Exposure to Near Infrared Light Decreases Osteoblast Apoptosis and Enhances Fracture Healing in Long Bones

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\textbf{Introduction:} Despite the societal burden and social consequences of long bone fractures, the healing process remains enigmatic and relatively uncharacterized. Recently, it has been shown that even brief application of near infrared light (NIR) in low doses has been capable of promoting healing in a variety of different tissues, including oral mucosa, tendons, and retina. Despite the exciting potential of these findings, little is known about the effects of NIR light on the healing of long bone fractures. Therefore, we hypothesized that NIR light would stimulate fracture healing in long bones, and that a potential mechanism of this action was through the alteration of apoptosis, leading to prolonged and enhanced anabolic effects in osteoblasts.

\textbf{Materials and Methods:} The effects of NIR light on apoptosis and fracture healing were evaluated both in vitro as well as in an animal model. The in vitro studies were performed using MC3T3-E1 osteoblasts in tissue culture. Light sources consisted of arrays of monochromatic light emitting diodes at specific wavelengths at an energy of 4J/cm\textsuperscript{2}. Effects of NIR light on apoptosis were assessed by determining alterations in the protein Bax, a marker of apoptosis, along with Bcl2 and Bclxl, two proteins associated with inhibition of apoptosis. Alterations in the gene expression of RANKL and OPG were determined by quantitative PCR, while cellular proliferation and total protein synthesis were evaluated by commercially available assays. Release of the protein cytochrome C, an early hallmark of apoptosis, was assessed by immunocytochemistry and fluorescence imaging. Nitric oxide release was determined by fluorescence as well as the Griess reaction. Controls for each experimental condition consisted of cells grown without exposure to the LED irradiation. The rat fracture model of Einhorn was used to assess the effects of NIR light on long bone fractures, with IACUC approval. Briefly, an intramedullary pin was placed in the femur of the animal, followed by creation of a fracture. Animals were exposed to NIR light on a daily basis, and then sacrificed at three weeks. The femora were dissected and sections were stained using a selection of histologic stains to evaluate the fracture healing using the ORS criteria. Controls were animals that had undergone fracture in the absence of NIR light treatment. Each slide was read in a blinded fashion. Statistical analysis was performed using the analysis of variance, with the Bonferroni-Dunn post hoc modification and a p value of ≤0.05 as significant.

\textbf{Results:} Exposure of osteoblasts to NIR light increased osteoblast proliferation compared to untreated controls [FIGURE 1]. The light also decreased the RANKL/OPG ratio in osteoblasts in the presence of PTH, as determined by quantitative PCR. The secretion of Bax was decreased in cells exposed to NIR light as compared to control osteoblasts grown in the absence of NIR irradiation. Additionally, the antiapoptotic proteins Bcl2 and Bclxl were increased in light treated cells as compared to controls. Release of cytochrome C was decreased in the light treated cells as compared to non-irradiated controls, consistent with delayed or diminished apoptosis, and NIR light also enhanced the secretion of nitric oxide into the conditioned media [FIGURE 2]. The rat long bone model demonstrated increased fracture callus formation and more rapid fracture healing using the ORS criteria. All of these evaluations were statistically significant.

\textbf{Discussion:} Exposure to near infrared light has been shown to have a broad range of biologic effects, including enhanced healing of apthous ulcers and laser induced retinal injuries. In this study we hypothesized that NIR light would enhance fracture healing, and that one possible mechanism was through the inhibition of osteoblast apoptosis. Using osteoblasts in tissue culture, we have shown that NIR light exposure decreased the effects of PTH on increasing the RANKL/OPG ratio, suggesting that NIR light inhibits bone resorption. Additionally, NIR light decreased levels of the apoptosis marker Bax, while simultaneously increasing the anti-apoptotic proteins Bcl2 and Bclxl. Application of light also enhanced osteoblast proliferation, while diminishing the release of cytochrome c, suggesting a delay or inhibition in apoptosis. Likewise, secretion of nitric oxide, which has a protective effect in response to oxidative stress and apoptosis, was increased in response to NIR light exposure. Finally, both callus volume as well as other parameters as reflected in the ORS fracture healing score were also increased in animals exposed to NIR light. Taken together, these findings demonstrate significant biologic effects to even brief exposures to NIR light, and suggest important applications of this technology to enhance fracture healing, block the generation of osteolysis in implants, and perhaps even prevent bone loss due to osteoporosis.

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