Research Article

Toxicology and Applied Pharmacology Insights

The Influence of Statins on Bone Tissue in Ovariectomized Rats

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Submitted: 16 Oct 2018; **Accepted**: 23 Oct 2018; **Published**: 31 Oct 2018

Abstract

Objectives: *Osteoporosis is a silent, progressive, systemic skeletal disease characterized by impaired bone architecture with decreased bone mass density, affecting mainly women. Statins, inhibitors of HMG-CoA-reductase, seem to interfere with bone formation exerting anabolic and anti-resorptive effect. The aim of the present study is to investigate the effect of simvastatin on size parameters and bone density of mandible and femur, in an ovariectomized rat model of osteoporosis.*

Methods: *50 Wistar female rats were randomized in five groups (n=10). Groups 1, 2,3 were ovariectomized, 4 and 5 serve as controls. Groups 2, 3 and 5 were treated with simvastatin (0.5mg/kg/daily per os). Size parameters of the isolated femur, mandible and uterus, BMD via dual energy X-Ray absorptiometry (DEXA) and serum interleukin-13 (IL-13) were estimated.*

Results: *DEXA results showed significant decrease of BMD in ovariectomized group / controls while statins treatment decreased the severity of BMD lose. The serum IL-13, as osteogenesis index, seems to be positively influenced by statins treatment. Conclusions: Statins treatment seems to restore the decreased femur and mandible parameters and uterus weight in experimental menopause following ovariectomy.*

Keywords: Mandible, Femur, Statins, Ovariectomy

Introduction

Osteoporosis is a silent, progressive, systematic bone tissue disease, characterized by disturbance of the architecture of the bone resulting in decreased bone mass density (BMD). It consists a major public health problem mainly affecting menopausal women or postmenopausal third age women and men, in a proportion of 6:1. Osteoporosis does not only affect the long bones of the skeleton, the vertebrae and the pelvis but also bone jaws. Osteoporosis not only increases a risk of fracture at the hip, spine, and wrist, but it is also associated with periodonditis [1-3]. The jaws that may be considered bones of minor importance play a major role in food intake, speech and overall self-esteem. Decreased BMD of the jaws implies increased incidence of periodontitis and tooth loss. In humans, the thickness of the bone of the jaws is critical for the application of dental implants, in case of endentulism.

Statins, which are 3-hydroxy-3 methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are used for the treatment of hypercholesterolaemia. Statins like nitrogen containing bisphosphonates, which are used for the treatment of osteoporosis, both inhibit the metabolic pathway of mevalonate acid. The mevalonate pathway leads to cholesterol synthesis and protein prenylation adding a lipid chain to transpeptidases in the cell membrane [4]. Statins interfere with HMG-CoA reductase early in this pathway reducing the mevalonate formation. The inhibition of mevalonate formation leads to the inhibition of formation of the ruffled border of osteoclasts and finally osteoclast apoptosis5. Furthermore, it seems that as far as metabolites of mevalonate acid are concerned, geranylgeraniol and not farnesol and squalene play an important role in the development and differentiation of osteoclasts [5]. It is suggested that statins may not only decrease the osteoclast absorption , but also enhance the production of new bone and increase bone mass by triggering the production of the bone morphogenetic protein -2 (BMP-2) in osteoblasts [6].

Up to date, some studies have shown that statins might reduce the fracture risk and may promote the formation of bone [6-10]. *In vitro* lovastatin administration in bone marrow cells that differentiate into osteoclasts under the influence of RANKL, M-CSF and vitamin D3, significantly decreased the production of the number of osteoclasts. On the other hand, other studies conducted did not show any benefit in the context of lower fracture incidence [11,12]. The divergence among the pre-clinical and clinical results might be attributed to the liver-specific action and the enterohepatic circulation of statins Statins exhibit low oral bioavailability, undergo an extensive first-pass metabolism that leads to low statin concentration in bone microenvironment [13-15].

The role of estrogen in bone was already known from Fuller Albright, who noticed the positive effect of hormone replacement therapy (HRT) on bone mass in postmenopausal women and the decrease of urine calcium excretion [16].

IL-13 has been shown to be an important T-helper 2 cytokine, able to both upregulate osteoprotegerin (OPG) expression and down-regulate RANKL expression in osteoblasts in a STAT6-dependent way [17,18]. IL-13 is implicated in inhibition of bone resorption also by decreasing prostaglandin production [19].

DEXA scans are used to evaluate bone mineral density in the spine, hip, peripheral limbs and the whole skeleton. It is considered the golden standard for the diagnosis and follow up of osteoporosis.

In this study, an ovariectomized (OVX) rat model of osteoporosis was used in order to test the effect of simvastatin on bone size parameters. The animals were divided into groups in order to test the preventive action of statins in the awaited osteoporosis as well as their possible reconstructive role in osteoporotic processes due to ovariectomy.

The modification of the formulation of statins and the optimization of the dosage and route of administration rendering statins bone-specific could highlight statins as an appropriate drug for osteoporosis when a concurrent therapy is indicated.

Material and methods

50 Wistar female rats aged 4-6 weeks, weighing 200-300g, from the animal colony of Athens Pasteur Institute, were randomized in five groups (n=10). The animals were housed under a 24h cycle of 12h darkness and 12h light, at 21 ± 1 °C, with food (rodent chow) and water *ad libitum*, and treated according to the "Guides for the care and use of laboratory animals" [20]. Animals in Groups 1, 2, and 3 were ovariectomized at 8 weeks of age and groups 4 and 5 served as controls. Surgical procedures were performed under general anesthesia with ketamine hydrochloride and diethyl ether. Groups 2, 3 and 5 were treated with simvastatin 0.5mg/kg/daily *per os* for three months. Group 2 received simvastatin daily after 20pm after three months from ovariectomy, whereas group 3 received simvastatin daily right after the ovariectomy. Two weeks after ovariectomy vaginal swabs were obtained for the confirmation of the loss of estrogen function. The vaginal smears were stained with Giemsa and examined under the microscope. Animals were anesthetized by ether in special cages and sacrificed by decapitation, blood was drawn from the jugular vein, centrifuged at 3000g for 10 minutes in order to obtain serum for the measurement of IL-13 by RAT IL-13 Elisa kit (Abcam Ltd, UK). Body weight was measured. Femur, mandible and uterus were isolated and weighed. The volume and the specific weight of each bone was estimated as well. Furthermore, BMD via DEXA was measured by Lunar DPX (Dual Energy X-ray Bone Densitometer). Femur ash was obtained after drying and heating for 24 h at 600°C in special porcelain cups. The experiment lasted 180 days.

Statistical analysis was performed by student's t-test.

Results

The results of the experiment are summarized in Table 1. Groups 1, 2 and 3, comprising the ovariectomized rats had decreased uterus weight and ratio of uterus weight/body weight compared to the control groups 4 and 5 (Table 1). The vaginal swab obtained 2 weeks after ovariectomy demonstrated low number of cells and a few phagocytes, in contrast to the controls that had a lot of epithelial cells. They also had the femur and mandible parameters reduced : weight ($p< 0.009$, $p< 0.058$), volume ($p<0.042$, $p< 0.084$), bone weight / body weight ($p<0.008$, $p<0.048$) respectively. However, the specific weight of the femur and mandible was not notably affected.

In group 3, representing the rats directly treated with simvastatin, although a trend to increase the weight of the femur and mandible as well as the ratio bone weight/ body weight was noted , no statistical significance was observed ($p<0.22$, $p<0.24$ and $p< 0.138$, $p<0.97$ respectively).

Table 1 Metric and biochemical results (* $p<0.05$ statistical significant)

Organs parameters	Group 1 mean±SD	Group 2 mean ±SD	Group 3 mean±SD	Group 4 mean ±SD	Group 5 mean±SD
Body weight (g)	291.11±65.28	303.57±51.37	276.67±47.08	256.67±.25.03	247,85±23,2
Femur weight (g)	2.7578±0.5368*	2.9943±0.5545	3.325±0.39	3.79±0.7785	3,85±0,8
Mandible (right) weight (g)	2.2089±0.2454	2.3229±0.1869	2.53±0.00775	2.4433±0.1499	2,5±0,12
Mandible (left) weight (g)	2.2078±0.2672*	2.3214±0.2253	2.44±0.1464	2.41±0.2373	2,43±0,2
Femur volume (cm^3)	2.4344±0.5865*	2.0329±0.6122*	2.33±0.3194*	3.15±0.6232	3,2±0,7
Mandible (right) volume (cm^3)	1.4278±0.3793	1.3625±0.0905*	1.4217±0.0979*	1.7467±0.2053	1,8±0,3
Mandible (left) volume (cm^3)	1.3767±0.2944*	1.32±0.1463*	1.3867±0.1213*	1.7083±0.2181	1,78±0,32
Femur specific weight (mg/cm^3)	1.1467±0.1190	1.5386±0.3079*	1.4283±0.1061*	1.2050±0.0841	1,38±0,05
Mandible (right) specific weight (mg/cm^3)	1,54706±0.2814	1.7171±0.0697*	1.7783±0.1565*	1.4117±0.1556	1,47±0,25
Mandible (left) specific weight	1.5022±0.2540	1.7614±0.046*	1.7633±0.0862*	1.4233±0.1646	1,43±0,17
Femur weight/ body weight (mg/g)	0.0097±0.0022*	0.0102±0.0028*	0.0124±0.0028	0.01467±0.0021	0,0147±0,2
Mandible (right) weight/body weight (mg/g)	0.0079±0.0017	0.0077±0.0013*	0.0093±0.0018	0.0093±0.0005	0,0095± 0,003

Mandible (left) weight/body weight (mg/g)	0.0078±0.0015*	0.0078±0.0016	0.0089±0.0013	0.0094±0.0012	0,0095±0, 0013
Uterus weight (mg)	2.855±0.8174	2.7714±1.245	2.8083±1.054	3,2189±0.6634	3,17±0,002
Uterus weight/body weight (mg/g)	0.0112±0.0037	0.0096±0.0051	0.0101±0.0048	0.0107±0.0044	0,127± 0,004
Femur ash (mg)	2.97±0.01	3.01±0.07	6.92±0.03	7.6±0.2*	8.2±0.07
Mandible (left) ash (mg)	3.05±0.02	3.08±0.02	3.7±0.03	4.85±0.13*	4.7±0.4
BMD Femur (gr/cm^2)	0.158±0.010	0.181±0.006	0.179±0,003	0.188±0.012	0.189±0.09
BMD Mandible (gr/cm^2)	0.198±0.011	0.215±0.008	0.211±0.009	0.208±0.010	0.21±0.09
IL-13 (pg/ml)	614,43±180,6	642,78±159,96	758,41±575,1	1275,87± 270	1264,85±540

In group 2, representing the rats with established osteoporosis before the treatment with simvastatin, the femur weight showed a tendency to increase in value under the influence of statins ($p<0.055$), whereas the ratio femur weight/ body weight significantly increased ($p<0.0083$). The weight of the mandible was not influenced by the administration of statins, but the ratio mandible weight/body weight was positively influenced ($p< 0.068$). The femur and mandible weight seems to have been positively influenced under the influence of statins ($p< 0.0077$ and $p<0.0004$). The specific weight of the femur and mandible was also significantly positively influenced ($p<0.0260$ and $p< 0.007$ respectively).

DEXA results showed a decreased tendency of BMD in the ovariectomized groups compared to the controls. Simvastatin treatment decreased the severity of bone lose. The serum IL-13 concentration was lower in the ovariectomized rats (groups 1, 2, 3) compared to the control group. The statin treated groups seem to have slightly elevated IL-13.

Discussion

Osteoporosis is a systematic disease, characterized by remodeling of the bone with a net result of increased bone resorption and decreased bone mass, increasing the possibility of fragility fractures [21]. 40% of Caucasian postmenopausal women have been affected by osteoporosis, imposing an economic and social burden [22].

The ovariectomized (OVX) rat model is the approved preclinical model by the Food and Drug Administration (FDA) for the study of the postmenopausal osteoporosis caused by the decrease in endogenous estrogen production by the ovaries [23]. The results demonstrate a clear decrease in the absolute weight of the uterus and the ratio between the uterus and the body weight after the ovariectomy, which clinically confirms the estrogen deficiency, in accordance with previous findings [24]. Furthermore, the body weight of the rats after ovariectomy was increased and abdominal fat accumulation was noticed obviously as a result of estrogen deprivation. The body weight presents negative correlation with bone loss and bone remodeling after menopause [25]. Therefore, obesity might play a protective role against osteoporosis via a mechanism that combines hormonal and mechanical factors. On the contrary, Revilla et al, proposed that the relation between bone density and obesity is independent of sex hormones in postmenopausal women [26].

Statins do not seem to exhibit any estrogen mimic effect, given that both the absolute weight of the uterus and the ratio between the uterus weight and the body weight was not influenced by their action (Table 1).

From the bone metric parameters, it arises that ovariectomy reduced both femur and mandible absolute weight, bone weight/body weight and bone volume. This is in accordance with previous findings [27,28].

As already mentioned, the preventive action of statins was tested in group 3. Although the metric parameters in femur and mandible showed a tendency to restore bone loss, this was not significant. The volume of the femur and the mandible did not show any significant difference compared to the controls, which is in contrast with the findings of other groups. Our results are in accordance in part with the findings of Saxlin et al [28-30].

Saxlin et al, report that statins do not improve periodontal infection, although a previous study has shown anti-inflammatory effect and amelioration of mandible bone mass under Freud's adjuvant arthritis [2]. Other studies report controversial results, that do not show maintenance of the mandible bone mass or of the height of the ridge of the jaws under the influence of statins. Another study indicates statins 'positive influence upon the integrity of the periodontal tissue [31]. Specifically, patients with hyperlipidemia are more susceptible to the appearance of periodontitis [32].

In group 2, in which osteoporosis had been established before simvastatin treatment, the specific weight of the femur and mandible was significantly influenced by statins, implying osteogenetic statin activity.

IL-13 blood levels have been reported to reflect alterations in bone metabolism [33]. Simvastatin has been reported to increase various interleukins, including IL-13 [34]. Probably IL-13 acts by inhibiting mechanisms involved in triggering osteoclast activity and bone resorption and by increasing the osteoprotegerin production [18].

DEXA bone density evaluation reflects that statins partly restore bone loss both in femur and mandible exerting osteogenetic activity, when they are administered after osteoporosis establishment, whereas they do not seem to exert a preventive action given in advance.

Therefore, statins can potentially play a vital role in bone remodeling and constitute an important indication in the application of dental implants, by prolonging their lifespan in the alveolar ridges of the jaws. It should be noted that a major prognostic factor for the osseointergration of the implants is the bone quality [35,36].

It may be concluded that the mandible and the femur affected by oestrogen deficiency seems to be repaired by statins , and if the

pharmacokinetic/ pharmacodynamic profile of statins is improved in order to be more bone- specific, they may serve as a useful tool promoting bone synthesis in parallel with their lipid lowering activity [37].

References

1. Darcey J, Horner K, Walsh T, Southern H, Marjanovic EJ, et al. (2013) Tooth loss and osteoporosis: to assess the association between osteoporosis status and tooth number. Br Dent J 214: E10.
2. Seferos N, Pantopoulou A, Kotsiou A, Rallis G, Tesseromatis C (2012) The influence of simvastatin in rats mandible and femur bone mass under Freund's adjuvant arthritis. Stomatologija 14: 46-52.
3. Tesseromatis C, Myrkou A, Dalles C, Speggos M (1989) [Metacarpal index in periodontal disease]. Stomatol DDR 39: 469-473.
4. Lips P (2002) Statins and bone turnover. Eur J Clin Invest 32: 543-544.
5. Fisher JE, Rogers MJ, Halasy JM, Luckman SP, Hughes DE, et al. (1999) Alendronate mechanism of action: geranylgeraniol, an intermediate in the mevalonate pathway, prevents inhibition of osteoclast formation, bone resorption, and kinase activation in vitro. Proc Natl Acad Sci USA 96: 133-138.
6. Bauer DC, Mundy GR, Jamal SA, Black DM, Cauley JA, et al. (2004) Use of statins and fracture: results of 4 prospective studies and cumulative meta-analysis of observational studies and controlled trials. Arch Intern Med 164: 146-152.
7. Chan KA, Andrade SE, Boles M, Buist DS, Chase GA, et al. (2000) Inhibitors of hydroxymethylglutaryl-coenzyme A reductase and risk of fracture among older women. Lancet 355: 2185-2188.
8. Meier CR, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H (2000) HMG-CoA reductase inhibitors and the risk of fractures. JAMA 283: 3205-3210.
9. Wang PS, Solomon DH, Mogun H, Avorn J (2000) HMG-CoA reductase inhibitors and the risk of hip fractures in elderly patients. JAMA. 283: 3211-3216.
10. Mundy G, Garrett R, Harris S, Chan J, Chen D, et al. (1999) Stimulation of bone formation in vitro and in rodents by statins. Science 286: 1946-1949.
11. Grasser WA, Baumann AP, Petras SF, Harwood HJ, Devalaraja R, et al. (2003) Regulation of osteoclast differentiation by statins. J Musculoskelet Neuronal Interact 3: 53-62.
12. Van Staa TP, Wegman S, de Vries F, Leufkens B, Cooper C (2001) Use of statins and risk of fractures. JAMA. 285: 1850-1855.
13. Jadhav SB, Jain GK (2006) Statins and osteoporosis: new role for old drugs. J Pharm Pharmacol 58: 3-18.
14. Reinoso RF, Sanchez Navarro A, Garcia MJ, Prous JR (2002) Preclinical pharmacokinetics of statins. Methods Find Exp Clin Pharmacol 24: 593-613.
15. Corsini A, Bellosta S, Baetta R, Fumagalli R, Paoletti R, et al. (1999) New insights into the pharmacodynamic and pharmacokinetic properties of statins. Pharmacol Ther 84: 413-428.
16. Forbes AP (1991) Fuller Albright. His concept of postmenopausal osteoporosis and what came of it. Clin Orthop Relat Res 269: 128-141.
17. Stein NC, Kreutzmann C, Zimmermann SP, Niebergall U, Hellmeyer L, et al. (2008) Interleukin-4 and interleukin-13 stimulate the osteoclast inhibitor osteoprotegerin by human endothelial cells through the STAT6 pathway. J Bone Miner Res 23: 750-758.
18. Palmqvist P, Lundberg P, Persson E, Johansson A, Lundgren I, et al. (2006) Inhibition of hormone and cytokine-stimulated osteoclastogenesis and bone resorption by interleukin-4 and interleukin-13 is associated with increased osteoprotegerin and decreased RANKL and RANK in a STAT6-dependent pathway. J Biol Chem 281: 2414-2429.
19. Trudeau J, Hu H, Chibana K, Hong Wei Chu, Jay Y. Westcott, et al. (2006) Selective downregulation of prostaglandin E2-related pathways by the Th2 cytokine IL-13. J Allergy Clin Immunol 117: 1446-1454.
20. Committee on Care and Use of Laboratory animals.Guide for the care and use of laboratory animals 1985: 83.
21. Manolagas SC (2000) Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev 21: 115-137.
22. Melton LJ (1997) 3rd. Epidemiology of spinal osteoporosis. Spine (Phila Pa 1976) 22: 2S-11S.
23. Thompson DD, Simmons HA, Pirie CM, Ke HZ (1995) FDA Guidelines and animal models for osteoporosis. Bone 17: 125S-133S.
24. Su SJ, Yeh YT, Shyu HW (2013) The preventive effect of biochanin a on bone loss in ovariectomized rats: involvement in regulation of growth and activity of osteoblasts and osteoclasts. Evid Based Complement Alternat Med 2013: 594857.
25. Lenchik L, Register TC, Hsu FC, Lohman K, Nicklas BJ, et al. Adiponectin as a novel determinant of bone mineral density and visceral fat. Bone 33: 646-651.
26. Revilla M, Villa LF, Hernandez ER, Sanchez-Atrio A, Cortes J, Rico H (1997) Influence of weight and gonadal status on total and regional bone mineral content and on weight-bearing and non-weight-bearing bones, measured by dual-energy X-ray absosorptiometry. Maturitas 28: 69-74.
27. Pytlik M, Janiec W, Cegiela U, Sliwinski L (1999) Influence of alpha-escin on the femoral bone strength in ovariectomized rats. Pol J Pharmacol 51: 511-515.
28. Pytlik M, Janiec W, Misiarz-Myrta M, Gubala I (2003) Effects of simvastatin on the development of osteopenia caused by ovariectomy in rats. Pol J Pharmacol 55: 63-71.
29. Kalu DN (1991) The ovariectomized rat model of postmenopausal bone loss. Bone Miner 15: 175-191.
30. Saxlin T, Suominen-Taipale L, Knuuttila M, Alha P, Ylostalo P (2009) Dual effect of statin medication on the periodontium. J Clin Periodontol 36: 997-1003.
31. Cunha-Cruz J, Saver B, Maupome G, Hujoel PP (2006) Statin use and tooth loss in chronic periodontitis patients. J Periodontol 77: 1061-1066.
32. Sangwan A, Tewari S, Singh H, Sharma RK, Narula SC (2013) Periodontal status and hyperlipidemia: statin users versus non-users. J Periodontol 84: 3-12.
33. Onoe Y, Miyaura C, Kaminakayashiki T, Nagai Y, Noguchi K, et al. (1996) IL-13 and IL-4 inhibit bone resorption by suppressing cyclooxygenase-2-dependent prostaglandin synthesis in osteoblasts. J Immunol 156: 758-764.
34. Arora M, Chen L, Paglia M, Gallagher I, Allen JE, et al. (2006) Simvastatin promotes Th2-type responses through the induction of the chitinase family member Ym1 in dendritic cells. Proc Natl Acad Sci USA 103: 7777-7782.
35. Mulligan R, Sobel S (2005) Osteoporosis: diagnostic testing,

interpretation, and correlations with oral health--implications for dentistry. Dent Clin North Am 49: 463-484.

36. von Wowern N (2001) General and oral aspects of osteoporosis: a review. Clin Oral Investig 5: 71-82.

37. Tian L, Yu X (2015) Lipid metabolism disorders and bone dysfunction - interrelated and mutually regulated (Review). Mol Med Rep 12: 783-794.