy to $\approx$ 120 keV and approximately halved the beam flux. Incident peak doses at 3 mm tissue depth were 74, 149, 190, 238, 297, and 594 Gy. Four SB rats were irradiated per dose group (exception: 8 rats in the 297 Gy dose group). Three other rats acted as sham irradiated controls.

Follow-up

The rats were monitored daily. MB: Each rat was lifted gently from its cage and pulled backward by its tail over a horizontal cage

cover so that it touched it only with its forelegs; it would normally grab a grid wire of the cover immediately and firmly resist the pull. Complete paralysis of both forelegs would invariably follow if a rat so tested repeatedly failed to grab a wire in the horizontal cover; such rats were killed (pentobarbital sodium 200 mg/ml, 0.7 ml/kg body weight, i.v.) before paralysis ensued. SB: Each rat was placed on a cage cover held vertically; if the rat could not cling to the cover for at least 30 s, the rat was killed as noted above.

Histopathology

MB: The cervicothoracic spinal cords were dissected from the vertebral column fixed, en bloc, in neutral buffered formalin. [[ALINEA PLEASE]] SB: Rats were fixed whole by transcardial perfusion with formalin, following which a cervicothoracic segment of the vertebral column was removed and decalcified in EDTA. All specimens were embedded in paraffin; subserial 5 μ m sections were stained by hemato-[[ALINEA PLEASE]] xylin-eosin (HE) or periodic acid Schiff (PAS) reagent.

Dosimetry

Absolute dosimetry for the SB was performed using an ionization chamber and Gafchromic^R films. Relative depth dose profiles and MB profiles can be reproduced using ion chambers and radiochromic film dosimeters combined with a scanner or a microdensitometer when high resolution is required [5]. The measured valley doses are typically higher than those calculated by Monte Carlo (MC) techniques, as they were in the present study. However, with average deviations of around 10% between those obtained by MC simulations and those measured directly by films, calculated and measured dose distributions agree and remain within the recommended acceptability criteria described in international codes of clinical radiotherapy practice [6].

The transverse widths of the structures of interest on the macroscopic cut surface of a transected neck of a rat were measured; peak and valley (nadir) doses versus depth in tissue were computed using MC statistical techniques and a virtual rat neck with dimensions and density factors approximating measured values. The quantal data for the incidence of forelimb paralysis were analyzed by one of us (JH) using probit analysis [7] and ED₅₀ ($\pm$ SE) values, the dose associated with a 50% incidence of paralysis, were derived for exposures to MBs (peak and valley doses) and to SB.

Results

Dose-effect relationships

MB: The depth dose curve showed a distinct build-up effect, mainly due to scattered photons [3]; as a result peak doses were maximal at a depth of 3 mm, where the incident peak doses were 334, 472, 669, and 945 Gy, with corresponding valley doses of $\approx$ 7, 10, 14 and 20 Gy; the peak/valley dose ratio (PVDR) was 47.5:1.

The peak doses in an approximately 11 mm long segment of the CSC, near its midsagittal plane, at approximately 19 mm depth, were of the order of 253, 357, 507, and 715 Gy, the corresponding mid-valley (nadir) doses 9, 12.7, 18, and 25.4 Gy (Fig. 1). A transversal dose profile, perpendicular to the propagation path, of the 11 mm long array of 52 MBs with an incident peak dose of 472 Gy is shown in Fig 2. The peak and valley doses in the center of the array were 357 Gy and 12.6 Gy, the PVDR 28:1. The valley dose in the center of the array decreased toward the edges of the field because of decreasing dose contributions from the other microbeams.

Depth dose profiles showed that the PVDR decreased rapidly to 21:1 in the first layer of bone as scattered photons were being

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