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RESEARCH ARTICLE

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Clinical and Histopathologic Effects of Radiotherapy on the Sciatic Nerve in a Rat Model

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ABSTRACT

Objective: Radiation-induced peripheral neuropathy (RIPN) is a debilitating effect of external beam radiation therapy, impacting 9-60% of treated patients. The exact pathogenesis and time to onset of RIPN are unknown, necessitating the creation of an animal model to explore radiation dose dependent sciatic nerve injuries. This study is aimed to (1) create a targeted radiation-induced sciatic nerve injury in a rat model and (2) analyze histological and functional impact across 3 radiation doses, 20/40/60 Gy, at 4-weeks and 16-weeks post-radiation.

Methods: 18 male Lewis rats were equally split into three treatment groups of differing radiation dosages. The left thigh of each rat was irradiated while the right served as a control. Primary outcomes were measured with histologic findings based on necrotic and vascular changes, with secondary outcome measures including sciatic functional index, tibialis anterior muscle mass, sciatic nerve conduction velocity, axon count, and axonal swelling.

Results: Overall, Sciatic Functional Index scores showed a decline in lower extremity function over time and a significant difference was found in the tibialis anterior muscle mass between the control and radiated sides ($p<0.001$). There was a significant difference in nerve conduction velocity between the control and irradiated sciatic nerves ($p<0.0001$), and for two of the dosage-specific subgroups. Furthermore, there was a significant difference in axon count per high powered field between the control and radiated sciatic nerves ($p<0.001$). Axonal swelling also significantly increased in the radiated side ($p<0.001$). Lastly, a significant difference was found in the severity of necrotic and vascular change between the control and irradiated sciatic nerves.

Conclusion: This study illustrated the viability of radiating small animals with single-fraction external beam radiation up to 60 Gy. We believe these findings begin to provide an explanation for post-radiation symptoms that patients experience, and will lay the groundwork for future RIPN animal studies.

ARTICLE HISTORY

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Introduction

Radiation therapy is commonly utilized in the management of more than 60% of cancer patients [1]. Radiation causes the generation of free radicals, including reactive oxygen species and reactive nitrogen species, leading to cellular damage and ultimately cell death [2]. Unfortunately, radiation affects healthy and cancerous cells alike, resulting in severe complications ranging from lymphedema and fibrosis to neuropathy, with occasional permanent motor and sensory deficits [3,4].

The degree of cell damage and death following radiation therapy directly correlates with the amount of radiation delivered to the tissue [5]. In the case of radiation-induced peripheral neuropathy, the time of onset is not immediate, as two unique stages must first occur [6]. The first stage includes the initial cellular damage, including the depletion of the cell's antioxidant system's ability to neutralize free radicals and reactive oxygen species. The second stage is the subsequent scarring and fibrosis of the nerve and surrounding tissues within the radiation field [1]. Peripheral

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nerves are particularly susceptible to the effects of radiation as the lipids found within the myelin sheath also undergo lipid peroxidation [7]. Within two days of irradiation, dose-dependent and irreversible changes in the nerve's vascular permeability, microtubule assembly, and bioelectric properties, including action potential and conduction velocity, can be observed [8,9]. A dosage of only 20 Gy to the sciatic nerve has been shown to impact myelin regeneration and increase collagen within the endoneurium within only two months [10]. Beyond these early histological findings, clinical symptoms can develop years later, with breast cancer patients reporting symptoms one to two years post-radiation and sarcoma patients reportedly developing peripheral neuropathy more than three years post-radiation [11,12].

The pathogenesis and timeline of radiation-induced neuropathy are not well understood. It remains a significant challenge for oncologists employing radiation therapy. In order to conduct experiments on potential interventions to prevent symptoms of radiation-induced neuropathy, it is necessary to develop a baseline model of radiation doses and administration methods similar to what occurs in clinical practice. While the current literature surrounding radiation-induced neuropathy is expanding, few studies are investigating the difference in radiation damage across various doses and at different time points. Therefore, the purpose of this study is to create a radiation-induced sciatic nerve injury model in a rat, analyzing the histological and functional impact of 20 Gy, 40 Gy, and 60 Gy treatments at one month and four months post-radiation.

Material and Methods: Level of Evidence 2

Animals

Eighteen adult male Lewis Rats (200-250g) were housed in pairs within our institution's animal facility with a twelve-hour light-dark cycle and given a rodent diet and water ad libitum.

Experimental Design

In 2020, we performed a prospective study utilizing Lewis rats following our institution's Institutional Animal Care and Use Committee (IACUC) approval. The study design involved 18 male Lewis rats, equally divided into three treatment groups: 20 Gy, 40 Gy, and 60 Gy (Figure1). A single-fraction radiotherapy dose corresponding to the rat's group was administered to the left thigh of each rat using a small animal external beam radiotherapy platform. A separate control (non-radiated) group was not included, as the right lower extremity of each rat was protected from the radiation field, and as such, served as the animal's own internal control. The number of rats included was based on this being a feasibility/pilot study, and a power analysis was not performed. Following the radiation, the rats were housed for either one month or four months. At the 4-week timepoint (Timepoint 1), half of the rats were tested and sacrificed; the second half underwent testing and sacrifice at the 16-week timepoint (Timepoint 2.) The live evaluation included a gait analysis, followed by bilateral nerve conduction velocity testing under anesthesia. The sciatic nerve and tibialis anterior muscle of both lower extremities were then harvested, and the animal was humanely euthanized. The primary outcome measure for this study was the histologic findings based on necrotic and vascular changes, with secondary outcome measures including sciatic functional index, tibialis anterior muscle mass, sciatic nerve conduction velocity, axon count, and axonal swelling. The researchers were not blinded during the dissection, weighing, nerve conduction velocity testing, or sciatic nerve functional index testing/scoring. However, they were blinded during the histologic analysis.

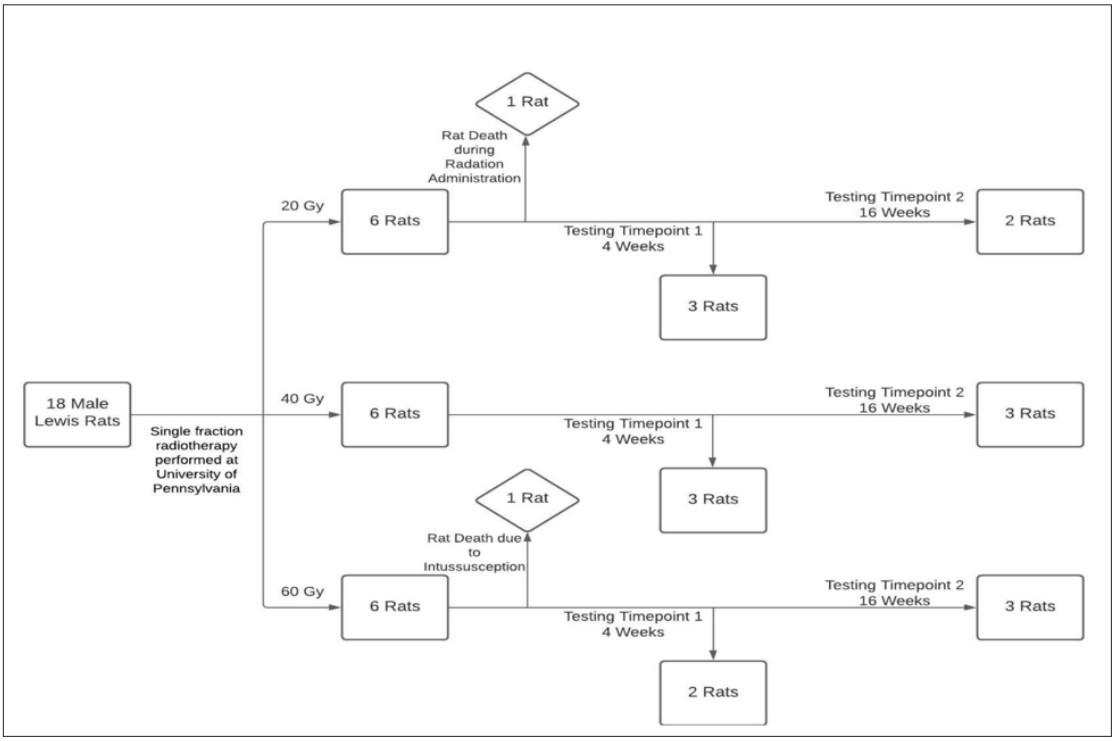

Figure 1: Flow diagram depicting blocking architecture and time points used to fulfill study design. 18 male Lewis rats were equally split into 3 treatment groups of 20/40/60 Gy radiation with data collected at 4 and 16 weeks within each treatment group.

Two rats ultimately were excluded from the study. One rat within the 20 Gy radiation group expired during radiation administration due to an anesthesia complication. One rat within the 60 Gy radiation group expired during the third week of the study due to bowel intussusception unrelated to the radiation experiment, and required humane euthanasia. Humane endpoints that necessitated early euthanasia included development of pain, infection, prolonged scratching of radiated skin, skin desquamation, weight loss (20% or more of body weight), or other disability that is not amenable to pain medications, local wound care, or nutritional care. Euthanasia was performed under deep anesthesia with induced bilateral pneumothoraxes to ensure death.

Irradiation Procedure

3 groups of six rats each received single-fraction radiation doses of 20Gy, 40Gy, or 60Gy to their left thigh. These doses were selected based on prior studies demonstrating single-fraction doses necessary to induce neurotoxicity in the brains of rats and peripheral nerves of rabbits [13,14]. Radiation was delivered with rats under inhaled 2% isoflurane anesthesia, using a collimated beam of x-rays with a tube potential of 220kVp, 13mA current, and a dose rate of ~2Gy/min.

Sciatic Nerve Functional Index

The sciatic functional index (SFI) is a validated scoring system described by Shabeeb et al. to assess rat sciatic nerve function by comparing multiple gait parameters between the control limb and experimental limb. The SFI is a composite score describing the relative dysfunction of the experimental (irradiated) limb vs. the control limb, with 0 being no difference in limb function and -100 being severe dysfunction. Measurements were taken from the rat footprints collected using ink on a walking track [1]. The three input variables for the composite score are derived from six measurements, including print length (distance from heel to third toe), toe spread (TS, distance from first to fifth toe), and intermediary toe spread (IT, distance from second to fourth toe.) The greater the sciatic nerve dysfunction and resultant motor neuropathy, the smaller the print length and distance between the toes (Figure 2).

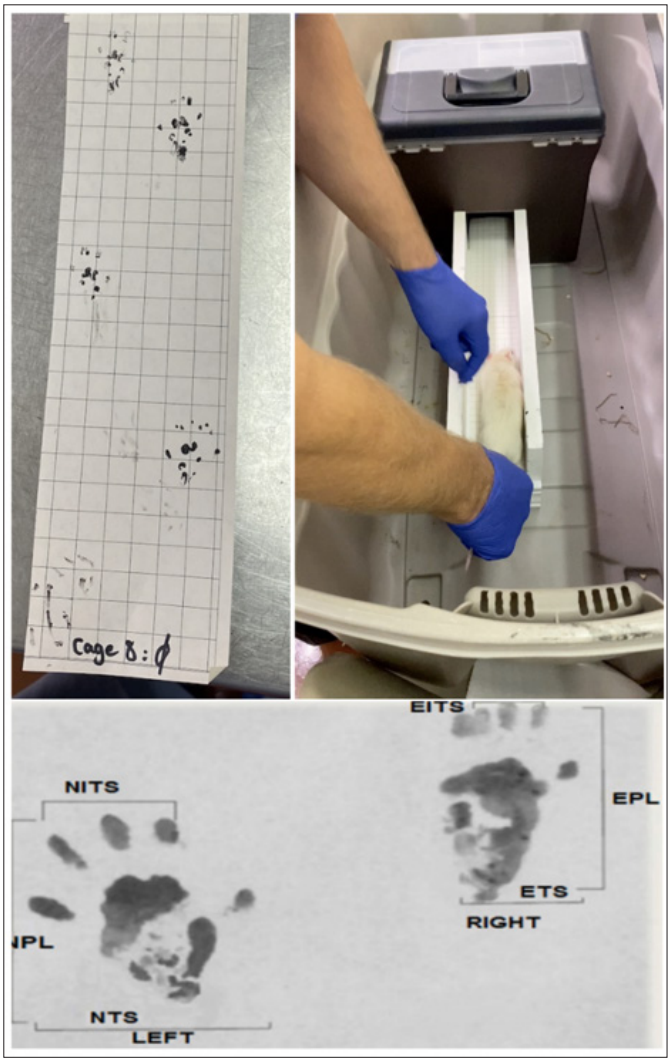

Figure 2: Functional sciatic nerve testing used to access rat sciatic nerve function. Various gait parameters including print length, toe spread, and intermediary toe spread were compared between experimental (radiated) and control (non-radiated) rat limbs to determine sciatic nerve dysfunction and motor neuropathy.

Nerve Conduction Velocity

The sciatic nerve was exposed using the dorsal approach to the rat's thigh. The sciatic nerve conduction velocity was then measured across the zone of injury utilizing a micro-hook electrode placed at the hip and knee (Figure 3). The conduction velocities were measured via a signal amplifier (AM3000 with Headstage (single channel) (AD Instruments, Colorado Springs, CO). The analog output from the amplifier was converted into digital input into a personal computer via a data acquisition unit (USB-6001 14-Bit 20 kS/s Multifunction I/O and NI-DAQmx, National Instruments, Austin, TX), and interpreted utilizing LabVIEW software (National Instruments, Austin, TX). Stimulation duration was 0.04 ms, frequency 1 Hz, and stimulus intensity of 0.4 mA to elicit an action potential (Figure 4).

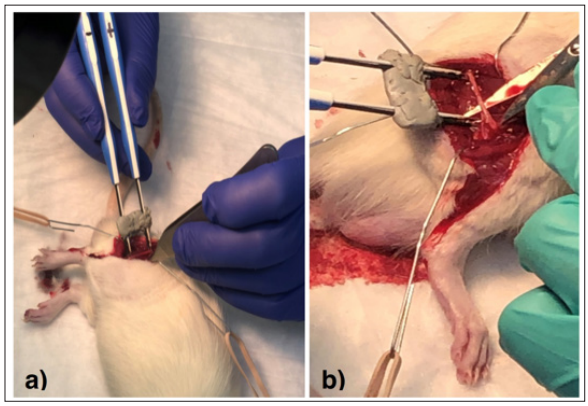

Figure 3: Electrophysiologic testing of sciatic nerve to measure nerve conduction velocity through the use of a micro-hook electrode and a signal amplifier.

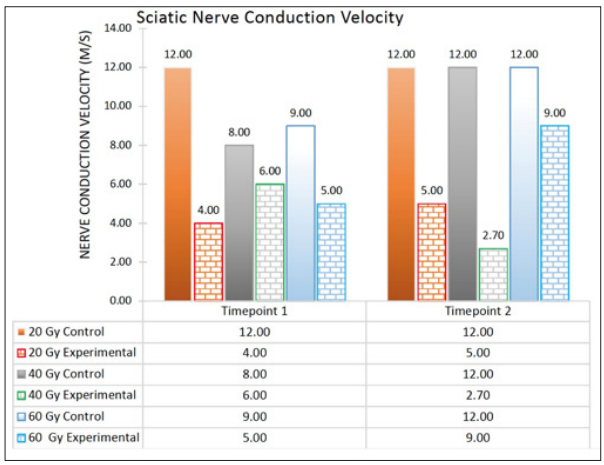

Figure 4: Graphic depicting sciatic nerve conduction velocities (M/S) at varying radiotherapy dosages collected at timepoint 1 and timepoint 2 during electrophysiologic testing of rat sciatic nerve.

Tibialis Anterior Muscle Weight

The tibialis anterior muscles on both the radiated and non-radiated sides were excised at the time of nerve harvest using identical surgical technique. The tibialis anterior muscle belly was then weighed (Figure 5).

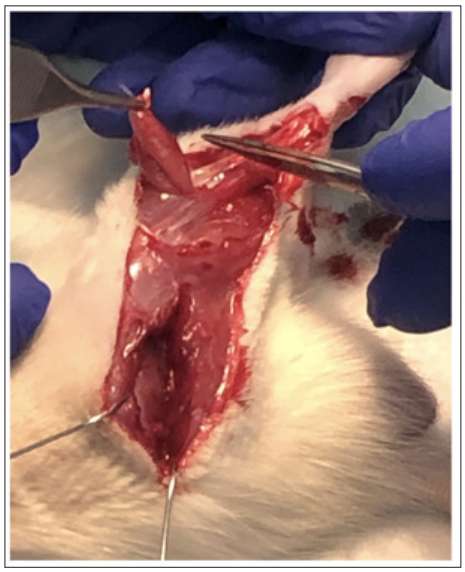

Figure 5: Harvest of rat tibialis anterior muscle belly

Sciatic Nerve Histological Examination

With the rat under anesthesia, the sciatic nerve was excised from both the radiated and non-radiated thighs after being isolated through a dorsal approach through the thigh. The proximal and distal ends were marked with different color inks. Nerve samples were prepared and stained in accordance with the methods described by Di Scipio et al [15]. After hematoxylin and eosin staining, both axonal and longitudinal cross-sections were analyzed.

Necrotic and Vascular Changes

The primary outcome measure for this study was the necrotic and vascular changes in the sciatic nerve following radiotherapy. To evaluate the impact of radiotherapy, a validated semi-quantitative scoring system to measure radiation-induced nerve damage described by Shirazi et al. was utilized [16]. A blinded oncology-trained pathologist and blinded orthopedic oncology surgeon scored each sciatic nerve section. The two scoring criteria were vascular changes and necrotic changes. Vascular changes were defined as the loss of epineural vessels as result of radiation. Necrotic changes were defined by increased interstitial edema and loss of axonal adhesion resultant from radiation (Figure 6). A score of 0-4 for vascular changes and necrotic changes was assigned to each specimen by the two reviewers, with a score of 0 representing no changes/healthy nerve, and 4 representing the most severe radiation-associated changes. The vascular and necrotic changes scores were averaged for a composite histological score.

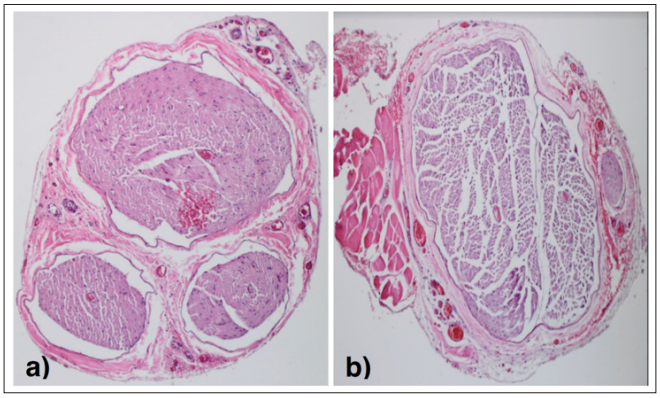

Figure 6: Necrotic changes are shown here. Figure 6b (radiated nerve) demonstrates interstitial edema, necrosis, and loss of axon density compared to Figure 6a (healthy nerve).

Axon Count and Axonal Swelling

For all 32 sciatic nerves harvested, an axonal nerve count was performed using Image J, a digital image processing program. The axon count was performed on axial slides under 100x power with oil immersion. Each axon was marked using Image J and manually counted, to obtain an axon count per high powered field (Figure 7). Then, the cross-sectional area of 10 randomly selected axons under 100x power was measured in micrometers. The 10 measurements were then averaged to determine the average cross-sectional area of the axons of that slide, a surrogate marker for axonal swelling (Figure 8).

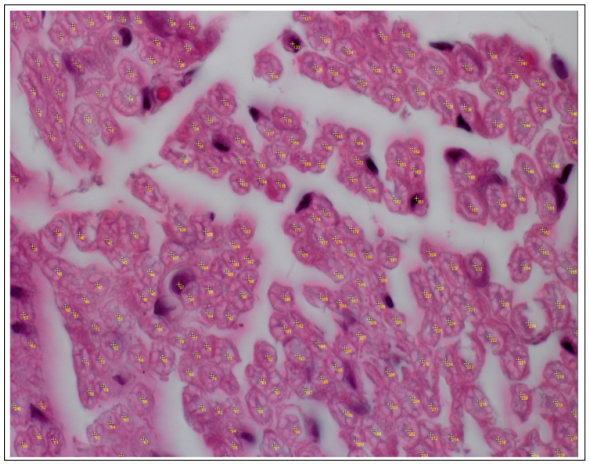

Figure 7: 100x axial cross section image of harvested rat 1 left (radiated) sciatic nerve. Hematoxylin and eosin staining in conjunction with Image J processing program were used to manually obtain axon count.

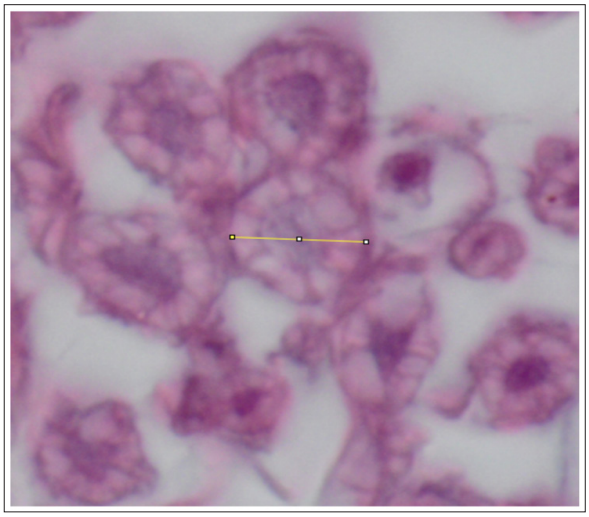

Figure 8: 100x axial cross section image of harvested rat 5 left (radiated) sciatic nerve. Hematoxylin and eosin staining in conjunction with Image J processing program were used to obtain cross-sectional area measurements of axons.

Statistical Analysis

The statistical analysis was performed utilizing SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). A paired t-test was used to compare the radiated and non-radiated results for: axon diameter, neuron count, tibialis anterior muscle mass, nerve conduction velocity, print length, toe spread, and intermediary toe spread. A Wilcoxon signed rank test was used to compare the necrotic changes, vascular changes, and average necrotic and vascular changes of radiated and nonradiated sciatic nerves. A linear regression analysis with time points and radiation dose, with radiation dose only, and with time only, was performed to determine the impact on each outcome variable with outcomes of radiation with 20Gy serving as the constant group.

Results

Necrotic Changes

There were significant differences in histologic necrotic changes between the radiated and nonradiated sciatic nerves overall, and when analyzed at both timepoints (Table 1). Additionally, the 20Gy and 60Gy dose-specific subgroups demonstrated significant differences at Timepoint 2. No other significant differences were found among the dose-specific subgroups.

Table 1: Results portraying significant necrotic changes between 20/40/60 Gy radiated and nonradiated sciatic rat nerves at timepoint 1, timepoint 2, and overall

Table 1	Necrotic Changes (Graded 0-4)		
	Right (Healthy)	Left (Radiated)	p-value
Overall	0.27	2.20	<0.001
Timepoint 1			
Overall	0.38	2.13	<0.001
20 Gy	0.33	1.67	0.057
40 Gy	0.33	2.33	0.074
60 Gy	0.50	2.50	0.295
Timepoint 2			
Overall	0.14	2.29	<0.001
20 Gy	0.00	2.00	<0.001
40 Gy	0.00	2.00	0.2952
60 Gy	0.33	2.67	0.0198

Vascular Changes

Similarly, statistically significant differences in histologic vascular changes were found in analyses of the radiated and nonradiated sciatic nerves overall, at both timepoints overall, for the 20Gy subgroup at Timepoint 1, and the 60Gy subgroup at Timepoint 2 (Table 2). No other significant differences were found among the dose-specific subgroups.

Table 2: Results portraying significant vascular changes between 20/40/60 Gy radiated and nonradiated sciatic rat nerves at timepoint 1, timepoint 2, and overall

Table 2	Vascular Changes (Graded 0-4)		
	Right (Healthy)	Left (Radiated)	p-value
Overall	0.20	2.47	<0.001
Timepoint 1			
Overall	0.25	2.00	<0.001
20 Gy	0.33	2.67	0.020
40 Gy	0.33	2.00	0.130
60 Gy	0.00	1.00	-
Timepoint 2			
Overall	0.14	3.00	<0.001
20 Gy	0.00	2.50	0.126
40 Gy	0.50	3.00	0.344
60 Gy	0.00	3.33	0.038

The above findings led to significant differences in the calculated average necrotic and vascular changes between the radiated and nonradiated sciatic nerves overall, at both timepoints overall, the 20Gy subgroup at Timepoint 1, and the 60Gy subgroup at Timepoint 2 (Table 3).

Table 3: Results portraying calculated average necrotic and vascular changes between 20/40/60 Gy radiated and nonradiated sciatic rat nerves at timepoint 1, timepoint 2, and overall

Table 3	Average Vascular and Necrotic Change Score (Graded 0-4)		
	Right (Healthy)	Left (Radiated)	p-value
Overall	0.23	2.33	<0.001
Timepoint 1			
Overall	0.3125	2.0625	<0.001
20 Gy	0.33	2.17	0.008
40 Gy	0.33	2.17	0.053
60 Gy	0.25	1.75	0.205
Timepoint 2			
Overall	0.14	2.64	<0.001
20 Gy	0.00	2.25	0.071
40 Gy	0.25	2.50	0.323
60 Gy	0.17	3.00	0.023

Axon Count

A significant difference in axon count was found between the radiated and nonradiated sciatic nerves overall, and between the two groups overall at Timepoint 1 (Table 4). No other significant differences were found among the dose-specific subgroups.

Table 4: Results portraying significant axon count differences between 20/40/60 Gy radiated and nonradiated sciatic rat nerves at timepoint 1, timepoint 2, and overall

Table 4	Axons per high powered field (100x)		
	Right (Healthy)	Left (Radiated)	p-value
Overall	196.8	167.27	<0.001
Timepoint 1			
Overall	214.13	183.38	0.022
20 Gy	248.00	211.67	0.344
40 Gy	177.00	152.67	0.140
60 Gy	219.00	187.00	0.137
Timepoint 2			
Overall	177.00	148.86	0.071
20 Gy	164.50	149.00	0.539
40 Gy	139.50	127.00	0.766
60 Gy	210.33	163.33	0.140

Axonal Swelling

A significant difference in axonal diameter was found between the radiated and nonradiated sciatic nerves of the entire cohort overall, as well as these groups at Timepoint 1 and Timepoint 2 (Table 5). No other significant differences were found among the dose-specific subgroups.

Table 5: Results portraying an overall significant axon diameter difference between 20/40/60 Gy radiated and nonradiated sciatic rat nerves as well as at timepoint 1 and timepoint 2

Table 5	Axon Diameter (micrometer)		
	Right (Healthy)	Left (Radiated)	p-value
Overall	3.331	5.011	<0.001
Timepoint 1			
Overall	2.880	4.389	0.006
20 Gy	2.708	4.012	0.250
40 Gy	2.921	5.010	0.080
60 Gy	3.075	4.021	0.277
Timepoint 2			
Overall	3.846	5.722	0.007
20 Gy	3.887	5.239	0.227
40 Gy	3.970	5.090	0.173
60 Gy	3.737	6.464	0.090

Nerve Conduction Velocities

A significant difference in the nerve conduction velocity was found between the radiated and nonradiated sciatic nerves of the entire cohort, these two groups at Timepoints 1 and 2, and among the 20Gy subgroup at Timepoint 1 (Table 6). No other significant differences were found among the dose-specific subgroups.

Table 6: Results portraying an overall significant nerve conduction velocity difference between 20/40/60 Gy radiated and nonradiated sciatic rat nerves as well as at timepoint 1 and timepoint 2

Table 6	Nerve Conduction Velocity (m/s)		
	Right (Healthy)	Left (Radiated)	p-value
Overall	10.71	5.24	<0.001
Timepoint 1			
Overall	9.75	5.00	0.008
20 Gy	12.00	4.00	0.020
40 Gy	8.00	6.00	0.423
60 Gy	9.00	5.00	0.295
Timepoint 2			
Overall	12.00	5.57	0.006
20 Gy	12.00	5.00	0.090
40 Gy	12.00	2.70	0.021
60 Gy	12.00	9.00	0.500

Tibialis Anterior Muscle Weight

A significant difference in muscle mass was found between the radiated and nonradiated tibialis anterior muscles overall, and at Timepoint 1 and 2; the radiated side demonstrated atrophy. We were unable to demonstrate significant differences in further subgroup analysis (Table 7).

Table 7: Results portraying significant tibialis anterior muscle mass difference between 20/40/60 Gy radiated and nonradiated sciatic rat nerves at timepoint 1, timepoint 2, and overall

Table 7	Tibialis Anterior Muscle Mass (grams)		
	Right (Healthy)	Left (Radiated)	p-value
Overall	0.92	0.78	<0.001
Timepoint 1			
Overall	0.82	0.70	0.002
20 Gy	0.87	0.78	0.141
40 Gy	0.78	0.67	0.094
60 Gy	0.80	0.63	0.253
Timepoint 2			
Overall	1.05	0.86	0.002
20 Gy	0.97	0.86	0.186
40 Gy	1.23	0.96	0.094
60 Gy	0.98	0.80	0.079

Sciatic Nerve Functional Index

There were no statistically significant differences found in our comparisons of radiated and healthy hindlimbs of the rats at any timepoint, at any radiation dosage (Table 8).

Table 8: Results portraying no significant differences found in the Sciatic Nerve Functional Index Scores between 20/40/60 Gy radiated and nonradiated sciatic rat nerves at timepoint 1, timepoint 2, and overall

Table 7	Sciatic Nerve Functional Index Score		
	Timepoint 1	Timepoint 2	P-Value
Overall	-16.63	-23.78	0.395
20 Gy	-32.59	-23.18	0.392
40 Gy	-15.58	-23.92	0.534
60 Gy	5.73	-24.08	0.104

Discussion

Radiation-induced peripheral neuropathy is a uniquely challenging complication that oncologists and their patients must contend with after radiotherapy. The etiology appears to be multifactorial, and the manifestation of the neuropathy itself seems variable and unpredictable. Our study is one of the first to develop a radiation-induced sciatic nerve injury model in a small animal and assess the histological and functional impact of graded doses of radiotherapy.

Our results were consistent with previous studies investigating histologic observations of radiated nerves, particularly the vascular and necrotic changes seen [4,16]. Significant vascular changes were detected across multiple radiotherapy dosages at both time points, with all specimens showing a greater degree of vascular changes at Timepoint 2 rather than Timepoint 1. There was obvious destruction of epineural vessels and this loss was more profound over time. Impaired vascular flow and limited delivery of oxygen to nerve may inhibit tissue repair and cellular function. We also observed necrotic changes within the radiated specimens: dramatic differences in levels of interstitial edema, swelling, and decreased axonal counts were detected. Previous literature has shown an association between worsening tissue damage and ischemia and the dose of radiation administered, and many dose-related trends are seen in our data on vascular and necrotic changes that suggest this phenomenon as well [7,8,13,14].

In terms of timing of these developments, radiation-induced peripheral neuropathies typically appear years following the initial radiation treatment despite evidence of cellular damage immediately following radiotherapy. This study supports this clinically-witnessed phenomenon. Axonal count was more conspicuously blunted at Timepoint 2 than at Timepoint 1, and the composite average score of necrotic and vascular changes was more severely affected at the later timepoint.

In only 4 months, this study was able to demonstrate Electrophysiologic and clinical changes in the nerves exposed to radiation. Overall, the nerve conduction velocity of the radiated nerves was approximately 50% of their matched pairs, an expected result following radiotherapy [9,12]. Previous studies have demonstrated that slowed motor nerve conduction velocity results in atrophy of the target musculature, and the authors hypothesize that this is the basis for the decrease in muscle mass observed here [17]. One of our most notable and clinically-relevant findings was the tibialis anterior muscle atrophy observed on the left hindlimb compared to the right in the same animal. Despite this finding, the atrophy did not translate to worse hindlimb function as quantified by the sciatic functional index. This is in line with previous studies, including the work of Aktas et al., who found no difference in motor function at six months following 20Gy radiotherapy to rat sciatic nerves, and Stoll et al. who describe the majority of neuropathies occurring 10 months after irradiation [8,18]. Similarly, Shabeeb et al. were unable able to demonstrate a statistically significant difference in SFI scores at four weeks after radiation, but were able to demonstrate significant differences at 12 and 20 weeks [1]. Our failure to demonstrate a significant difference in SFI may also be due to the low power of our study, as two of the three individual components of the SFI did indeed demonstrate a significant difference, or it may be that the animal was able to compensate for weak tibialis anterior (at least at the 16-week timeframe) and a difference truly did not exist between right and left hindlimb function.

Despite these important findings, we recognize that our study is not without limitations, and the results should be interpreted with these limitations in mind. First and foremost, the biggest limitation is the power of our study, as it was designed with the intention of serving as a pilot/feasibility study. As such, 3 rats per sub-group were included in the initial design, of which 2 were lost to early attrition, and this limited the meaningful statistical analysis that could be performed. To mitigate this low power, a paired t-test using the contralateral limb as an internal control was performed, but this did not completely eliminate the risk of committing a Type II error. Additionally, the inclusion of only 2 discrete time points and lack of follow up beyond 16 weeks may limit the conclusions that can be drawn from this study, especially considering that the onset of radiation-induced peripheral neuropathy occurs months to years following radiotherapy [4].

Despite limitations, we believe our findings allow this study to meaningfully contribute to current literature regarding radiation-induced neuropathies. One important strength of this investigation is that it proves feasibility and safety of

single-fraction radiation administration to the rat hindlimb; we have addressed safety concerns posed by previous studies that delivered radiation in 10 fractions attempting to minimize toxicity [18]. Our report establishes a rat model for the administration of various radiation doses to the sciatic nerve, as well as offers a framework of multiple outcome measures of radiation's effect on the health and function of peripheral nerve. We hope that future studies will utilize this fundamental understanding of the underlying pathologic mechanisms of radiation-induced neuropathy to further explore strategies to minimize this complication in patients.

This study established a rat model of radiation-induced sciatic neuropathy and studied the functional, histopathologic, and electrophysiologic effects of various radiation dosages on the nerve and target muscle. Furthermore, this study demonstrates the safety and viability of administering single-fraction radiation doses up to 60Gy to Lewis rats' hindlimb. Despite our small sample size, we found statistically significant changes in many parameters in as little as 4 weeks. We believe these findings represent the underlying basic science explanations for the symptoms patients suffer after radiation. They will lay the groundwork for future animal studies on radiation-induced peripheral neuropathy.

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