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A Reproducible Radiation Delivery Method for Unanesthetized Rodents during Periods of Hind Limb Unloading

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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 cm 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 LINAC's at academic medical centers and the reliability of their dosimetry properties, this method may find great utility in the

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implementation of future ground-based studies that examine the combined spaceflight challenges of reduced loading and radiation while using the HLU rodent model.

Keywords

Hind limb unloading; tail suspension; radiation; dosimetry; spaceflight

INTRODUCTION

Exposure to the spaceflight environment has long been a known health challenge concerning many body systems [1]. For instance, the damaging effects of microgravity on muscle and bone with reduced loading are well documented [2–8]. The mission-critical and post-mission effects of microgravity on many organ systems are urgent 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 [9–14]. 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 [15–17]. The HLU model is a standard approach in part because it also can induce other physiologic effects observed during spaceflight, such as a cephalic fluid shift [18]. Thus, ground-based rodent studies (particularly those with a musculoskeletal focus) have necessarily combined HLU with radiation exposure to assess combined biologic effects [19–23].

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 [20, 24]. 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 [21–23, 25, 26]. 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 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.

MATERIALS & METHODS

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 (Figure 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 (Figure 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 irradiated. These sheets remained upright as they were each adhered to 5 cm of water-equivalent material (Gammex RMI, Middleton, WI, United States) [27, 28]. This material was necessary for dosimetric purposes in addition to limiting the movement of the animal.

Radiation Delivery & 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 [11]. 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 (Figure 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%, were utilized as a second dosimetric check. The location for each dosimetric measure were matched to a coordinate system (Table 2), with the origin at the bottom left corner of the irradiation chamber (Figure 2). To clarify, along the lateral axis (X), the left side is considered 0 cm, while the right PMMA wall is considered 8.0 cm. Along the longitudinal axis (Y), the far wall of the irradiation chamber is considered 0 cm,

while the wall closest to the observer is considered 30.5 cm. Finally, in the vertical direction (Z), the table surface is established as 0 cm while the top of the PMMA sheet is 30.5 cm. Initially, single beam measurements were performed before deciding that two parallel and opposed beams, each delivering half the dose, ensured greater dose homogeneity. An important consideration in this setup is the possible dose inhomogeneity due to scatter from metal components of the cage within the radiation field. In our irradiation protocol, we positioned the cage to minimize this effect. Radiation inhomogeneities due to scatter effects could be assessed by measuring dose at different points within the irradiated volume, and the setup of these measurements is detailed below. Gantry position during dosimetry measurements were identical to the setup displayed in Figure 3, which provides an image of the cage occupied by an animal with the gantry oriented laterally.

A variation in dose in an attenuating medium in the plane perpendicular to the beam is to be expected due to the “horn effect” [29]. This is a result of the linear accelerator’s flattening filter geometry and at shallow depths it can lead to “hot spots” of radiation intensity at locations off the central axis. The placement of the water-equivalent material outside of each of the PMMA insert compensated for this effect and permitted the desired radiation dose buildup (electronic equilibrium) and helped to achieve a flatter dose profile (Figure 1). This material is used routinely in medical physics applications to achieve reproducible dosimetry results when practicing quality assurance measurements of clinical linear accelerators and isotopic radiation sources [27, 28].

RESULTS

Table 2 provides the results of our dosimetry measurements and confirms a uniform dose distribution for this setup. Here monitor units (MU) correspond to dose deposited to detectors in the treatment head of the machine, and are used to calculate target dose based on target composition, beam parameters, and depth of the target region. For example, the linear accelerator used in this study is calibrated to deliver 1 cGy/MU to the isocenter at the depth of maximum dose for a 10 cm × 10 cm field in water with a radiation source to surface distance equal to 100 cm. From this knowledge, dosimetry at any depth and location can be calculated.

All ion chamber measurements are <3% variation from central axis (CAX), within the accepted tolerance of 5%, and the OSLD measurements are within their manufacturer specified accuracy of 10%. This ensures that the unanesthetized rodent will receive a uniform dose regardless of its location within the chamber (Figure 3). Additionally, this confirms that possible inhomogeneities from scattering elements of the cage are not significantly present.

DISCUSSION

A chamber was constructed to allow for total-body irradiation of unanesthetized, hind limb unloaded rodents for the purpose of improving ground-based studies that use the HLU to simulate both microgravity and interplanetary radiation environments. Dosimetry was performed using a calibrated ionization chamber that verified a homogenous dose

distribution. The maintenance of clinical sources of radiation and the characterization of radiation beams generated from these sources is highly standardized within the United States, making this protocol highly adaptable to use at other academic cancer centers. The use of this setup could be further explored at proton treatment centers, where radiation conditions could be further matched to a space radiation environment. Thus, this setup and protocol gives the ability to confidently irradiate small animal models to a desired dose without the additional complication of anesthesia and could be easily adapted to be used in any radiation oncology clinic associated with a research institution.

The use of megavolt radiation generated from a clinical linear accelerator may have some advantages for examining spaceflight-relevant biologic effects during periods of unloading than exposure to standard isotopic sources of ionizing radiation. ^{137}Cs is distinct from the radiation encountered in low earth orbit, and certainly varies from radiation encountered outside of the Van Allen belt. This difference is even more pronounced when a solar particle event (SPE) occurs [30, 31]. This difference principally lies in ^{137}Cs quality. Its radiation output is comprised of a spectrum of homogeneous, monoenergetic photons with energy of 662 keV. Space radiation, in contrast, has a broad energy spectrum ranging from 10^1 to 10^6 MeV per particle. Moreover, low earth orbit is almost completely comprised of a heterogeneous mixture of particles, principally protons, but also consisting of neutrons as well as charged particles including helium, oxygen, and iron ions. This environment changes based on solar activity, with significant flux variances measured at different time points between 1970 and 1990 [32]. The argument that photon irradiation is comparable has been made, and is rooted in the similarity of the accepted RBE of photons (1.0) and protons (1.1–1.3) [33]. It should be noted, however, the RBE of protons has been contested with models arising involving an RBE dependent on depth of dose deposition [34–36].

Our protocol for dose delivery using megavolt X-rays from a LINAC during hind limb unloading circumvents some potential problems, and may employ more spaceflight-relevant radiation conditions than use of ^{137}Cs . The use of the LINAC allows us to control the mean energy, field size, and dose rate. Moreover radiation fields produced with a clinical linear accelerator are polychromatic in nature, with known energy spectra. For example, in this study our accelerating potential of 10 MV produces photons with a maximum energy of 10 MeV, but an average energy of 2.9 MeV [37]. This, taken in conjunction with the higher number of clinical linear accelerators present at research hospitals, leaves us with a more practical protocol for the delivery of ionizing radiation to non-anesthetized rodents that are hind limb unloaded.

This chamber design and irradiation protocol for rodents during periods of HLU can be implemented at most academic medical centers that utilize a standard medical linear accelerator source that is available for research purposes. The dosimetry measurements confirmed variances typically within 3%, with maximum variance <10% indicated through OSLD measurements, thus delivering reliable spaceflight-relevant total body exposures. Thus 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.

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Highlights

- We developed a method to irradiate unanesthetized rodents during periods of hind limb unloading.
- The total body irradiation (TBI) dose is homogenous.
- The HLU + TBI protocol can be utilized at any academic medical center using a clinical linear accelerator.

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 [17].

FIG. 2.

A photograph of our irradiation chamber as it was used to obtain dosimetry measurements. The ion chamber is in a hanging position to mimic a HLU rat during dosimetric measurements. The dosimetry was performed using both the ion chamber in multiple positions, as well as optically stimulated luminescent dosimeters distributed throughout the chamber. Absorbed doses were identified at specific locations using a three dimensional coordinate system, illustrated by the white arrows in the three orthogonal planes (X, Y, and Z) superimposed on the image of the hanging ion chamber. The central axis laser (CAX) is indicated by the dashed white arrow. Both blocks of water-equivalent material are indicated by the white stars.

FIG. 3.

The experimental setup that includes a hind limb unloaded rat positioned in the clinical linear accelerator using the verified dosimetric setup. Each total radiation dose is delivered in two parallel opposed beams.

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

Table 2

Verification parameters used for assurance of dose uniformity

Calculated Dosimetry Data			
Dosimeter	Dose Rate (cGy/MU)	Deviation from CAX	Location (X,Y,Z) (cm)
Ion chamber	0.987	--	(4, 15, 2)
Ion chamber	0.999	1.16%	(6, 23, 2)
Ion chamber	1.009	2.23%	(2, 8, 2)
Ion chamber	1.002	1.48%	(4, 22, 5)
Ion chamber	0.989	0.14%	(4, 21, 7)
Ion chamber	0.982	-0.55%	(4, 23, 3)
Ion chamber	0.992	0.52%	(6, 2, 8)
OSLD	1.017	3.00%	(8, 15, 5)
OSLD	1.019	3.26%	(0, 15, 5)
OSLD	1.071	8.44%	(8, 1, 15)
OSLD	1.084	9.80%	(0, 15, 15)

Notes: CAX = Central axis laser; MU = monitor unit.