THE EFFECTS OF NEAR INFRARED LIGHT IRRADIATION ON THE GENE EXPRESSION AND SECRETION OF RANKL AND OPG IN OSTEOBLASTS

Takenishi JA; Struve JA; Whelan HT; Buchmann EV; +Ninomiya JT
Department of Orthopaedic Surgery, Medical College of Wisconsin, Milwaukee, WI, USA.

The study of visible and infrared radiation on wound healing has become an exciting area of interest, since exposure to light in the near infrared and visible range have been shown to exhibit a wide range of biologic effects. Using light emitting diodes(LED), exposure to near infrared light promotes rapid healing of apthous ulcers and other skin lesions. Other studies have demonstrated the efficacy of low energy light on intact limbs, which can penetrate distances up to 23 cm through surface tissue and muscle, producing enhanced cellular activity, as demonstrated by increases in proliferation and incorporation of tritiated thymidine. Several wound healing models have also shown that growth factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) are also increased following exposure to near infrared light (NIR). While previous studies have demonstrated that NIR light increased the proliferation and rate of tritiated thymidine incorporation in osteoblasts, little is known about the effects of NIR light on bone cells and their responsiveness to hormones such as parathyroid hormone (PTH) that regulate bone resorption. Due to the wide range of observed biologic effects, we hypothesized that NIR light might also act on bone cells, altering bone resorption by modulating the responsiveness of osteoblasts to PTH. Therefore, we chose to evaluate the effects of NIR light from LED's on the gene expression and secretion of the proteins RANKL and osteoprotogerin (OPG), two modulators of osteoclast maturation and ultimately bone resorption.

Murine MC3t3-E1 osteoblasts were grown in tissue culture in ∀-MEM. For the initial studies, three different wavelengths were evaluated, using arrays of LEDS (670, 728, and 880nm) all at a constant energy. The cells were irradiated on a daily basis until confluent. Parathyroid hormone (bovine 1-34) was added to the wells, and following twelve further hours of further incubation the conditioned media was collected and frozen. The effects of NIR light on the gene expression of RANKL, OPG, and IL-6 were assessed by RT-PCR using specific primer pairs for each protein of interest. All results were obtained following amplification of submaximal amounts of cDNA, and each sample was analyzed with at least triplicate determinations for each experimental condition. In an independent series of experiments, cells were exposed to NIR light, and were then lysed in sucrose. Total cell lysates were then added to an osteoclast maturation assay, consisting of murine bone marrow cells in culture. Following incubation, the marrow cells were stained for TRAP activity, and the number of multinucleate cells were counted for each condition.

Following stimulation of the osteoblasts with NIR light, it was determined that 880nm produced the largest biologic effects, compared to light of the other wavelengths evaluated. Specifically, light of this wavelength at 4J/cm^2 produced the greatest increase in the ratio of RANKL/OPG, suggesting that light of this wavelength was most effective in enhancing bone resorption. (Figure 1)

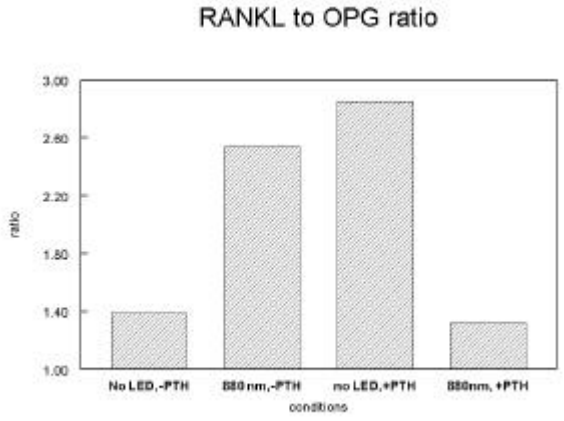

Figure 1. Ratio of RANKL/OPG gene expression following exposure to 880nm light in the presence or absence of PTH. NIR light and PTH alone increased the ratio, while in combination RANKL gene expression was decreased.

Results from the osteoclast maturation assay demonstrated an increase in the formation of multinucleate cells in the presence of NIR light compared to unstimulated controls (Figure 2)

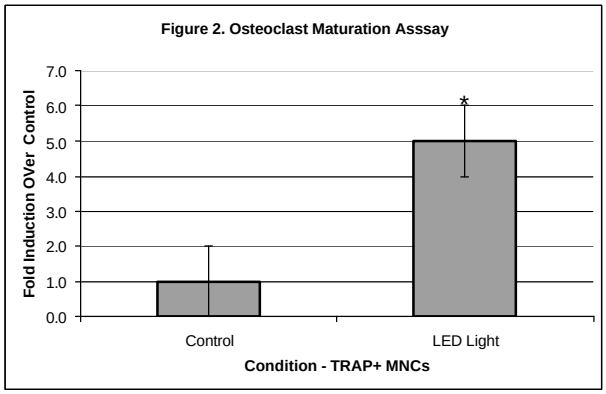

Figure 2. Osteoblasts were exposed to NIR light (880nm, 4J/cm^2), and following incubation the cells were lysed in hypertonic sucrose. Aliqouts of the cell lysates were added to the osteoclast maturation assay, and cells were stained for trap activity.

Our studies demonstrate that NIR light has important biologic effects on the gene expression of RANKL, an important protein in the regulation of bone mass. Following exposure to NIR light of 880nm, RANKL gene expression was increased, while no effects were observed for either OPG or IL-6. Interestingly, NIR light and PTH alone increased the gene expression of RANKL, while in combination it was decreased. This suggests that NIR light may function by multiple distinct pathways. Compared to basal control levels, no alterations were seen in the secretion of IL-6 in the conditioned media, as determined by specific ELISA. These increases in RANKL gene expression were also reflected by increases in osteoclast maturation in the murine assay.

These findings suggest that NIR light may play an important role in the regulation of bone resorption through the regulation of the gene expression and secretion of RANKL, and have important implications in such diverse applications as fracture healing, treatment of osteoporosis, and the prevention of bone loss following prolonged space flight.

49th Annual Meeting of the Orthopaedic Research Society
Poster #0684