An Examination of the Effects of Atorvastatin and Parathyroid Hormone on Osteoblast Activity

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Abstract

HMG-CoA reductase inhibitors, also known as statins, are a ubiquitous class of medication used for lowering cholesterol. In-vitro and animal studies have suggested that statins can activate osteoblast differentiation and have anabolic effects on bones; however, observational and experimental studies in humans have shown conflicting results.1-5 The exact mechanism of statins on bone growth is unknown; however, there are several hypotheses. The “Lipid Hypothesis” (Figure 1) suggests that lipid oxidation leads to activation of PPARγ, and production of isoprostanes including isoPGF2α and isoPGEα. PPARγ is associated with inhibition of osteoblast differentiation, while isoprostanes markers are associated with the induction of osteoclast differentiation and inhibition of osteoblast differentiation. This led to the hypothesis that statins can decrease lipid oxidation, which can inhibit the action of PPARγ and isoprostane-mediated bone loss.6 The “statins hypothesis” (Figure 2) suggests that the anabolic bone activity of statins is due to the induction of osteoblast differentiation, suppression of osteoblast apoptosis and inhibiting osteoclastogenesis. Statins inhibits HMG-CoA reductase, which decrease the productions of isoprenoids farnesyl pyrophosphate (FPP) and Geranylgeranyl pyrophosphate (GGPP). The decrease in FPP and GGPP leads to upregulation of bone morphogenetic protein-2 (BMP-2) downstream, stimulating bone formation by increasing mesenchymal condensation. Statins inhibit osteoblast apoptosis by upregulating TGFβ/Smad3 kinases signaling. It also decreases osteoclastogenesis by upregulating osteoprotegerin (OPG), a decoy receptor that binds to RANKL to inhibit osteoclast differentiation.6

Disciplines

Pharmacy and Pharmaceutical Sciences

Comments

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OBJECTIVE
To investigate whether statins have an effect on osteoblast growth when used alone and whether they have an anabolic effect on osteoblastic activity when used with parathyroid hormone.

BACKGROUND
HMG-CoA reductase inhibitors, also known as statins, are a ubiquitous class of medication used for lowering cholesterol. In vitro and animal studies have suggested that statins can alter osteoblast differentiation and have anabolic effects on bones; however, observational and experimental studies in humans have shown conflicting results.6

The exact mechanism of statins on bone growth is unknown; however, there are several hypotheses. The “Lipid Hypothesis” (Figure 1) suggests that lipid oxidation leads to activation of PPARγ, and production of isoprostanes including isoPGF2α and isoPGEα. PPARγ is associated with osteoclastogenesis by upregulating osteoprotegerin (OPG), a decoy receptor that binds to RANKL and inhibit osteoblast apoptosis, which leads to an increased bone turnover. This hypothesis states that statins decrease lipid oxidation, which can inhibit the action of PPARγ and isoprostane-mediated bone loss.6

The “statins hypothesis” (Figure 2) suggests that the anabolic bone activity of statins is due to the induction of osteoblast differentiation, suppression of osteoblast apoptosis and inhibiting osteoclastogenesis. Statins inhibit HMG-CoA reductase, which decrease the productions of isoprostanes farnesyl pyrophosphate (FPP) and Geranylgeranyl pyrophosphate (GGPP). The decrease in FPP and GGPP lead to upregulation of bone morphogenetic protein-2 (BMP-2) downstream, stimulating bone formation by increasing mesenchymal condensation. Statins inhibit osteoblast apoptosis by upregulating TGFβ/Smad3 kinases signaling. It also decreases osteoclastogenesis by upregulating osteoprotegerin (OPG), a decoy receptor that binds to RANKL to inhibit osteoclast differentiation.6

METHODS
A pre-osteoblast cell line, mouse MC3T3-E1 subclone 4 (“ATCC CRL-2593™”), was used in this study. Fetal bovine serum (10%), penicillin-streptomycin (1%) and Alpha Minimum Essential Medium (aMEM) were combined to make growth media. Differentiation media was prepared by adding ascorbic acid (50 μg/mL) and β-glycerol phosphate (10mM concentration) to the growth media. Atorvastatin treatment solution was prepared by dissolving atorvastatin in dimethyl sulfoxide (DMSO), then further diluted with differentiation media. Parathyroid hormone was dissolved in water and further diluted with differentiation media.

Experiments
Pre-osteoblast cells were incubated in growth media at 37°C for 2 days to allow for proliferation, and then they were incubated in differentiation media at 37°C to allow for differentiation into osteoblast cells. After two days of incubation, cells were treated with atorvastatin (10^-6 M), PTH (10^-6 M) or both in differentiation media. Osteoblast activity was assessed using three different tests: an MTT proliferation assay to determine cell survival after eight days of treatment, Alizarin Red staining to determine calcium deposition after 16 days of treatment, and an alkaline phosphate assay (Abnova Corporation) to determine alkaline phosphatase activity after 3 days of treatment.

Results

Alizarin Red Stain - Calcium Deposition

Figure 4. Calcium deposition in cells treated with atorvastatin, parathyroid hormone or both. Cell deposition was significantly reduced in cells treated with parathyroid hormone with and without atorvastatin compared to control and atorvastatin alone. PTH treatment was continuous in this study. * denotes p<0.05. Parathyroid hormone is abbreviated “PTH”.

RESULTS

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Alkaline Phosphatase - Osteoblast activity

Figure 3. Osteoblast proliferation in cells treated with atorvastatin, parathyroid hormone or both. Cell proliferation was significantly reduced in cells treated with parathyroid hormone with and without atorvastatin compared to control and atorvastatin alone. PTH treatment was continuous in this study. * denotes p<0.05. Parathyroid hormone is abbreviated “PTH”.

REFERENCES

CONCLUSION

• Atorvastatin alone does not have an anabolic effect on osteoblast activity, as measured by cell survival, calcium staining, and alkaline phosphatase activity.
• Additive effects of atorvastatin on alkaline phosphatase activity may be present when treated in combination with pulsatile PTH.
• Due to the assumed absence of osteoclasts in culture, continuous PTH suppressed osteoblast activity. Further test will be needed to investigate this effect.
• Further studies are needed to test for additive effects of atorvastatin and pulsatile PTH on cell survival and calcium deposition.

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