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A reproducible radiation delivery method for unanesthetized rodents during periods of hind limb unloading

M.C. Walb [a,d,1], P.J. Black [a,1], V.S. Payne [a], M.T. Munley [a,c,d], J.S. Willey [a,b,c,*]

[a] Department of Radiation Oncology, Wake Forest School of Medicine, Winston-Salem, NC 27157, United States
[b] Department of Orthopedic Surgery, Wake Forest School of Medicine, Winston-Salem, NC 27157, United States
[c] The Comprehensive Cancer Center, Wake Forest School of Medicine, Winston-Salem, NC 27157, United States
[d] Department of Physics, Wake Forest University, Winston-Salem, NC 27109, United States

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ABSTRACT

Exposure to the spaceflight environment has long been known to be a health challenge concerning many body systems. Both microgravity and/or ionizing radiation can cause acute and chronic effects in multiple body systems. The hind limb unloaded (HLU) rodent model is a ground-based analogue for microgravity that can be used to simulate and study the combined biologic effects of reduced loading with spaceflight radiation exposure. However, studies delivering radiation to rodents during periods of HLU are rare. Herein we report the development of an irradiation protocol using a clinical linear accelerator that can be used with hind limb unloaded, unanesthetized rodents that is capable of being performed at most academic medical centers. A 30.5 cm × 30.5 cm × 40.6 cm rectangular chamber was constructed out of polymethyl methacrylate (PMMA) sheets (0.64 cm thickness). Five centimeters of water-equivalent material were placed outside of two PMMA inserts on either side of the rodent that permitted the desired radiation dose buildup (electronic equilibrium) and helped to achieve a flatter dose profile. Perforated aluminum strips permitted the suspension dowel to be placed at varying heights depending on the rodent size. Radiation was delivered using a medical linear accelerator at an accelerating potential of 10 MV. A calibrated PTW Farmer ionization chamber, wrapped in appropriately thick tissue-equivalent bolus material to simulate the volume of the rodent, was used to verify a uniform dose distribution at various regions of the chamber. The dosimetry measurements confirmed variances typically within 3%, with maximum variance <10% indicated through optically stimulated luminescent dosimeter (OSLD) measurements, thus delivering reliable spaceflight-relevant total body doses and ensuring a uniform dose regardless of its location within the chamber. Due to the relative abundance of LINACs at academic medical centers and the reliability of their dosimetry properties, this method may find great utility in the implementation of future ground-based studies that examine the combined spaceflight challenges of reduced loading and radiation while using the HLU rodent model.

1. Introduction

Exposure to the spaceflight environment has long been a known health challenge concerning many body systems (Blaber et al., 2010). For instance, the damaging effects of microgravity on muscle and bone with reduced loading are well documented (Fitts et al., 2010; Harrison et al., 2003; Keyak et al., 2009; Lang et al., 2004; Tilton et al., 1980; Trappe et al., 2009; Lang, 2006). The consequences of microgravity on many organ systems, including mission-critical and post-mission effects, are primary concerns as NASA directs more resources towards extended stays aboard the International Space Station and exploration beyond low-Earth orbit. Likewise, radiation present in the spaceflight environment represents a substantial health challenge for many organ systems, including bone, muscle, and joints (Hamilton et al., 2006; Kondo et al., 2009; Willey et al., 2011, 2013; Bandstra et al., 2009; Hutchinson et al., 2014). The hind limb unloading (HLU) rodent model was developed as a ground-based analogue for the microgravity of spaceflight to be used as a platform for studying unloading effects on various systems (Morey, 1979; Morey-Holton and Globus, 1998, 2002). The HLU model is a standard approach in part because it also can induce other physiologic

* Corresponding author at: Departments of Radiation Oncology, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, United States.
E-mail address: jwilley@wakehealth.edu (J.S. Willey).
[1] These authors contributed equally to this manuscript.

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effects observed during spaceflight, such as a cephalic fluid shift (Summers et al., 2009). Thus, ground-based rodent studies (particularly those with a musculoskeletal focus) have necessarily combined HLU with radiation exposure to assess combined biologic effects (Zhou et al., 2012; Alwood et al., 2010; Li et al., 2014; Lloyd et al., 2012; Sanzari et al., 2013).

Ideally, studies that examine the combined effects of reduced weight bearing and radiation deliver that radiation at some point during the period of HLU in order to better model spaceflight conditions (Alwood et al., 2010; Yumoto et al., 2010). To date, most of these studies have been performed using resources at the NASA Space Radiation Laboratory (NSRL, at Brookhaven National Laboratory), where galactic cosmic ray (GCR) exposures are simulated using heavy charged particle (HZE) radiation. For studies not performed using resources at the NSRL, radiation is often delivered to rodents that are either restrained or have fully loaded limbs prior to or after initiating HLU (Li et al., 2014; Lloyd et al., 2012; Sanzari et al., 2013; Kondo et al., 2010; Xu et al., 2014). The irradiation of unanesthetized rodents during HLU can be technically challenging for several reasons, including but not limited to the size restrictions of both the suspension apparatus and the irradiator, and because rodents may be housed at different locations than where they are irradiated. Regardless of any procedural limitations, all of these investigations provide novel and important information regarding the combined effects of simulated microgravity and radiation on body systems.

Herein we report the development of a whole-body irradiation protocol using a clinical linear accelerator (LINAC) that can be used with hind limb unloaded, unanesthetized rodents and which can be adapted to, and performed at, most academic medical centers. This improved method is not confounded by movement of the unanesthetized rodent, and more accurately simulates the whole-body exposure that would be received during spaceflight. The use of clinical linear accelerators for the delivery of radiation to hind limb unloaded rodents is advantageous in terms of i) the relative abundant access researchers at medical centers have to this common radiation source, and ii) dosimetric conditions that we consider to be more relevant to space radiation environments. This report describes the experimental enclosure we developed for this purpose, as well as the detailed dosimetry of the radiation field used in these experiments.

2. Materials and methods

2.1. Chamber construction

A 30.5 cm × 30.5 cm × 40.6 cm rectangular chamber was constructed out of polymethyl methacrylate (PMMA) sheets (0.64 cm thickness) and stainless steel L-brackets (Fig. 1). Aluminum strips with regularly spaced holes were affixed to opposite sides of the chamber to allow for the suspension dowel to be placed at varying heights depending on the rodent size. Additional single sheets of 30.5 cm × 30.5 cm PMMA were inserted 16.5 cm inward from both sides in order to further limit the area to which the rat had access (Fig. 1). For future use of this irradiation setup, it is desirable to minimize the space between the animal and the PMMA inserts, in order to minimize animal movement and maximize dose homogeneity. This setup, therefore, must be tailored to the size of the rodent to be irradiated. These sheets remained upright as they were each adhered to 5 cm of water-equivalent material (Gammex RMI, Middleton, WI, United States) (Constantinou et al., 1982; Ho and Paliwal, 1986). This material was necessary for dosimetric purposes in addition to limiting the movement of the animal. All procedures involving rodents were approved by the Institutional Animal Care and Use Committee.

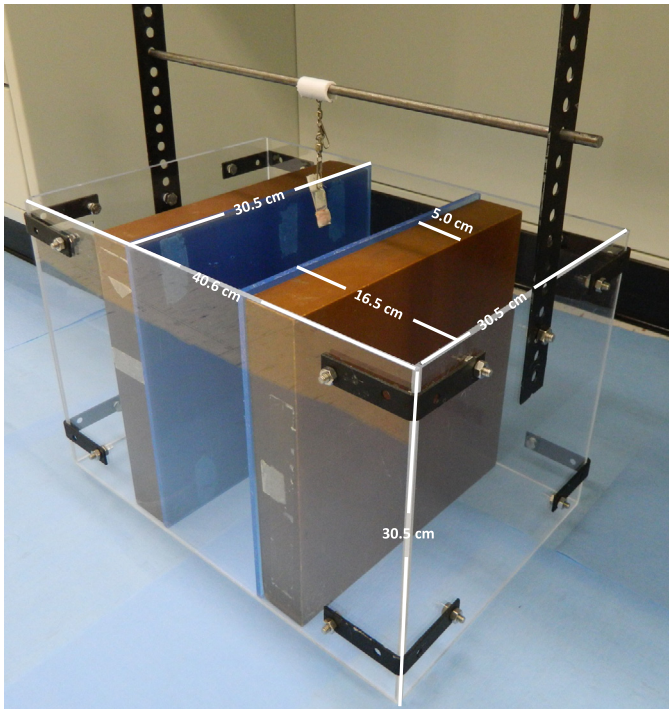

Fig. 1. The rat suspension apparatus that permitted radiation exposure from a clinical LINAC while simultaneously tail-suspending non-anesthetized rodents. The design for the cage was based on apparatus detailed by Morey-Holton and Globus (2002).

Table 1
Parameters for radiation delivery.

Linear accelerator parameters	
Beam energy	10 MV X-rays
Field size	40 × 40 cm^2 (at isocenter)
Buildup region	5 cm water-equivalent material on each side
Gantry angles	90° and 270°
Dose	100 cGy
Dose rate	∼0.98 Gy/min

2.2. Radiation delivery and dosimetry

Radiation was delivered using a Varian 2100 SC medical linear accelerator at an accelerating potential of 10 MV. The highest available beam energy of 10 MV was selected to minimize the depth-dependent dose fall off during exposure. The beam area was 40 cm × 40 cm, which encompassed the entire chamber. The total dose of whole-body radiation exposure selected for dosimetric purposes was 1 Gy, as it is relevant to a possible exposure scenario resulting from a solar particle event and has been shown to induce musculoskeletal deficiencies (Willey et al., 2011) The lowest available dose rate of ∼1 Gy/min was selected in order to minimize dose heterogeneity, specifically to the rodent due to animal movement during irradiation. All linear accelerator parameters used to deliver the radiation are provided in Table 1.

A calibrated PTW Farmer ionization chamber (PTW, Freiburg, DE), wrapped in appropriately thick tissue-equivalent bolus material to simulate the volume of a rat, was used to verify a uniform dose distribution at various regions of the chamber (Fig. 2), and determined the central axis (CAX; Table 2) dose calibration factor in order to ensure off-axis dose uniformity. The readings taken at multiple chamber positions mimicked different rodent orientations, to verify that all variances remained within the accepted <5% tolerance. Furthermore, optically stimulated luminescent dosimeters (OSLDs), with a lesser, intrinsic accuracy of ±10%,