CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

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Project Number

31641

Project Title

The Skeleton as an Endocrine Organ: The Effect of Insulin and L-Sulforaphane on Osteogenesis in MC3T3 Preosteoblasts

Abstract

Objectives/Goals

Diabetes is a devastating disease with formidable impact on personal and societal health. Research has indicated that when osteoblasts are treated with insulin (INS), bone is mineralized, producing osteocalcin which is vital to blood sugar control. The purpose of this study is to investigate insulin#s mechanism of effect on osteogenesis in MC3T3-E1 osteoblasts and the influence of PI3K/Akt cellular transcription pathway blocking agent,L-sulforaphane (SFN), on these cells.

Methods/Materials

A pilot study was first done to determine the effective range INS and SFN in MC3T3s. Baseline cell counts were taken. Treatment with SFN was done at concentrations of 0uM (control), 1uM, 3uM, 5uM, 10uM, and 15uM. Treatments with INS at 0nM (control), 1nM, 3nM, and 10nM were performed separately. Final counts for each treatment type were performed for proliferation and differentiation at 2 weeks. The results indicated a negative response for all concentrations of SFN and a highest positive dose response for INS at 3nM. Results were used to design the Main Project where six different concentrations of SFN from the pilot were tested against 0nM and 3nM INS. Four replicates were done for each treatment type, with controls for each variable. Amount of differentiating cells was measured weekly and compared to controls and baseline. Relative mineralization at endpoint (21 day) for each treatment was determined by Von Kossa stain and extraction of Alizarin Red.

Results

For the Main Project, differentiation was higher in the INS groups with no SFN. The 3nM insulin group had more differentiation than the 0nM insulin group. The additional of SFN to both 3nM and 0nM insulin groups caused a dose-dependent reduction in differentiation. Mineralization and bone formation were also higher in the 3nM INS group compared to the 0nM insulin group. SFN decreased bone formation and mineralization for both INS dosages. No change in proliferation counts were seen from baseline.

Conclusions/Discussion

INS increases differentiation and mineralization in MC3T3-E1 osteoblasts. PI3K/Akt transcription pathway blocking agent SFN diminishes these effects in a dose dependent manner. Increasing INS concentration over the control only partially mitigated this effect. The results support the hypothesis that INS is osteogenic in MC3T3-E1 osteoblasts, and that the mechanism of this osteogenesis is through the PI3K/Akt transcription pathway.

Summary Statement

This novel study is an investigation into the osteogenic effect of insulin, and the ability of a PI3K/Akt pathway blocking agent, L-Sulforaphane, to diminish this effect.

Help Received

Dr. Gardiner provided lab facilities; Jeffery Hoshiko assisted with calculations.