12-2014

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 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

Poster presented at the American Society of Health Systems Pharmacy Midyear Clinical Meeting in Anaheim, California, December 2014.

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OBJECTIVE

To investigate whether statins have an effect on osteoblast growth when used alone and whether they have an additive effect on anabolic activities 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 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 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]

METHODS

Materials

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 (α-MEM) were combined to make growth media. Differentiation media was prepared by adding ascorbic acid (50 ug/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 37ºC for two days to allow for proliferation, and then they were incubated in differentiation media at 37ºC to allow for differentiation into osteoblast cells. After two days of incubation, cells were treated with atorvastatin (10^{-6} or 10^{-7} M), PTH (10^{-8} 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, Alazarin Red S staining to determine calcium deposition after 16 days of treatment, and an alkaline phosphatase assay (Abnova Corporation) to determine alkaline phosphatase activity after 3 days of treatment.

Statistical Analysis

Statistical analysis was performed with one way ANOVA and a Bonferroni post-hoc test; p values < 0.05 were considered statistically significant.

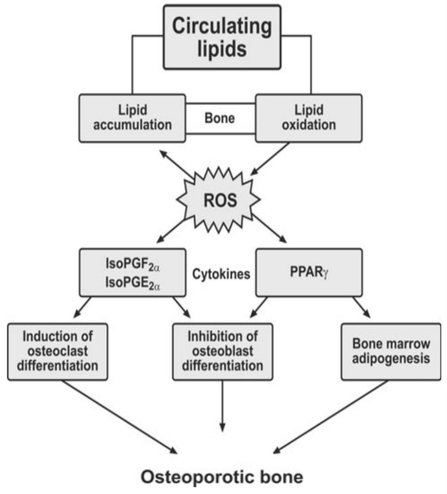

Figure 1. The "Lipid Hypothesis" states that statins decrease circulating lipids, which can reduce the generation of reactive oxidative species-induced cytokines that can lead to osteoporotic bone. This image is adapted from Esposito et al.[6]

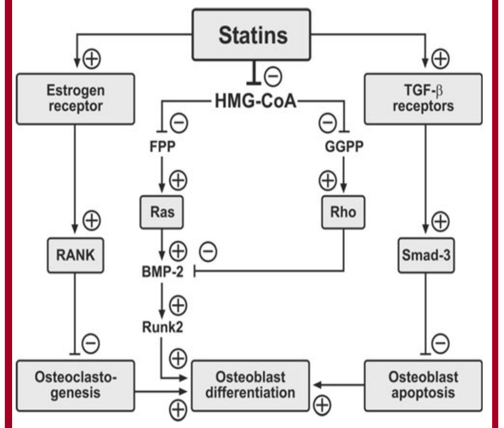

Figure 2. The "Statins Hypothesis" states that its action on the estrogen receptor, HMG-CoA, and the TGFβ receptors can lead to the inhibition of osteoclast differentiation, induction of osteoblast differentiation and the prevention of osteoblast apoptosis, which leads to an increased bone density. This image is adapted from Esposito et al.[6]

RESULTS

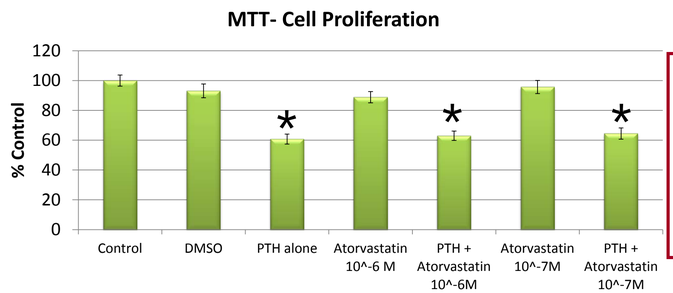

Figure 3. Osteoblast proliferation in cells treated with atorvastatin, parathyroid hormone or both. Cell proliferation was significantly reduced in cells treated with parathyroid hormone with and without atorvastatin compared to control and atorvastatin alone. PTH treatment was continuous in this study. * denotes p<0.001. Parathyroid hormone is abbreviated "PTH".

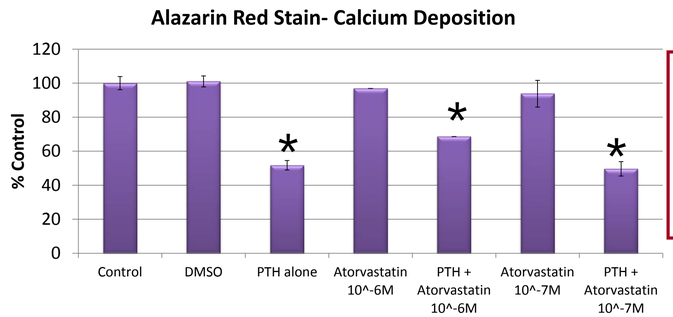

Figure 4. Calcium deposition in cells treated with atorvastatin, parathyroid hormone or both. Calcium deposition was significantly reduced in cells treated with parathyroid hormone with and without atorvastatin compared to control and atorvastatin alone. PTH treatment was continuous in this study. * denotes p<0.001. Parathyroid hormone is abbreviated "PTH".

RESULTS (continued)

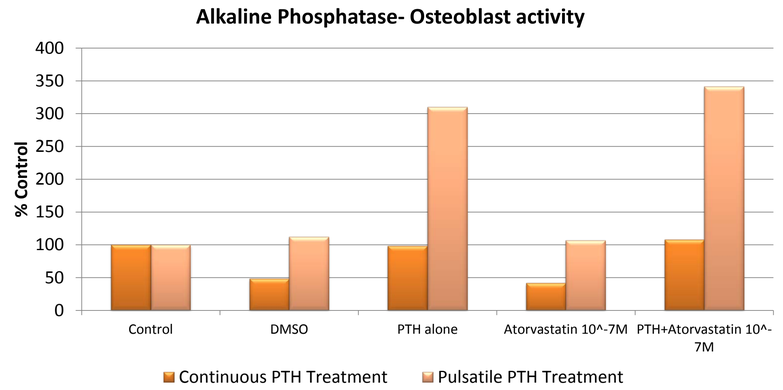

Figure 5. Osteoblast activity in cells treated with atorvastatin, parathyroid hormone or both. There was an increase in osteoblast activity when parathyroid hormone was administered in a pulsatile fashion compared to the continuous treatment. Atorvastatin alone did not increase alkaline phosphatase activity; however, there appears to be an additive effect on alkaline phosphatase activity when cells were treated with atorvastatin and parathyroid hormone in combination compared to either agent alone. More replicates are needed to determine statistical significance. Parathyroid hormone is abbreviated "PTH".

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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Author Disclosure Panel: All authors of this presentation have nothing to disclose concerning possible financial or personal relationships with commercial entities that may have a direct or indirect interest in the subject matter of this presentation.