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Effects of atorvastatin on bone metabolism and bone mineral density in Wistar rats

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Objective: To investigate the effects of atorvastatin on bone formation, bone resorption and bone mineral density in Wistar rats. *Methods:* Sixty healthy male Wistar rats were randomly divided into one control group treated with vehicle alone and three drug treatment groups, which were treated with atorvastatin at 5 mg/kg·d, 25 mg/kg·d and 50 mg/kg·d respectively. Left femur BMD and bone metabolic parameters were measured after 8 weeks of treatment. In high dose of atorvastatin group, 20 rats were randomly allocated into persistent treatment group or atorvastatin washout group for another 4 weeks; bone metabolic parameters were retested. *Results:* Compared with vehicle alone, atorvastatin treatment significantly increased serum levels of ALP and BGP, but had no effects on serum Ca or P levels. Moreover, atorvastatin significantly decreased bone resorption markers including 24 h urinary Ca/Cr ratio, P/Cr ratio and serum IL-6 level. There was no significant difference among atorvastatin treatment groups. After 4 weeks of washout period, the effects of atorvastatin on bone formation and resorption markers decreased. Atorvastatin treatment did not alter BMD compared with the control group, even in the highest dose of atorvastatin group. *Conclusion:* Atorvastatin treatment in a certain extent inhibits bone resorption and promotes bone formation, but has no significant effects on bone mineral density in healthy rats.

1. Introduction

The hydroxymethylglutaryl coenzyme A reductase inhibitors (statins) are widely used in the treatment of dyslipidemia in an age group that has an increased prevalence of osteoporosis. The statins, apart from reducing the intracellular cholesterol pool, also reduce other products of the mevalonate pathway, including the isoprenoids farnesyl diphosphate and geranylgeranyl diphosphate (GGPP). Farnesyl diphosphate and geranylgeranyl diphosphate are attached to the carboxy terminal of numerous monomeric, such as small GTP-binding proteins to form cytosolic prenylated proteins. Prenylation is essential for the membrane localization and function of these prenylated proteins, including Rac and Rho (Zhang et al. 1996). Rac and Rho are pivotal in mediating the cytoskeletal changes initiated by growth factors and integrins, leading to membrane ruffling, the formation of lamellipodia and stress fibers, and resulting in the activation of polarized and motile cells including macrophages and osteoclasts (Burridge et al. 1996; Zigmond 1996). Alendronate, a nitrogen-containing bisphosphonate used in the treatment of osteoporosis, inhibits prenylation and thereby inhibits the osteoclast function. By reducing substrate availability, statins also inhibit prenylation and have been shown to inhibit osteoclastic bone resorption in a fashion similar to alendronate (van Beek et al. 1999). Recently, statins was also proved to have a role in increasing bone mineral density and decreased 45–55% risk of bone fractures in some cross-sectional and retrospective case-control studies (Yaturu 2003; Tang 2008). But in 4S and LIPID study, no significant differences were found between statin treatment and control groups in terms of the risk for bone fractures (Hatzigeorgiou et al. 2005). However, in these two studies, bone fracture occurred in few patients, so further studies are needed to ascertain whether statins play an important role in protection of bone health. In the present study, both bone formation and resorption biomarkers were measured to investigate the roles of atorvastatin in bone metabolism and BMD in rats.

2. Investigations and results

2.1. Effects of atorvastatin on bone formation biomarkers

Compared to the control group, none of the three atorvastatin treatment groups showed significant changes in the bone formation markers 4 weeks after treatment. Eight weeks after treatment, serum ALP and BGP levels were greatly increased in atorvastatin treatment groups compared with control group, with no significant differences among the 3 atorvastatin groups.However, atorvastatin treatment did not change the levels of serum Ca and P even at 8th week (Table 1). In high-dose of atorvastatin group, after 4 weeks of washout period, the levels of serum ALP and BGP decreased greatly compared with those in atorvastatin persistent group (Table 2).

2.2. Effects of atorvastatin on bone resorption biomarkers

There was no significant change between control group and different dose of atorvastatin groups about all the bone resorption biomarkers after 4 weeks. At week 8, compared with control group, atorvastatin treatment significantly decreased the values

Table 1: Effects of atorvastatin on serum bone formation biomarkers at week 8 ($\chi \pm s$, $n = 10$)

Group	Ca (mmol/L)	P (mmol/L)	ALP (μmol/L)	BGP (μg/L)
Control	2.18 ± 0.03	2.23 ± 0.03	2.47 ± 0.02	6.86 ± 0.65
Atorvastatin low-dose	2.17 ± 0.02	2.20 ± 0.02	5.10 ± 0.03*	7.24 ± 0.82
Atorvastatin mid-dose	2.16 ± 0.2	2.18 ± 0.03	5.12 ± 0.03*	7.41 ± 0.71*
Atorvastatin high-dose	2.16 ± 0.19	2.17 ± 0.02	5.20 ± 0.02*	7.52 ± 0.68*

Note: * $P<0.05$ *vs.* control group

Table 2: Changes of ALP and BGP levels after 4 weeks of washout period in the high dose atorvastatin group (n=10)

Group	ALP(μmol/L)	BGP(μg/L)
Atorvastatin persistent	5.21 ± 0.02	7.81 ± 0.73
Atorvastatin washout	2.59 ± 0.02*	7.30 ± 0.69*

Note: * $P<0.05$ *vs.* Atorvastatin persistent group

Table 4: Changes of bone resorption biomarkers after 4 weeks of washout period in high dose atorvastatin group (n=10)

Group	IL-6(pg/ml)	24h urinary Ca/Cr	24h urinary P/Cr
Atorvastatin persistent	63.1 ± 10.17	0.12 ± 0.01	0.32 ± 0.02
Atorvastatin washout	69.5 ± 11.56*	0.17 ± 0.02*	0.34 ± 0.02*

Note: * $P<0.05$ *vs.* Atorvastatin persistent group

of 24 h urinary Ca/Cr ratio, P/Cr ratio and serum IL-6 level; no differences were found among treatment groups (Table 3), but there was a decreasing trend about the bone resorption biomarkers among different doses of atorvastatin. In high-dose atorvastatin groups, after 4 weeks of washout period, the values of 24 h urinary Ca/Cr ratio, P/Cr ratio and serum IL-6 level were greatly increased compared with those in the atorvastatin persistent group (Table 4).

2.3. Effects of atorvastatin on bone mineral density

After 8 weeks of treatment, there were no significant differences in femur BMD among low, high dose atorvastatin groups (0.249 ± 0.01 g/cm^2, 0.251 ± 0.02 g/cm^2) and the control group (0.254 ± 0.01 g/cm^2) ($P>0.05$).

3. Discussion

The balance between osteoblast cells and osteoclast cells is the major determinant of BMD, and is regulated by substantial factors such as Ca, cytokines and hormones etc (Yang et al. 2009). In the present study, both bone formation and resorption biomarkers were examined in order to investigate the effects of atorvastatin on bone metabolism and BMD in healthy Wistar rats.

There are sound reasons to believe that statins may have beneficial effects on bone health. Existing data show that prenylation is important for osteoclast function and that inhibition of prenylation impairs osteoclast function (Chuengsamarn et al. 2010; Luisetto et al. 2009; Kretz et al. 2006; Luckman et al. 1998). The inhibiting effects of alendronate on osteoclasts may be related to its suppression of GGPP modified proteins. Statins could also inhibit GGPP production, thereby potentially existing a similar effect on bone to alendronate. Urinary calcium/creatinine (Ca/Cr) ratio and urinary phosphorus/creatinine (P/Cr) ratio are two important biomarkers for bone resorption. Our study indicated that both 24 h urinary Ca/Cr and P/Cr ratios were decreased after atorvastatin treatment. Moreover, we found that atorvastatin treatment for 8 weeks significantly decreased IL-6 level. IL-6 is an important cytokine in stimulating bone resorption, and may regulate the function of osteoclast cells by binding with IL-6 receptor located on the surface of osteoclast cells (Ralston 1994). Therefore, the inhibiting effects of atorvastatin on bone resorption may be related to its role in decreasing the level of IL-6.

Further evidence supporting a beneficial effect of statins on bone comes from the data demonstrating that statins increase bone formation (Mundy et al. 1999; Laufs et al. 2000; Mundy et al. 1998; Yamashita et al. 2008). Mundy et al. (1998) first reported that statins stimulated osteoblast-derived BMP-2 expression and subsequently enhanced osteoblastic bone formation. Since then, this enhancing effect of statins on bone formation has been repeatedly confirmed by numerous *in vitro* studies. It was additionally reported that statin-mediated activation of BMP-2 promoter was completely inhibited by the downstream metabolite of HMG-CoA reductase, mevalonate, indicating that the activation was a result of the inhibition of the enzyme (VanAelst and DSouza-Schorey et al. 1997). Our study showed that atorvastatin treatment for 8 weeks greatly increased BGP and ALP levels, which inferred that atorvastatin also has a role in promoting bone turnover.

It is still controversial whether statins affect BMD. In retrospective clinical analysis, statins was found to decrease the incidence of bone fracture in aged patients with osteoporosis (Meier et al. 2000); long term treatment of statins in postmenopausal patients and diabetic patients significantly increased BMD (Chung et al. 2000). But in animal studies, Maritz et al. (2001) reported that

Table 3: Effects of atorvastatin on bone resorption biomarkers at week 8 ($\chi \pm s$, n=10)

Group	Serum IL-6(pg/ml)	24h urinary Ca/Cr	24h urinary P/Cr
Control	84.16 ± 12.59	0.18 ± 0.02	0.34 ± 0.02
Atorvastatin low-dose	77.31 ± 13.02*	0.15 ± 0.02*	0.32 ± 0.02*
Atorvastatin mid-dose	72.76 ± 12.82*	0.14 ± 0.01*	0.32 ± 0.03*
Atorvastatin high-dose	70.31 ± 11.43*	0.12 ± 0.02*	0.30 ± 0.01*

Note: * $P<0.05$ *vs.* control group

statins decreased BMD in rodents, while Kawane et al. (2004) reported that chronic administration of atorvastatin, along with submaximal doses of E_2 and hPTH(1–34), appeared to modestly enhance the BMD of the lumbar vertebrae and femoral metaphysis of OVX rats, with no effect on BMD with atorvastatin alone. Moreover, different doses of atorvastatin and simvastatin were found to have different effects on BMD in rats (Maritz et al. 2001; Kawane et al. 2004). In the present study, we found that atorvastatin, even at the highest dose, did not affect BMD after 8 weeks of treatment. Our results were in accordance with another study reported by Bone et al. (2007). 626 postmenopausal women were enrolled in this randomized, double-blind, placebo-controlled clinical trial, and were treated by 10, 20, 40, or 80 mg atorvastatin for 52 weeks. No significant difference was found among all the atorvastatin and placebo groups in lumbar (L1-L4) spine BMD. It is not clear why atorvastatin did not alter BMD in spite of its effects on bone biomarkers. Further study is needed to clarify the underlying mechanism.

In conclusion, atorvastatin in a certain extent inhibits bone resorption and promotes bone formation, but has no significant effects on BMD.

4. Experimental

4.1. Animals

Eight-weeks-old male Wistar rats were acquired from the Radiology Institute of Chinese Academy of Sciences. The experimental protocols were approved by local Ethics Committee and the Animal Research Committee. For all studies, 8-weeks-old male Wistar rats weighing 300–350 g were obtained from similarly raised and weaned litters and housed at 5 rats per cage in a light- (12 h) and temperature- (23–25 °C) controlled environment. The rats were allowed free access to water, were pair-fed, and were weighed weekly.

4.2. Methods

All rats were randomly allocated into a control group (n=10) and three atorvastatin treatment groups (atorvastain doses: 5 mg/kg.d, n=10; 25 mg/kg.d, n=10; and 50 mg/kg.d, n=30). Dosages of atorvastatin were based on the equation described in *Experimental Methodology in Pharmacology*. Atorvastatin was dissolved in 2 ml 0.9% NaCl and was lavaged to rats on a daily basis for 8 weeks. Control rats received the same amount of 0.9% NaCl alone. Bone biomarkers including serum calcium (Ca), phosphorus (P), alkaline phosphatase (ALP), IL-6, Bone Gla-protein (BGP) were measured at 4th week and 8th week after treatment; Urine collected continuously for 24 h was analyzed for the levels of urinary Ca, P and creatinine (Cr), which were used for calculation of urinary Ca/Cr ratio and P/Cr ratio at 8th week post-treatment. Levels of serum or urinary Ca, serum or urinary P, serum ALP and urinary creatinine levels were measured by automatic biochemistry analyzer; Blood IL-6 and BGP levels were measured by radio-immunologic analysis method. At the end of 8th week, all rats in control group, low and middle dose of atorvastatin groups and 10 rats in high dose of atorvastatin group were humanely killed. Left femurs were harvested after the rats were killed, and were preserved in 70% alcohol. Femur BMD for each rat was measured by dual-energy x-ray absorptiometry (OsteoSys), with the equipment, software, and methodology provided by Hologic Inc. Another twenty rats in high dose of atorvastatin group were randomly divided into continuing atorvastatin treatment group (50 mg/kg.d atorvastatin, n=10) and atorvastatin washout group (0.9% NaCl, n=10) for another 4 weeks. At 12th week, blood and urine were collected and analyzed for bone biomarkers as described above.

4.3. Statistical analysis

SPSS15.0 statistical software was used for data processing. Data were expressed as mean ± SD. Comparison of numerical variable data was conducted with the single factor analysis of variance (ANOVA); differences between individual groups were analyzed by *SNK-q* test. Independent-sample T-test was used for comparison between two groups. Differences were considered statistically significant at $P < 0.05$.

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