SATELLITE CELLS SAY NO TO IRRADIATION

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INTRODUCTION

Satellite cells are myogenic stem cells that play a fundamental role in dictating: i) the growth of skeletal muscle during development; ii) the regeneration of skeletal muscle in response to myopathies or trauma; and iii) growth in the adult state as might occur following muscle atrophy or in response to resistance training.

Skeletal muscles are commonly exposed to irradiation for diagnostic procedures and the treatment of cancers and heterotopic bone formation. While skeletal muscle fibers (cells) are post mitotic and, as a result, thought to be highly radioresistant, few studies have considered the impact of clinical doses of irradiation on the ability of SCs to proliferate, differentiate, and contribute to recovering/maintaining muscle mass.

We performed a series of baseline experiments in a previous study (1) where we observed that both 1 and 5 Gy reduced NO levels in SCs by approximately 50-55% and this corresponded to large decreases in SC proliferation (i.e., 30 and 70% decreases, respectively).

Given the background above, we performed a more mechanistic study exploring the role of NO further as it relates to the irradiation of SCs. Specifically, we hypothesized that SC proliferation might be effectively rescued from the harmful effects of \(\gamma\)-irradiation using an NO donor.

METHODS

**Proliferation Assays with SNP and/or PTIO.** Satellite cells were plated onto 24-well Costar plastic plates coated with matrigel in a 1:100 dilution with PBS-minus. Cells from passages 1-3 were used, and 2500 cells were plated per well in 1 mL of media. The cells were \(\gamma\)-irradiated with various doses (0, 1, 2, and 5 Gy) one day after plating. SNP (NO donor; Calbiochem, La Jolla, CA) and/or PTIO (NO scavenger; MP Biomedicals, Irvine, CA) were added immediately after irradiation in various concentrations (0, 0.3, 1, 3 \(\mu\)M SNP; 0 and 5 \(\mu\)M PTIO). Daily media changes with fresh drug were performed over the course of 72 and 96 hours.

**Cell Counting.** Satellite cells were counted in the 24-well plates using a Biotek microplate reader. After the media was removed from each well, the cells were washed once with PBS-minus and placed in a -80°C freezer for a minimum of 30 minutes. Cell lysis was achieved using mammalian protein extraction reagent (Thermo Fisher Scientific, Waltham, MA) containing 1x SYBRGreen dye (Invitrogen, Carlsbad, CA) to quantitate nucleic acids. Fluorescence intensity was detected at an excitation wavelength of 497 ± 9 nm and an emission wavelength of 520 ± 13.5 nm. The mean relative
fluorescence units obtained from the plate reader were calibrated against a standard curve in order to determine absolute cell counts.

**FACs Analysis of MyoD and Myogenin Expression.** Satellite cells from passage 1 were grown on matrigel-coated T75 flasks. The cells were γ−irradiated with various doses (0, 1, 2, and 5 Gy). Fresh media ± 1 µM SNP was added immediately after irradiation, and daily media changes with fresh SNP were performed over the course of 96 hours. At 96 hours, the cells were harvested and fixed as single cells in 4% paraformaldehyde in PBS-minus. The cells were then incubated with primary MyoD and myogenin antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) at a concentration of 1:20 in an antibody diluent (Dako, Carpinteria, CA) at 4°C overnight. Following incubation with immunofluorescent secondary antibodies for 1 hour, the cells were immediately subject to FACS analysis.

**RESULTS**

*Rescuing satellite cells from the harmful effects of γ-irradiation.* When SCs were irradiated using 1 Gy, cell count was reduced approximately 25-28% at each time-point (i.e., 48, 72, and 96 hrs). At the earliest time-point, only the highest dose of SNP appeared to be effective in rescuing SC proliferation. However, at 96 hr time-point (see Figure 1), it is clear that each dose of SNP was highly effective in rescuing SC proliferation. For instance, the lowest dose of SNP (0.3 µM) more than doubled SC proliferation (~160% of the control condition) above the 1 Gy condition (75% of the control condition). SNP doses of 1.0 and 3.0 µM produced rescuing effects that were ~2.3 fold higher than the 1 Gy condition.

![Figure 1. Effects of NO donor (SNP) on SC proliferation 96 hrs following various doses of γ-irradiation.](image-url)
Inhibiting the cellular response to NO through the use of specific scavengers. When the NO scavenger PTIO was used in conjunction with SNP, we observed that the rescue effect of SNP was completely abolished, providing further evidence confirming the potential role of NO in mediating the effects of γ-irradiation.

Figure 2. Effects of SNP (NO donor) and PTIO (NO scavenger). Values along the x-axis are expressed represent dosage of each expressed as μM value.

Effects of γ-irradiation and NO on myogenic transcription factors. SNP alone had little effect on MyoD and myogenin. In contrast, when SCs were treated with 2 Gy of γ-irradiation there was a large and consistent increase in both MyoD and myogenin levels. Interestingly, both the MyoD and myogenin levels partially returned to baseline levels when irradiated cells were also given 1 μM SNP.

DISCUSSION
Skeletal muscles are frequently injured due to trauma, ischemia, or unusually high loading forces. Typically, such injuries produce a robust regenerative response that is highly dependent on the proliferative capacity of SCs. Unlike these forms of injury, irradiation of skeletal muscle has the potential to be a “silent” killer that strikes at the very heart of skeletal muscle’s impressive regenerative capacity, i.e., the activation and proliferation of myogenic stem cells. The findings of this study are unique in at least three ways. First, we observed that NO donors were effective in rescuing the proliferation of irradiated SCs, but this appears to be limited to doses of irradiation less than 5 Gy. Second, we found that NO scavengers were effective in blocking the rescue effects of NO donors. Finally, we observed that irradiation alone produced large increases in key myogenic transcription factors and that this could be reversed by treating SCs with a NO donor following γ-irradiation.