Pharmacological Reports
2006, 58, 60–66
ISSN 1734-1140

Fluvastatin does not elevate periosteal osteogenesis induced by Moloney sarcoma virus (MSV) in mice

Ryszard Galus[1,2], Paweł Włodarski[1], Krzysztof H. Włodarski[1]

[1] Chair and Department of Histology and Embryology, Center for Biostructure, Medical University in Warszawa, Chałubińskiego 5, PL 02-004 Warszawa, Poland

[2] Department of Dermatology, Military Institute of Health, Szaserów 128, PL 00-909 Warszawa, Poland

Correspondence: Krzysztof Włodarski, e-mail: kwlodar@ib.amwaw.edu.pl

Abstract:
Several studies have demonstrated the pleiotropic effects of statins. Since Wang and associates reported that in rabbits lovastatin reduced steroid-induced bone loss, numerous authors have confirmed these data, however, others have reported conflicting results. In this study, the effects of fluvastatin on bone formation were investigated in early and late phase of osteogenesis. In the first set of experiments (early phase of osteogenesis) CFW/Ll mice were randomly divided into three groups. Two groups were injected with Moloney–murine sarcoma virus (Mo-MSV) into right thighs to induce orthotopic bone formation. Mice in the experimental group received fluvastatin for 11 consecutive days. Thirty days after Mo-MSV inoculation, total serum cholesterol, triglycerides, high- and low-density lipoprotein cholesterol, alkaline phosphatase (AP) were measured and bone mineral increase was calculated. In the second set of experiments (late phase of osteogenesis), fluvastatin was administered from day 11 after Mo-MSV inoculation for 20 consecutive days. Fluvastatin administration in the early phase of osteogenesis made no significant difference in average bone increase compared with mice receiving placebo. Lipid profile and AP were not significantly affected. During late phase of osteogenesis, the average increase in femural dry mass was significantly lower in the group of mice receiving fluvastatin than in the control group. Also, Mo-MSV-initiated tumors disappeared earlier in mice treated with fluvastatin. This may be attributed to the antioncogenic potential of fluvastatin. These results also point out that orthotopic bone formation at the sites of Mo-MSV inoculation in mice seems to be a useful model to examine the pleiotropic effects of statins.

Key words:
fluvastatin, mice, periosteum, orthotopic bone formation, antitumor effect, Mo-MSV

Abbreviations: AP – alkaline phosphatase, HDL-C – high density lipoprotein cholesterol, HMG-CoA – 3-hydroxy-3-methylglutaryl coenzyme A, LDL-C – low density lipoprotein cholesterol, Mo-MSV – Moloney-murine sarcoma virus, TC – total cholesterol, TG – triglycerides

Introduction

Statins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are a well-established class of drugs that effectively lower serum cholesterol levels. Since Wang et al. [30] reported that lovastatin reduced steroid-induced bone loss in rabbits, several articles have confirmed the positive impact of statins on bone formation.

However, data from experiments with HMG-CoA inhibitors on bone formation are conflicting. Among numerous *in vitro* and *in vivo* studies demonstrating the anabolic effect of statins and/or the reduction of bone loss and improved bone fracture healing [4, 14, 15, 17, 23, 26] there are reports which deny the influence of statins on bones [3, 5, 11, 25, 29].

Mundy and coworkers reported that this stimulatory effect is secondary to the increased expression of bone morphogenetic protein-2 gene in bone cells [17]. The aim of the present work was to examine the effects of fluvastatin, a widely used synthetic statin that effectively lowers serum cholesterol levels, on the orthotopically induced osteogenesis by Moloney-murine sarcoma virus (Mo-MSV) in mice. Orthotopically induced osteogenesis by Mo-MSV used in this study served as a technically simple yet a valuable model for the local regulation of bone growth. Mo-MSV induces development of pleomorphic tumors at the site of inoculation, named incorrectly rhabdomyosarcoma [22, 33]. Spontaneous regression of such tumors is followed by periosteal membrane proliferation of bones adjacent to the tumors and subsequent periosteal bone formation [31, 32].

According to the literature, in rodents daily administrated doses of statins vary between 5–10 mg/kg. Here we examine whether fluvastatin, at doses sufficient to lower cholesterol level in humans, would affect bone metabolism in early and late phases of viral bone induction in mice.

Materials and Methods

Animals

Mature male mice of inbred CFW/Ll strain weighing 21.73 ± 3.27 g were used in accordance with the Medical Academy Ethics Committe guidelines for the care and use of laboratory animals. The mice were kept up to five per cage with free access to mouse chow and water. Fluvastatin (Novartis, Basel, Switzerland) was dissolved in water and phosphate buffer to obtain stock solution pH 7.3, osmolality 337 mOsm/kg H_2O. The placebo was phosphate buffer adjusted to pH 7.3 and osmolality was 335 mOsm/kg H_2O. Two sets of administration protocols were applied. Injections of fluvastatin or its solvent were administered subcutaneously once a day for 11 and 20 consecutive days, respectively. In the first group of experiments, fluvastatin was administered during the early phase of osteogenesis, i.e. when periosteal membrane proliferates. In the second group, the statin was administered during the late phase of periosteal osteogenesis, i.e. when periosteal membrane proliferation is ceased.

Schemes of fluvastatin administration

Group 1 – early phase of osteogenesis

For this mode of fluvastatin administration 37 mice were used. Thirty mice were injected 0.2 ml of standard Mo-MSV stock into right thigh muscles, seven mice were untreated and served as the control. Standard Moloney murine sarcoma virus stock was obtained according to Włodarski et al. [32], aliquoted and stored at –80°C until the time of experiment.

The mice injected with Mo-MSV were randomly divided into two groups (19 and 11 mice/group). Nineteen mice (fluvastatin group) injected with Mo-MSV received fluvastatin at 1.2 mg/kg/day for 11 consecutive post inoculation days, whilst 11 mice (control group) received placebo (Fig. 1). The remaining 7 mice were not treated with Mo-MSV or with fluvastatin. Animals were analyzed on day 30.

Group 2 – late phase of osteogenesis

For this experiment a total of 101 mice were used of which 94 received Mo-MSV into the right thigh muscles. Of these, 48 mice received fluvastatin whilst 46 mice received placebo. The remaining 7 mice were not inoculated with Mo-MSV and served as a control of the experimental model. Fluvastatin was administered daily for 20 days since day 11 after Mo-MSV injection (Fig. 1). Subjects were analyzed on day 30.

Analysis of serum lipid levels, alkaline phosphatase and bone mass

Thirty days after Mo-MSV inoculation, animals from both groups were anesthetized with an intraperitoneal injection of Rompun (Bayer AG, Leverkusen, Holland) and Calypsol (Richter Gedeon, Budapest,

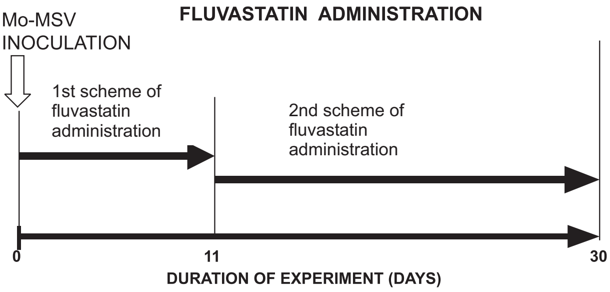

Fig. 1. Scheme of fluvastatin administration

Hungary), blood samples were collected from the retroorbital sinus to measure total serum cholesterol (TC), triglycerides (TG), high- and low-density lipoprotein cholesterol (HDL-C and LDL-C, respectively) and alkaline phosphatase (AP). To obtain the required volume of serum (0.7 ml) necessary to read measurements on Cobas Integra 700 analyzer (Roche Diagnostics, Hoffmann-La Roche Ltd., Basel, Switzerland) two or three serum samples from the same group of mice were pooled.

After blood collection, mice were immediately sacrificed by cervical dislocation and both hind legs were excised and hydrolyzed in 0.1 M NaOH at 65°C for 24 h. During this procedure soft tissues are dissolved and bones can be easily removed without affecting the mineralized tissue mass. The isolated femurs were washed in distilled water and dried at 65°C overnight. The dry bones were weighted on an analytical balance with an accuracy of 0.1 mg and results are presented as a percentage of increase ($\Delta\%$) of stimulated femural mass *vs.* unstimulated femur, calculated using the following formula:

$$\frac{\text{weight of Mo-MSV-stimulated (right) femur} - \text{weight of not treated with Mo-MSV (left) femur} \times 100\%}{\text{weight of not treated with Mo-MSV (left) femur}}$$

All measurements were read by an experimenter unaware of treatment and time.

Statistical analysis

The significant differences between bone mass ratios were analyzed using Student's *t*-test at $p < 0.05$ and ANOVA with Scheffé's *post-hoc* test was used for blood tests. For data that were not normally distributed, the nonparametric Wilcoxon signed rank test and the Mann-Whitney test were applied. A p value < 0.05 was considered statistically significant. Statistical analysis was performed using SAS V.6.12 for Windows software (SAS Institute, USA).

Tab. 1. Effects of fluvastatin administered during the early phase of osteogenesis (days 1–11 after Mo-MSV inoculation) and during the late phase of osteogenesis (days 11–30 after Mo-MSV inoculation) on the average time of appearance and disappearance of the tumors in male CFW/Ll mice

Treatment		Time of appearance of the tumors (days)	Time of disappearance of the tumors (days)
Early phase of osteogenesis	fluvastatin	4	21 ± 1
	placebo	4	21 ± 1
Late phase of osteogenesis	fluvastatin	4	19 ± 1
	placebo	4	21 ± 1

Results

We did not observe extravasation or swelling and no bacterial infection was found related to subcutaneously administered fluvastatin.

Fluvastatin effects on Mo-MSV-induced tumorogenesis

In the group 1, where mice were treated with fluvastatin in early phase of osteogenesis, the time of appearance and disappearance of the tumors was similar in placebo- and fluvastatin-treated mice (4 and 21days after Mo-MSV inoculation, respectively). In the group 2, when fluvastatin was administered in the late phase of osteogenesis, although tumors appeared in all of Mo-MSV-treated mice simultaneously, the tumors in fluvastatin-treated mice disappeared a few days earlier (day 19 *vs.* day 21) (Tab. 1).

Fluvastatin lowers Mo-MSV-induced bone formation and AP serum concentrations

The Mo-MSV inoculations have provoked a substantial increase in femoral mass within the group of placebo-treated and fluvastatin-treated animals in the group 1 and 2 (Tab. 2, 3), so the average right femur weight of all mice inoculated with Mo-MSV was increased when compared to contralateral bone. We did not observe significant differences in the average dry weight increase in femurs between fluvastatin-treated and placebo-treated mice in group 1 (Tab. 2). In the group 2 the average femural dry weight increase was significantly lower in fluvastatin-treated mice than in placebo-treated mice (Tab. 3). Serum levels of AP increased significantly after Mo-MSV inoculation in placebo-treated mice. In the fluvastatin-treated group this regulation was not observed (Tab. 3).

Tab. 2. Effects of fluvastatin administered during the early phase of osteogenesis (days 1–11 after Mo-MSV inoculation) on the femur dry mass and the serum alkaline phosphatase activities in male CFW/LI mice. Data represent the mean ± SD

Treatment	n	Effect of fluvastatin on bone mass					Alkaline phosphatase (U/l)	p values
		Dry weight (mg)		p values	The average increment of dry bones mass (%)	p values		
Fluvastatin (Mo-MSV)	19	Left femur	27.6 ± 3.5	p < 0.05	12.5 ± 11.4	p > 0.05	150.4 ± 18.6	p > 0.05
		Right femur	30.9 ± 3.8					
Placebo (Mo-MSV)	11	Left femur	29.5 ± 2.5	p < 0.05	7.05 ± 8.0		155.4 ± 15.5	
		Right femur	31.5 ± 2.8					
Mice not treated with Mo-MSV	7	Left femur	28.6 ± 3.5	p > 0.05			152.7 ± 17.2	
		Right femur	28.3 ± 5.4					

Tab. 3. Effects of fluvastatin administered during the late phase of osteogenesis (days 11–30 after Mo-MSV inoculation) on the femur dry mass and the serum alkaline phosphatase activities in male CFW/LI mice. Data represent the mean ± SD

Treatment	n	Effect of fluvastatin on bone mass					Alkaline phosphatase (U/l)	p values
		Dry weight (mg)		p values	The average increment of dry bones mass (%)	p values		
Fluvastatin (Mo-MSV)	48	Left femur	27.6 ± 6.6	p < 0.05	19.8 ± 17.7	p < 0.05	90.9 ± 35.6	p < 0.05
		Right femur	33.0 ± 8.9					
Placebo (Mo-MSV)	46	Left femur	28.4 ± 5.2	p < 0.05	27.3 ± 19.9		112.3 ± 27.3	
		Right femur	36.2 ± 8.7					
Mice not treated with Mo-MSV	7	Left femur	27.3 ± 6.3	p > 0.05			89.5 ± 30.3	
		Right femur	28.9 ± 5.4					

Tab. 4. Effects of fluvastatin administered during the early phase of osteogenesis (days 1–11 after Mo-MSV inoculation) on the lipid profile in male CFW/LI mice. Data represent the mean ± SD

Treatment	n	Lipid profile			
		Total cholesterol (mg/dl)	Low-density lipoprotein cholesterol (mg/dl)	High-density lipoprotein cholesterol (mg/dl)	Triglycerides (mg/dl)
Fluvastatin (Mo-MSV)	19	65.1 ± 3.5	6.5 ± 3.4	46.6 ± 3.3	150.2 ± 40.3
Placebo (Mo-MSV)	11	63.2 ± 3.4	8.4 ± 2.1	46.8 ± 3.0	146.8 ± 33.7
Mice not treated with Mo-MSV	7	63.2 ± 2.9	7.6 ± 3.0	47.0 ± 3.0	148.7 ± 38.7
p values		p > 0.05	p > 0.05	p > 0.05	p > 0.05

Table 5. Effects of fluvastatin administered during the late phase osteogenesis (days 11–30 after Mo-MSV inoculation) on the lipid profile of male CFW/LI mice. Data represent the mean ± SD

Treatment	n	Lipid profile			
		Total cholesterol (mg/dl)	Low-density lipoprotein cholesterol (mg/dl)	High-density lipoprotein cholesterol (mg/dl)	Triglycerides (mg/dl)
Fluvastatin (Mo-MSV)	48	76.1 ± 12.2	12.3 ± 6.9	42.2 ± 13.2	158.6 ± 48.5
Placebo (Mo-MSV)	46	78.9 ± 14.2	13.5 ± 10.0	48.5 ± 17.0	150.4 ± 28.5
Mice not treated with Mo-MSV	7	79.3 ± 14.0	13.5 ± 9.8	47.5 ± 16.6	157.3 ± 40.8
p values		p > 0.05	p > 0.05	p > 0.05	p > 0.05

Low doses of fluvastatin did not affect serum lipid profile

The results of lipid profile determination in the group 1 and 2 are presented in Table 4 and 5, respectively.

In the group 1 receiving fluvastatin, the data showed a tendency to a lower total cholesterol concentration, but this difference was not statistically significant when compared with placebo-Mo-MSV-treated and Mo-MSV-untreated mice. In group 2, serum lipid profile did not change significantly in either group of mice when compared to untreated Mo-MSV mice.

Discussion

In the present study, fluvastatin administered in early phase of periosteal osteogenesis (injections of fluvastatin 1– 11 days after Mo-MSV inoculation) showed no significant effect on femoral dry mass increase. These results indicate that fluvastatin at doses adequate to lower cholesterol levels in humans have no proosteogenic potential at the time of early osteogenesis. Lack of significant serum lipid modification in mice receiving fluvastatin, compared to mice receiving placebo, may be explained by a very short period (11 days) of fluvastatin administration and by the relatively low doses of statin used in this study.

Of particular interest are the results of the second set of experiments in late phase of osteogenesis. Fluvastatin administration during intensive periosteal osteogenesis (daily injections of fluvastatin 11–30 days after Mo-MSV inoculation) lowered new bone formation, indicating the pleiotropic effects of fluvastatin. In this study, we were unable to determine the precise reason for this inhibition. However, the tendency to the accelerated regression of tumors in fluvastatin-treated mice implies that inhibitory effect of fluvastatin on bone formation was caused by its antitumor activity [2, 6–8, 10, 18]. Mo-MSV-induced tumors locally stimulate periosteal membrane to proliferate and to produce new bone [32]. In this experiment, fluvastatin administration could reduce the release of cytokines, involved in stimulating the periosteum by the Mo-MSV-tumor. The depletion of such agents stimulating the periosteum could antagonize the proosteogenic effect of fluvastatin. Further work is needed to determine whether the antitumor properties of fluvastatin, noted in these studies, are related to the triggering of apoptosis [1]. Although the pro-apoptotic potency of fluvastatin, simvastatin and lovastatin, has been considered to be of medium magnitude [19], it is proven that the lovastatin concentration required to induce apoptosis *in vitro* might be too high to be applied in the animal model [9, 16]. Phase 1 study in patients with cancer has revealed that the administration of lovastatin at doses 2 to 25 mg/kg daily resulted in achieving a plasma drug concentrations in the range between 0.1 and 3.9 μM, sufficient to induce antiproliferative and proapoptotic effects [27].

Lack of the expected increase in bone induction by fluvastatin was consistent with serum AP level, which was lower than in placebo-treated mice. Lack of the lipid lowering effect of fluvastatin strengthens the previous results indicating the pleiotropic effects of certain statins beyond their effect on plasma lipid levels [12, 20, 21, 24, 28]. The AP levels in mice not treated with Mo-MSV, differed in groups 1 and 2. This may suggest a certain degree of variability between pools of animals used, or miscalibration of apparatus, since both sets of experiments were done at different times. We were unable, however, to determine if there was any miscalibration, however the blood samples of control and of fluvastatin-treated sera were measured at the same time for group 1 and 2. No comparison of the results between group 1 and 2 was done. We believe that the absolute AP level is less important than the degree of its regulation.

The administration of fluvastatin during orthotopic bone formation at sites of tumors evoked by Mo-MSV inoculation in mice seems to be an interesting example of the pleiotropic effect of statins. In our paper statins do not enhance bone formation in rodents, but are sufficient to reduce the growth of Moloney sarcoma. In our experiment the dose 1.2 mg/kg of fluvastatin applied for two weeks in mice have no effect on serum lipid profile, while in human such dose applied for 4 and 12 weeks decreased TC and LDL-C [20]. Li et al., however, using a dose of fluvastatin similar to doses used by us were unable to reduce the level of cholesterol in humans [13]. Our results support the findings about a relatively weak effect of statin therapy on bone metabolism and partially explain contradictory results of other studies on the effect of statins on bone. There is a need for further analysis of the effect of statins on bone with the use of different doses effectively lowering serum cholesterol level.

Acknowledgments:
The technical assistance of Mrs. Ewa Wiśniewska is gratefully acknowledged. This work was supported in part by grant 1M15/W2/2004 from Medical University of Warszawa.

References:

1. Agarwal B, Bhendwal S, Halmos B, Moss SF, Ramey WG, Holt PR: Lovastatin augments apoptosis induced by chemotherapeutic agents in colon cancer cells. Clin Cancer Res, 1999, 5, 2223–2229.
2. Alonso DF, Farina HG, Skilton G, Gabri MR, De Lorenz MS, Gomez DE: Reduction of mouse mammary tumor formation and metastasis by lovastatin, an inhibitor of the mevalonate pathway of cholesterol synthesis. Breast Cancer Res Treat, 1998, 50, 83–93.
3. Banu J, Kalu DN: Effects of cerivastatin and parathyroid hormone on the lumbar vertebra of aging male Sprague-Dawley rats. Bone, 2002, 31, 173–179.
4. Bauer DC, Mundy GR, Jamal SA, Black DM, Cauley JA, Ensrud KE, van der Klift M et al.: Use of statins and fracture: results of 4 prospective studies and cumulative meta-analysis of observational studies and controlled trials. Arch Inter Med, 2004, 164, 146–152.
5. Bjarnason NH, Riis BJ, Christiansen C: The effect of fluvastatin on parameters of bone remodeling. Osteoporos Int, 2001, 12, 380–384.
6. Broitman SA, Wilkinson J 4th, Cerda S, Branch SK: Effects of monoterpens and mevinolin on murine colon tumor CT-26 *in vitro* and its hepatic "metastases" *in vivo*. Adv Exp Med Biol, 1996, 401, 111–130.
7. Hawk MA, Cesen KT, Siglin JC, Stoner GD, Ruch RJ: Inhibition of lung tumor cell growth *in vitro* and mouse lung tumor formation by lovastatin. Cancer Lett, 1996, 109, 217–222.
8. Jani JP, Specht S, Stemmler M, Blanock K, Singh SV, Gupta V, Katoh A: Metastasis of B16F10 mouse melanoma inhibited by lovastatin, an inhibitor of cholesterol biosynthesis. Invasion Metastasis, 1993, 13, 314–324.
9. Jones KD, Couldwell WT, Hinton DR, Su Y, He S, Anker L, Law RE: Lovastatin induces growth inhibition and apoptosis in human glioma cells. Biochem Biophys Res Commun, 1994, 205, 1681–1687.
10. Kikuchi T, Nagata Y, Abe T: *In vitro* and *in vivo* antiproliferative effects of simvastatin, an HMG-CoA reductase inhibitor, on human glioma cells. J Neurooncol, 1997, 34, 233–239.
11. LaCroix AZ, Cauley JA, Pettinger M, Hsia J, Bauer DC, McGowan J, Chen Z et al.: Statin use, clinical fracture, and bone density in postmenopausal women: results from the Women's Health Initiative Observational Study. Ann Intern Med, 2003, 139, 97–104.
12. Laufs U, Wassmann S, Hilgers S, Ribaudo N, Bohm M, Nickenig G: Rapid effects on vascular function after initiation and withdrawal of atorvastatin in healthy, normocholesterolemic men. Am J Cardiol, 2001, 88, 1306–1307.
13. Li Z, Iwai M, Wu L, Liu HW, Chen R, Jinno T, Suzuki J et al.: Fluvastatin enhances the inhibitory effects of a selective AT1 receptor blocker, valsartan, on atherosclerosis. Hypertension, 2004, 44, 758–763.
14. Lupattelli G, Scarponi AM, Vaudo G, Siepi D, Roscini AR, Gemelli F, Pirro M et al.: Simvastatin increases bone mineral density in hypercholesterolemic postmenopausal women. Metabolism, 2004, 53, 744–748.
15. Maeda T, Matsunuma A, Kawane T, Horiuchi N: Simwastatin promotes osteoblast differentiation and mineralization in MC3T3 cells. Biochem Biophys Res Commun, 2001, 280, 874–877.
16. Marcelli M, Cunningham GR, Haidacher SJ, Padayatty SJ, Sturgis L, Kagan C, Denner L: Caspase-7 is activated during lovastatin-induced apoptosis of the prostate cancer cell line LNCAP. Cancer Res, 1998, 58, 76–83.
17. Mundy GR, Garret R, Harris S, Chan J, Chen D, Rossini G, Boyce B et al.: Stimulation of bone formation *in vitro* and in rodents by statins. Science, 1999, 286, 1946–1949.
18. Narisawa T, Fukarua Y, Tanida N, Hasebe M, Ito M, Aizawa R: Chemopreventive efficacy of low dose of pravastatin, an HMG-CoA reductase inhibitor, on 1,2-dimethylhydrazine-induced colon carcinogenesis in ICR mice. Tohoku J Exp Med, 1996, 180, 131–138.
19. Negre-Aminou P, van Vliet AK, van Erck M, van Thiel GC, van Leeuwen RE, Cohen LH: Inhibition of proliferation of human smooth muscle cells by various HMG-CoA reductase inhibitors: comparison with other human cell types. Biochim Biophys Acta, 1997, 1345, 259–268.
20. Okopien B, Krysiak R, Madej A, Belowski D, Zielinski M, Kowalski J, Herman ZS: Effect of simvastatin and fluvastatin on plasma fibrinogen levels in patients with primary hypercholesterolemia. Pol J Pharmacol, 2004, 56, 781–787.
21. Owczarek J, Jasinska M, Orszulak-Michalak D: Drug-induced myopathies. An overview of the possible mechanisms. Pharmacol Rep, 2005, 57, 23-34.
22. Perk K, Moloney JB: Pathogenesis of a virus-induced rhabdomyosarcoma in mice. J Natl Cancer Inst, 1966, 37, 581–599.
23. Phillips B, Belmonte N, Vernochet C, Ailhaud G, Dani Ch: Compactin enhances osteogenesis in murine embryonic stem cells. Biochem Biophys Res Commun, 2001, 284, 478–484.
24. Ridker PM, Rifai N, Lowenthal SP: Rapid reduction in C-reactive protein with cerivastatin among 785 patients with primary hypercholesterolemia. Circulation, 2001, 103, 1191–1193.
25. Sirola J, Honkanen R, Kroger H, Jurvelin JS, Maenpaa P, Saarikoski S: Relation of statin use and bone loss: a prospective population-based cohort study in early postmenopausal women. Osteoporos Int, 2002, 13, 537–541.
26. Skoglund B, Forslund C, Aspenberg P: Simvastatin improves fracture healing in mice. J Bone Miner Res, 2002, 17, 2004–2008.
27. Thibault A, Samid D, Tompkins AC, Figg WD, Cooper MR, Hohl RJ, Trepel J et al.: Phase I study of lovastatin, an inhibitor of the mevalonate pathway, in patients with cancer. Clin Cancer Res, 1996, 2, 483–491.
28. Tsunekawa T, Hayashi T, Kano H, Sumi D, Matsui-Hirai H, Thakur NK, Egashira K et al.: Cerivastatin, a hydroxymethylglutaryl coenzyme A reductase inhibitor,

improves endothelial function in elderly diabetic patients within 3 days. Circulation, 2001, 104, 376–379.

29. Van Staa TP, Wegman SL, de Vries F, Leufkens HG, Cooper C: Use of statins and risk of fractures. JAMA, 2001, 285, 1850–1855.
30. Wang GJ, Chung KC, Shen WJ: Lipid clearing agents in steroid-induced osteoporosis. J Formos Med Assoc, 1995, 94, 589–592.
31. Wlodarski K, Kobus M, Łuczak M: Orthotopic bone induction at sites of Moloney murine sarcoma virus inoculation in mice. Nature, 1979, 281, 386–387.
32. Wlodarski K, Kobus M, Łuczak M, Dołowy J: Virally induced periosteal osteogenesis in mice. Calcif Tissue Int, 1981, 33, 135–142.
33. Wlodarski K, Marciniak M, Reddi AH: *In vitro* establishment of a murine Moloney sarcoma cell line. Its morphology and transplantation. Folia Biol (Kraków), 1986, 34, 227–234.

Received:
June 2, 2005; in revised form: October 25, 2005.