Intermittent Exposure to Simvastatin Enhances Osteoblastic Activity

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Introduction

Daily injections of the cholesterol synthesis inhibitor simvastatin have been reported to stimulate bone formation [*Science* 286:1946, 1999]. Use of controlled release methods can provide local, intermittent concentrations and thereby avoid the need for injections.

Blends of cellulose acetate phthalate (CAP) and Pluronic F-127 (PF-127) undergo surface erosion and show zero order release. Therefore, alternating blank and drug-loaded layers will give intermittent release of the drug.

In this study, devices for intermittently releasing simvastatin were made, and *in vitro* osteoblastic responses to intermittent exposure were evaluated.

Materials and Methods

Release Devices

CAP and PF-127 were blended at different weight fractions (5:5, 6:4, and 7:3), and simvastatin-loaded microspheres were prepared by an acetone-oil-water (W/A/O/W) triple emulsion process. UV-sterilized microspheres were placed in a mold and consolidated under 20 Pa pressure for 5 seconds. Next, blank microspheres were added on the top of the first layer and pressure was applied. In the same manner, 6, 8, and 10 layer devices were prepared. To provide directional control of drug release, the bottom and sides were coated three times with 10% poly(lactic-co-glycolic acid) solution in methylene chloride.

In Vitro Release

Samples were immersed in PBS, pH 7.4, and incubated at 37°C statically or dynamically (shaken at 80 rpm). Supernatant was collected and replaced every day.

In Vitro Bioactivity

MC3T3-E1 osteoblastic cells (ATCC CRL-2593) were cultured in α-MEM containing 10% FBS, 50 μg/ml ascorbic acid, and 5 mM ß-glycerophosphate. Suspensions containing 12,500 cells were seeded into 24-well plates and cultured for up to two weeks with 0 to 1 μM simvastatin. Each day, medium was replaced to maintain either constant or alternating concentration. After 3, 7, 10, and 14 days of culture, cells were harvested to determine bioactivity by measuring DNA and alkaline phosphatase (AP) activity.

Results and Discussion

Intermittent Release Profile

Figure 1 shows that 6 layer devices had three release peaks, 8 layer devices had four, and 10 layer devices had under dynamic conditions. During static release, 10 layer devices showed the same five discreet peaks, but the devices lasted 22 days.

In Vitro Bioactivity

For most treatments, DNA content increased over the first week of culture. Whereas DNA remained low in constant 1 μM cultures, those with alternating 1 μM recovered to some extent but never reached the levels found for other treatments. Alternating 0.1 and 10 nM simvastatin produced significantly higher DNA contents compared to control and constant treatments at 7 and 10 days.

AP activity in control cultures slowly increased over the two-week period (Figure 2). The response of cells to constant simvastatin was similar to that for control (unexposed) cells. In contrast, alternating simvastatin had a significant effect in elevating AP activity. At 3 days, alternating concentrations of 10 nM and 1 μM produced significantly higher activity. Similarly, at 10 days, cells responded significantly better to alternating 0.1 and 10 nM simvastatin.

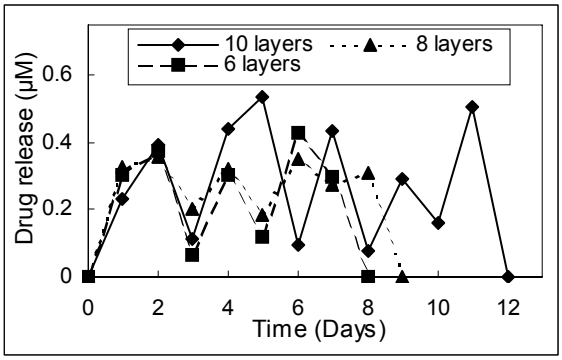

Figure 1. Layer effect on simvastatin release.

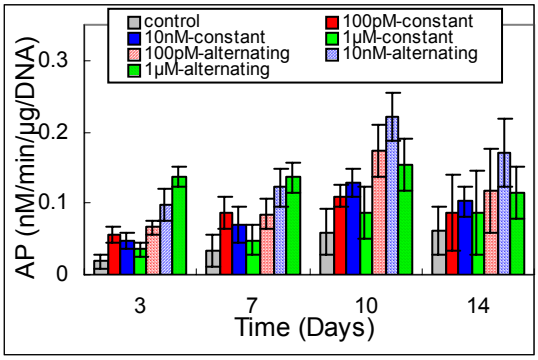

Figure 2. AP activity in cultures exposed to constant and alternating simvastatin.

Conclusions

Intermittent release of simvastatin was achieved using the association polymer system of CAP-PF-127. *In vitro* bioactivity experiments show that alternating exposure of osteoblastic cells to simvastatin significantly enhanced their activity. These devices may be useful for stimulating local bone formation.

Acknowledgement

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