CALIFORNIA STATE SCIENCE FAIR
2009 PROJECT SUMMARY

Name(s)
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Project Number
S1809

Project Title
Implications in Osteoporosis: Caffeine Impairs, Dexamethasone Increases Osteogenesis in MC3T3 Osteoblast Cells

Abstract

Objectives/Goals
To study the effect of caffeine and dexamethasone on osteoblast cell line MC3T3-E1, and determine the toxicity level of caffeine in these cells in vitro, so as to better understand the pathogenesis of osteoporosis.
Hypotheses: 1)Caffeine will interfere with MC3T3-E1 osteogenesis in a dose-responsive manner as evidenced by decreased amounts of mineralized nodules on Von Kossa staining. 2)Osteoblasts grown in culture media supplemented with dexamethasone will show greater mineralization as compared to cultures grown without dexamethasone, but this will also be negatively influenced by caffeine.
3)Caffeine will affect osteoblast growth in a dose-responsive manner as seen by the toxicity study.

Methods/Materials
Cell line MC3T3-E1 was maintained in DMEM with 10%FBS and gentamicin at 37 deg C., humidified and 5%CO2. For Investigations #1 and #2, culture solutions with or without the inducers glycerol phosphate and ascorbic acid were compared to cultures treated with various concentrations of caffeine, and a subset of cells enhanced with dexamethasone. At 19 days, these cells were stained by Von Kossa technique to determine formation of nodules. For investigation # 3, caffeine (0 mM control; 0.1mM; 0.3mM; 1mM; 3mM; and 10mM) was added to nonconfluent cells. Cells were fixed at days 3, 4, and 5. Cell counts were done to determine caffeine toxicity and effect on growth.

Results
Investigations #1 and #2 show osteoblasts grown with caffeine form fewer nodules, regardless of induction (10mM Caffeinated=238, vs. Non-caffeinated Control=937). Cells grown with dexamethasone show much greater matrix nodule formation, but this, too, is diminished by caffeine(10mM Caffeinated=726, vs. Non-caffeinated Control=10,436). A novel finding was that dexamethasone was seen to act as an independent inducer of osteogenesis. Investigation #3 shows that caffeine is toxic to osteoblasts and negatively affects growth,primarily occurring at levels of 1.0 mM and above.

Conclusions/Discussion
The results support my original hypotheses. Caffeine interferes with osteogenesis in a dose-responsive manner shown by decreased amounts of mineralized nodules. Cells grown in culture media supplemented with dexamethasone show greater mineralization as compared to cultures grown without dexamethasone, but were also negatively influenced by caffeine. Caffeine did affect osteoblast growth in a dose-responsive manner as seen by the toxicity study.

Summary Statement
This study investigates the effect of caffeine and dexamethasone on osteoblast cell line MC3T3-E1, and determines the toxicity level of caffeine in these cells in vitro, so as to better understand the pathogenesis of osteoporosis.

Help Received
Used lab equipment at UC Irvine under supervision of Dr. Gardiner; however, the design and work was all my own and not part of any university research program. My parents drove me and helped in typing and proof reading.