Cellular Response to Simvastatin Released from Calcium Sulfate

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Introduction

Calcium sulfate (CS) has a long history of use as a bone void filler. It serves as a biodegradable material on which osteogenesis occurs. Because of its relatively rapid rate of biodegradation, it appears well-suited as a vehicle for local delivery of osteotropic agents that can enhance bone formation.

Simvastatin was identified as an osteogenic compound based on its ability to stimulate activation of the BMP-2. Furthermore, simvastatin has been shown to stimulate bone formation both *in vitro* and *in vivo* [*Science* 286:1946, 1999].

The objective of this study was to investigate cellular reaction to simvastatin as released from calcium sulfate.

Materials and Methods

Delivery Devices

Calcium sulfate hemihydrate was used to fabricate hemispherical samples having diameter of 6 mm and height of 4 mm. CS was set overnight at 40ºC. Simvastatin (Sim) was loaded by adsorption at different concentrations and for different durations in an attempt to modulate release profiles. For most experiments, CS was treated with Sim solutions of 4.2 mg/mL.

In Vitro Release

Loaded samples were incubated in physiological saline at 37ºC with shaking at 80 rpm, and supernatant was collected every 1-3 days. The amount of released Sim was determined by UV spectroscopy.

In Vitro Cellular Reaction

MC3T3-E1 cells (ATCC CRL-2593) were cultured in α-Minimum Essential Medium containing 10% fetal bovine serum and 1mM sodium pyruvate. Cells were harvested and seeded into sterile 24-well plates at an initial concentration of 12,500 cells per well. After 24 hours, medium was replaced with medium containing 5 mM beta glycerophosphate, 5 µg/ml ascorbic acid, and simvastatin. Simvastatin solutions tested were from the supernatants collected from the release study. Supernatants were tested by diluting in cell culture medium at 1:2.5, 1:10, and 1:100. Medium was replaced at 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, and 20 days to mimic the release profile from the CS elution experiments. Cells were harvested at 7, 10, 14, and 20 days. DNA contents were measured by a Hoechst assay to assess cell growth. Alkaline phosphatase was determined by measuring cleavage of p-nitrophenyl phosphate.

Results and Discussion

For CS adsorbed with Sim (Figure 1), nonlinear profiles were found, with an initial burst of 25-35 µg. The higher the concentration of drug in the adsorbing solution, the larger the initial burst. Typical of diffusive release, the amount of Sim released decrease as a function of time. Increasing the duration of adsorption did not have a significant effect on the release profile and amount of drug released. Adsorption for 30 min was selected for further experiments.

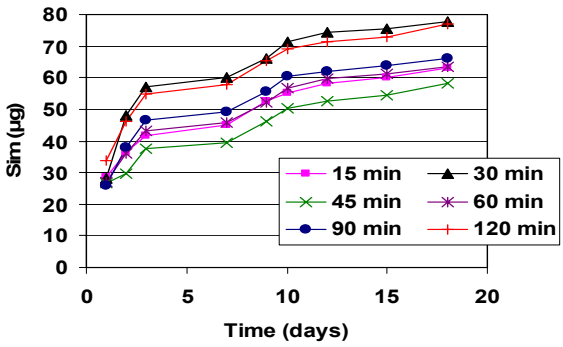

Figure 1. Release of Sim from CS.

As shown in Figure 2, steady cell growth was observed over the 20 day period for control cultures. Addition of release supernatant at 1:2.5 dilution, however, resulted in formation of a fine precipitate in the cultures and significant cell death. Dilution at 1:10 and 1:100 did not have as large an adverse effect, and DNA contents were comparable to those in controls. AP activity was relatively steady over the culture period.

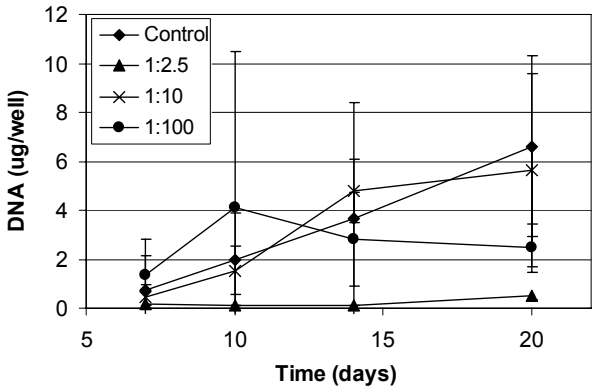

Figure 2. Effect of released Sim on cell growth.

Conclusions

CS can serve as a vehicle for localized release of Sim for the purpose of repairing localized bone defects. Release can be modified by changing adsorption concentration, and drug released from the CS delivery vehicle promotes cell growth. Ongoing experiments are investigating other measures of cell activity.

Acknowledgment

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