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Skin wound trauma, following high-dose radiation exposure, amplifies and prolongs skeletal tissue loss

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ABSTRACT

The present study investigated the detrimental effects of non-lethal, high-dose (whole body) γ-irradiation on bone, and the impact that radiation combined with skin trauma (i.e. combined injury) has on long-term skeletal tissue health. Recovery of bone after an acute dose of radiation (RI; 8 Gy), skin wounding (15–20% of total body skin surface), or combined injury (RI + Wound; CI) was determined 3, 7, 30, and 120 days post-irradiation in female B6D2F1 mice and compared to non-irradiated mice (SHAM) at each time-point. CI mice demonstrated long-term (day 120) elevations in serum TRAP 5b (osteoclast number) and sclerostin (bone formation inhibitor), and suppression of osteocalcin levels through 30 days as compared to SHAM ($p < 0.05$). Radiation-induced reductions in distal femur trabecular bone volume fraction and trabecular number through 120 days post-exposure were significantly greater than non-irradiated mice ($p < 0.05$) and were exacerbated in CI mice by day 30 ($p < 0.05$). Negative alterations in trabecular bone microarchitecture were coupled with extended reductions in cancellous bone formation rate in both RI and CI mice as compared to Sham ($p < 0.05$). Increased osteoclast surface in CI animals was observed for 3 days after irradiation and remained elevated through 120 days ($p < 0.01$). These results demonstrate a long-term, exacerbated response of bone to radiation when coupled with non-lethal wound trauma. Changes in cancellous bone after combined trauma were derived from extended reductions in osteoblast-driven bone formation and increases in osteoclast activity.

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1. Introduction

The recently expanded use of radioactive materials in medicine, industry, agriculture, and research increases the opportunity for radiation combined injuries (CI) to occur [1]. These combined injuries, which include exposure to ionizing radiation in addition to another trauma (i.e. skin wounds, thermal burns, hemorrhage, skeletal fractures, and traumatic brain injuries), have been prevalent in the survivors of recent and historical radiation accidents (~10% of the 237 victims at Chernobyl) and bombings (~60–70% of all victims after the bombings at Hiroshima and Nagasaki) [2–4]. First responders and survivors of a nuclear attack, nuclear accident, or exposure to a radioactive dispersal device (RDD) will likely also suffer secondary consequences related to combined injuries [5,6]. In addition, long-duration space exploration or missions involving astronaut time on terrestrial surfaces with little to no atmosphere to shield space-relevant radiation (i.e. galactic cosmic rays or solar particle events), will expose travelers to unavoidable radiation [7,8]. In these events, any type of traumatic injury that is sustained by a crew member, in addition to ionizing radiation exposure, could limit or impede mission success [9]. Long-term consequences of these radiation combined traumas may exacerbate the effects of the individual insult on skeletal tissue, putting those individuals at greater risk for bone atrophy and risk of skeletal fracture, yet details of the interaction between radiation and injury are unclear.

The majority of literature detailing the effects of radiation on bone illustrates the consequences to skeletal tissue following radiation therapy as a treatment for various types of cancers. Radiation therapy induces severe long-term consequences to bone, which include increased bone loss and incidence of skeletal fracture [10–12]. Previous investigations have demonstrated that focalized radiation treatment to the pelvis results in a demineralization of the bone matrix, and increases risk of hip fracture in women receiving radiation therapy for cervical cancers (65%, 66%, and 214%, respectively) as compared to patients who received other therapies (i.e. chemotherapy or surgery) [10,13]. The predominant bone damage following whole body γ-irradiation originates from a rapid loss of trabecular bone tissue, due to rapid elevations in

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osteoclast activation/activity coupled with decreased osteoblast activity, resulting in reduced bone formation activity and volume lasting for months following exposure [14–19]. Although investigations into the effects of radiation on skeletal tissue have uncovered significant evidence, to our knowledge, no known investigations detailing the effects of ionizing radiation combined with skin wound trauma on bone have been undertaken. An understanding of the long-term consequences of these two traumas on skeletal tissue is necessary to determine preventative measures that can be utilized to reduce subsequent bone loss and risk of fracture.

The goal of the present study was to define the temporal impact of high-level radiation injury, wounding, and combined injury on bone properties using a mouse model. Most importantly, our aim was to define whether the long-term effects of combined injury were more detrimental than either wounding or irradiation alone. We hypothesized that irradiation followed by skin wounding would lead to a greater degree of destruction to bone microarchitecture and enhanced deleterious effects on bone cell activity than radiation exposure or wounding alone.

2. Methods

2.1. Ethics statement

The health status of animals was monitored daily and the research was conducted in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care-International (AAALACI). All procedures involving animals were reviewed and approved by the Armed Forces Radiobiology Research Institute (AFRRI) Institutional Animal Care and Use Committee (IACUC). Euthanasia was carried out in accordance with the recommendations and guidelines of the American Veterinary Medical Association.

2.2. Animals and experimental design

Female, B6D2F1/J mice (12 weeks old), were obtained from Jackson Laboratories (Bar Harbor, Maine), placed in quarantine for 2 weeks after arrive, and then allowed to acclimate to their surroundings for 14 days prior to initiation of the study (age was 16 weeks at start of study). All animals were group housed (3–4 mice/cage) in a temperature- (72 ± 2 °F) and light-controlled room (12-h light–dark cycle) and rank ordered by body mass to one of 4 experimental groups (n = 40/group): Sham (0 Gy), Wound (W; 15% total body surface area), Radiation Injury (RI, 8 Gy), or Combined Injury (CI; RI + W). Mice from each group were then euthanized at the following time points after irradiation to determine the time course of changes to bone (n = 10/group): Day 3, 7, 30, 120.

2.3. Radiation injury (RI)

Mice were placed in well-ventilated acrylic restrainers and one single whole-body dose of 8 Gy ^{60}Co γ-photon irradiation was delivered at a dose rate of approximately 0.4 Gy/min. This radiation dose has previously been established as the highest non-lethal dose of radiation when combined with wounding [20]. Dosimetry was performed using the alanine/electron paramagnetic resonance system. Calibration of the dose rate with alanine was traceable to the National Institute of Standards and Technology and the National Physics Laboratory of the United Kingdom. Sham-irradiated mice were placed in the same acrylic restrainers, taken to the radiation facility, and restrained for the time required for irradiation.

2.4. Wound trauma (W)

Two to three days before initiation of the experiment, hair from the dorsal surface was removed using electric clippers under isoflurane inhalation anesthesia. Just prior to wounding, all mice, including controls, received fluid therapy of 0.5 ml sterile isotonic 0.9% NaCl solution (i.p.) combined with 0.05 mg/kg buprenorphine (sac.; 0.5 mol) immediately after irradiation and before wound injury to alleviate pain associated with the wounding process and to avoid radiation-induced dehydration. While under isoflurane anesthesia, a 15–20% non-lethal, total body skin surface experimental wound was inflicted on the shaved dorsal surface between the shoulder blades of mice within 1 h after RI as previously described [20,21]. In brief, a 5/16″ diameter non-surgical stainless steel punch was used to remove the skin on a Teflon-covered board (both cleansed with 70% alcohol after each use). Subsequent to wounding, the panniculus carnosus muscle and overlying skin adhere to the inside of the punch and are easily dislodged by light tapping on clean gauze. Sham-wounded mice were treated identically to other groups except without wounding. The wound was left open and allowed to heal on its own during the investigation. After wounding, mice were assigned to clean cages and provided with proper food (standard rodent chow, Harlan Teklad 8604) and acidified water ad libitum. No other analgesics were administered after the wound trauma during the course of the investigation. Animals resumed normal cage activity within 24 h after being returned to their cages.

2.5. Serum biomarker analysis

Whole blood was collected by terminal cardiac puncture from mice anesthetized by isoflurane and serum was separated into separate aliquots in 1.5 mol Eppendorf tubes for each biomarker, and stored at −80 °C until assayed. TRAP 5b (IDS; Fountain Hills, AZ), osteocalcin (ALPCO Diagnostics; Salem, NH), and sclerostin (SOST; ALPCO Diagnostics; Salem, NH) were determined according to the manufacturer's instructions. The interassay coefficient of variation for each biomarker assay was found to be 1.6–4.9%.

2.6. Micro-computed tomography (μCT) analysis

Right distal femur metaphyses were analyzed by μCT (SkyScan 1172; SkyScan, Kontich, Belgium) to quantify microarchitecture and geometry of trabecular and cortical bone. Ex vivo scans were conducted using an x-ray source set at 60 kV over an angular range of 180° with rotational steps of 0.70°. Projection images were attained at 6 μm resolution and then reconstructed and analyzed using manufacturer provided software to determine cancellous bone properties including bone volume fraction (BV/TV; %), trabecular thickness (Tb.Th; mm^{-1}), and trabecular number (Tb.N; mm^{-1}) (NRecon and CTAn; SkyScan). Cortical bone morphology was determined at a standardized diaphyseal site located 3 mm proximal to the trabecular bone region of interest (ROI). The cortical bone was manually segmented to determine properties including cortical bone area (Ct.Ar; mm^2), cortical bone thickness (Ct.Th, mm), and polar moment of inertia (pMOI; mm^4). All nomenclature follows currently accepted guidelines for μCT evaluation of bone [22].

2.7. Mechanical testing

The mechanical properties of the right femora were determined by 3 point bending. Bones were thawed to room temperature, hydrated on saline gauze, placed in a bending fixture with the upper contact point on the midshaft of the anterior surface and the lower supports separated by 8 mm, and loaded to failure at 2 mm/min. Load vs. displacement data were collected at 10 Hz and analyzed for structural properties including ultimate force (N), stiffness (N/mm), and energy to failure (mJ).

2.8. Histomorphometry

Fluorochrome labeling was performed by intraperitoneal injection of calcium (15 mg/kg body mass) 7 and 2 days prior to euthanasia. Undemineralized left distal femora were subjected to serial dehydration