Pharmacological Reports
2007, 59, 58–63
ISSN 1734-1140

Effect of pamidronate on the action of catecholamines on blood pressure in the marrow cavity of long bones in rats with prednisolone-induced osteoporosis

Ilona Kaczmarczyk-Sedlak, Joanna Folwarczna, Urszula Cegieła, Barbara Nowińska

Department of Pharmacology, Medical University of Silesia, Jagiellońska 4, PL 41-200 Sosnowiec, Poland

Correspondence: Ilona Kaczmarczyk-Sedlak, e-mail: farmak@slam.katowice.pl

Abstract:
Pamidronate is a representative of bisphosphonates, which are effectively used in the treatment of bone diseases. Although a number of properties of pamidronate have been recognized which influence the metabolic process in bones, the issue of the effect of bisphosphonates on the function of blood circulation and autonomic nervous system in osteoporotic bones remains open. In order to clarify this problem, the present study concentrated on the effects of pamidronate on catecholamine action on blood pressure in the marrow cavity in rats with prednisolone-induced osteoporosis. The animals were divided into 3 groups: I – control rats; II – rats which were given prednisolone at the dose of 5 mg/kg, *im*, for 3 weeks; III – rats which were given prednisolone at the dose of 5 mg/kg, *im* and pamidronate at the dose of 3 mg/kg, *sc* together, for 3 weeks.
The experiments demonstrated that rats with prednisolone-induced osteoporosis displayed a decreased blood pressure in the marrow cavity. In addition, a disordered action of catecholamines (norepinephrine and epinephrine) on blood pressure in the marrow cavity of osteoporotic bone was observed. Pamidronate administration in osteoporotic rats resulted in smaller increases in the blood pressure caused by norepinephrine and epinephrine in the marrow cavity of long bones.

Key words:
blood pressure, catecholamines, osteoporosis, pamidronate, prednisolone, rat

Introduction

Bisphosphonates are potent inhibitors of osteoclastic bone resorption, furthermore, can bind avidly to hydroxyapatite, and for these reasons, they are used in the treatment of osteoporosis. Pamidronate's antiresorptive action was proved in *in vitro* tests, in experimental animal models, as well as in clinical research. However, the effect of pamidronate on autonomic nervous system and processes affecting blood circulation in bones has still not been recognized.

Metabolic and vascular control of bone is influenced by the nervous system. Potential transmitters of this influence include catecholamines [9]. Catecholamines act as inducers of osteoclast maturation *in vitro* and as stimulators of osteoclast activity [4]. Disorders of the peripheral nerves may have substantial influence on bone remodeling. Specifically considered are potential neural influences operating under such con-

ditions as osteoporosis. Hence, this study is aimed at examining the effect of pamidronate on the action of catecholamines (norepinephrine, epinephrine and isoprenaline) on blood pressure in rats with prednisolone-induced osteoporosis.

Materials and Methods

The experiments were carried out on male Wistar rats (205–230 g). The permission for the animal tests and experiments was granted by the Bioethical Board of the Silesian Medical University.

The animals were divided into 3 groups (18 animals each): I (C) – control rats; II (O) – rats which were given prednisolone at the dose of 5 mg/kg, *im*, for 3 weeks; III (P) – rats which were given prednisolone at the dose of 5 mg/kg, *im* and pamidronate at the dose of 3 mg/kg, *sc*, for 3 weeks.

The animals in each of the three main groups (C, O and P) were additionally divided into three subgroups administered adrenoceptor agonists:
1. Subgroup of rats (n = 6), which were administered norepinephrine (10 μg/kg, *iv*, 20 μg/kg, *iv* and 40 μg/kg, *iv*),
2. Subgroup of rats (n = 6), which were administered epinephrine (10 μg/kg, *iv*, 20 μg/kg, *iv* and 40 μg/kg, *iv*),
3. Subgroup of rats (n = 6), which were administered isoprenaline (3 μg/kg, *iv* and 6 μg/kg, *iv*).

Catecholamines (norepinephrine, epinephrine, isoprenaline) were administered at increasing doses at 10-min intervals.

After the lapse of 24 h from the last administration of 0.9% NaCl (C group), prednisolone (O group) or prednisolone + pamidronate (P group), the animals were anesthetized with urethane at the dose of 1.9 g/kg, *ip*. To prevent blood clotting, the animals received an *iv* injection of heparin at the dose of 2000 IU/kg.

Arterial blood pressure was measured in the left carotid artery. This was done by inserting a polyethylene cannula (d = 2 mm) into the artery, which was connected with a pressure meter through a membrane sensor.

A method described by Ficat and Arlet [3] was used to examine blood pressure in the femoral marrow cavity. For the measurement to be carried out, the anesthetized rats had the left femoral diaphysis revealed and a hole of d = 1.2 mm was drilled using a dental drill 1/3 of the distance from the proximal epiphysis. A metal cannula was fixed tight in the hole. The end of the cannula was in the marrow cavity where the osseous tissue contacted the marrow. The cannula was connected to the blood pressure measuring sensor *via* a drain filled with 0.9% NaCl supplemented with heparin, which was linked to the pressure meter.

A 15-min period was allowed after a rat had been connected to the set, in order to obtain stable parameters. Following the recording of initial parameters, the examined compounds were administered *iv* (as described above). The right carotid vein had previously been exposed and the polyethylene cannula of d = 1 mm was inserted into it.

The examined parameters (mean blood pressure in the carotid artery and blood pressure in the marrow cavity) were recorded continuously. The parameters were read using the recorder immediately following the administration (0–0.25 min), and after the lapse of 0.5, 1, 2, 3, 5 and 10 min from the administration of catecholamines.

Unpaired Student's *t*-test was applied for statistical evaluation of the results (the arithmetic mean values ± SEM).

Results

In the control (C group), the mean blood pressure measured in the carotid artery was 117.1 ± 2.2 mmHg. The pressure in the femoral marrow cavity measured in parallel was 15.1 ± 0.2 mmHg (Fig. 1).

In rats with osteoporosis induced by a 3-week administration of prednisolone (O group) and osteoporotic rats receiving pamidronate (P group), the pressure in the carotid artery was similar to the results obtained for the controls (data not shown). In the osteoporotic rats, blood pressure in the marrow cavity was statistically significantly lower ($p < 0.01$) by 45.33% when compared to the control (Fig. 1). In the rats that were administered pamidronate and prednisolone concurrently, blood pressure in the marrow cavity was statistically significantly lower ($p < 0.05$), by 20%, when compared to the controls, and was statistically significantly higher ($p < 0.05$), by 46.34%, when compared to the osteoporotic group (Fig. 1).

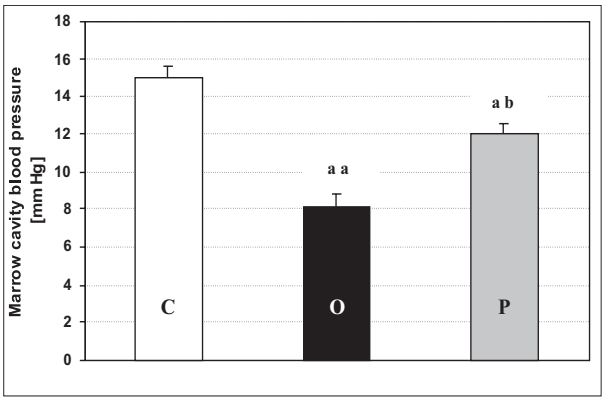

Fig. 1. Blood pressure in the marrow cavity in control (C), osteoporotic (O) and osteoporotic rats, which were given pamidronate (P). [a] $p < 0.05$, [aa] $p < 0.01$ – differences statistically significant compared to the results obtained for control rats; [b] $p < 0.05$ – differences statistically significant compared to the results obtained for osteoporotic rats

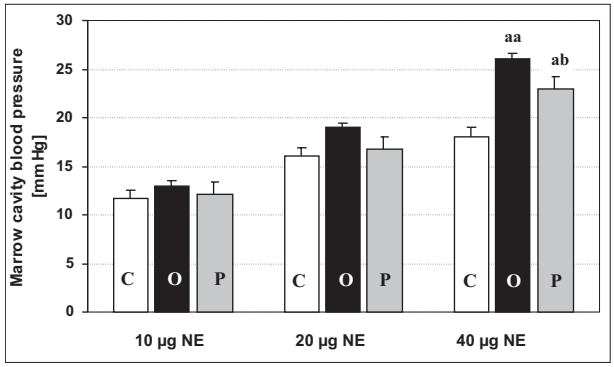

Fig. 2. The difference after the administration of norepinephrine (NE) in relation to the initial blood pressure in the marrow cavity in control (C), osteoporotic (O) and osteoporotic rats, which were given pamidronate (P). [a] $p < 0.05$, [aa] $p < 0.01$ – differences statistically significant compared to the results obtained for control rats; [b] $p < 0.05$ – differences statistically significant compared to the results obtained for osteoporotic rats

The effect of norepinephrine (NE) on arterial blood pressure and bone marrow cavity blood pressure

The administration of NE to the control rats at increasing doses of 10, 20 or 40 µg/kg, *iv* resulted in a rise in blood pressure in the carotid artery by 35.02%, 65.11% and 82.98% of the initial value, respectively, when measured immediately following NE administration. Subsequently, the carotid artery blood pressure fell gradually and at 10 min following the NE administration, it reached the initial value. Parallelly to the increase in arterial blood pressure, the blood pressure in the femoral marrow cavity was also rising. Compared to the initial value, the increase was by 78.15% (at the dose of 10 µg/kg, *iv*), 105.96% (at the dose of 20 µg/kg, *iv*) and 119.20% (at the dose 40 g/kg, *iv*; $p < 0.01$) (Fig. 2).

After NE administration at increasing doses of 10, 20 or 40 µg/kg, *iv*, the osteoporotic rats displayed an increase in carotid artery blood pressure similar to the controls. In relation to the initial value, the rise in marrow cavity blood pressure following NE administration was higher than in the controls, and immediately after the administration it equaled 158.53% (10 µg/kg), 213.70% (20 µg/kg) and 317.07% (40 µg/kg) (Fig. 2).

Administration of NE to osteoporotic rats which received pamidronate, resulted in an increased marrow cavity blood pressure, which reached 101.67% (10 µg/kg), 140.00% (20 µg/kg) and 191.67% (40 µg/kg) when compared to the initial value. This group of animals displayed a statistically significantly higher ($p < 0.05$) increase in marrow cavity blood pressure after NE administration at 40 µg/kg than the controls, and statistically significantly lower ($p < 0.05$) than the group of osteoporotic rats (Fig. 2).

The effect of epinephrine (E) on arterial blood pressure and marrow cavity blood pressure

The administration of E at 10 µg/kg, *iv* to the control rats resulted in an increase in blood pressure in the carotid artery by 39.89%. E administration at 20 or 40 µg/kg, *iv* resulted in an initial increase in carotid artery blood pressure by 65.00% and 91.02%, respectively, followed by a decrease by 5.11% (20 µg/kg) and 10.24% (40 µg/kg). Simultaneously to the increase in carotid artery blood pressure, an increase in marrow cavity pressure by 27.81% (10 µg/kg), 59.60% (20 µg/kg) and 72.84% (40 µg/kg) was recorded (Fig. 3). Subsequently, a drop of marrow cavity pressure was observed despite still elevated carotid artery pressure. The maximum drop in marrow cavity pressure was 8.16% (10 µg/kg), 12.78% (20 µg/kg) and 35.12% (40 µg/kg).

The osteoporotic group having been administered E at the increasing doses demonstrated changes in the carotid artery similar to those observed in the controls. E also had a biphasic effect on marrow cavity blood pressure in the osteoporotic rats, but the in-

creases after the administration were statistically significantly higher than in the control rats, and immediately after the administration they equaled 112.20% (10 μg/kg), 190.24% (20 μg/kg) and 229.26% (40 μg/kg) when compared to the initial value (Fig. 3).

When pamidronate was injected to the osteoporotic group, the administration of E resulted in similar blood pressure changes in the carotid artery as those observed in the controls. Following E administration at 10 μg/kg and 20 μg/kg, the blood pressure in the marrow cavity increased by 38.33% and 83.33%, respectively, compared to the initial value. The increases were similar to those observed in the controls, and they were statistically significantly lower ($p < 0.05$) when compared to the osteoporotic rats. The administration of E at 40 μg/kg resulted in a marrow cavity blood pressure increase by 136.66% when compared to the initial value (the increase was higher than in the controls, but lower than in the osteoporotic group) (Fig. 3).

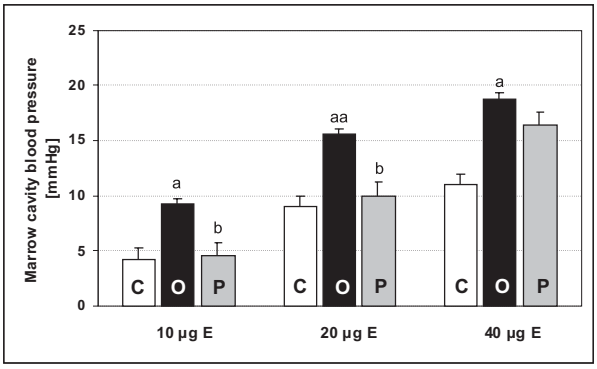

Fig. 3. The difference after the administration of epinephrine (E) in relation to the initial blood pressure in the marrow cavity in control (C), osteoporotic (O) and osteoporotic rats, which were given pamidronate (P). [a] $p < 0.05$, [aa] $p < 0.01$ – differences statistically significant compared to the results obtained for control rats; [b] $p < 0.05$ – differences statistically significant compared to the results obtained for osteoporotic rats

The effect of isoprenaline (IP) on arterial blood pressure and marrow cavity blood pressure

IP given to the controls at 3 or 6 μg/kg, *iv* caused a decrease in carotid artery blood pressure by 10.04% and 18.66%, respectively. This decrease was parallelled by a drop in marrow cavity blood pressure by 16.98% and 50.22%, respectively (data not shown). The osteoporotic rats displayed a drop in blood pressure in the carotid artery and marrow cavity similar to the controls after IP administration. In osteoporotic rats, which were administered pamidronate, IP elicited similar effect on carotid artery and marrow cavity blood pressure as was observed in the controls.

Discussion

Earlier studies demonstrated that the administration of prednisolone to rats, at a dose of 5 mg/kg, *im* daily, for three weeks, resulted in osteoporosis. Prednisolone induced the following changes: a decreased width of bone trabeculae in the bone epiphysis, reduced area of transverse cross-section of the cortical part of the bone diaphysis, increased area of the transverse cross-section of the bone marrow cavity, as well as a reduced mass of long bones, and reduced content of mineral substances and calcium in bones. In addition, we observed a reduced transverse growth and reduced width of the osteoid in the bone in prednisolone-treated rats. Structural and morphological changes in animals were accompanied by an increased brittleness of bones [21]. In rats with prednisolone-induced osteoporosis, the reduced blood pressure in the marrow cavity and the intensified effect of NE and E on blood pressure in the marrow cavity were observed, without any effect on systemic blood pressure [10].

In our studies, no significant changes in mean blood pressure measured in the carotid artery were observed in rats with prednisolone-induced osteoporosis (prednisolone 5 mg/kg, *im* daily, for 3 weeks) when compared to the controls. Moreover, the effects of catecholamines (NE, E and IP) on blood pressure in the carotid artery were similar to those observed in the controls. This was acknowledged in earlier tests on animals administered prednisolone at 5 mg/kg, *im* for three weeks [10]. Similarly, no systolic and diastolic blood pressure changes were observed in healthy postmenopausal women after prolonged prednisolone administration. However, a long-term administration of prednisone reduced bone repair or renewal in such patients, which consequently led to osteoporosis [22].

The effect of glucocorticosteroids on cardiovascular system still remains controversial. The majority of experiments proved that glucocorticosteroids admin-

istered to healthy human subjects caused an increase in blood pressure [7, 19, 24]. Hypertensive effect of glucorticosteroids was demonstrated by some experiments performed on animals [16], whereas in patients, the treatment of asthma and rheumatoid arthritis with prednisolone did not cause hypertension [8]. Some scientific reports indicate a potential blood pressure decrease after administering glucocorticosteroids [5, 14, 15]. Glucocorticosteroids may modulate the function of cardiovascular system by affecting adrenergic receptors (α i β) [12, 18, 20] or catecholamine reactivity [23]. The autonomic nervous system and catecholamines modulate the blood circulation in bones. Adrenoceptors (α and β) are present in blood vessels both in the marrow cavity [1] and the cortex of bone [6].

As we previously reported [10], the administration of prednisolone to rats, revealed a decreased blood pressure in the femoral marrow cavity despite the lack of any effect on systemic blood pressure. The decreased blood pressure in the bone may result from the increase in the marrow cavity as a result of prednisolone-induced osteoporotic changes. Other causes may also underlie the decrease in the marrow cavity blood pressure in prednisolone-administered rats. Prednisolone affects microcirculation [17] and neovascularization [2] in various tissues, and it may also change the blood vessel geometry [13].

In our studies, the administration of NE (α-stimulator) to the controls resulted in a parallel increase in carotid artery blood pressure and marrow cavity blood pressure. Osteoporotic rats, which were administered NE, displayed a more significant marrow cavity blood pressure increase than the controls. E had a biphasic effect on the carotid artery and long bone marrow cavity blood pressure in the controls, *viz*. the initial increase (most probably resulting from the activation of α-receptors) was followed by a decrease in blood pressure (probably following the activation of β-receptors). In rats showing osteoporotic changes in the femur, the initial hypertensive phase was intensified, while the blood pressure decrease was similar to the controls. IP (β-stimulator) affected the marrow cavity blood pressure in a manner similar in the osteoporotic rats and the controls. The results obtained in this study with respect to catecholamine effect on marrow cavity blood pressure in osteoporotic bones are similar to those reported earlier [10]. The intensified effect of NE and E on α-receptors may be related to prednisolone-induced osteoporotic changes in the bone tissue.

In our study, the animals which were administered pamidronate at 3 mg/kg, *sc*, for the period of three weeks, concurrently with prednisolone, displayed a higher marrow cavity blood pressure when compared to rats, in which experimental osteoporosis was induced by prednisolone. Pamidronate administration in osteoporotic rats resulted in smaller increases in marrow cavity blood pressure following NE and E administration. This normalizing effect of pamidronate on marrow cavity blood pressure (also after catecholamine administration) in rats with prednisolone-induced osteoporosis might have resulted from pamidronate-induced inhibition of the destructive effect of prednisolone on the bone. As demonstrated in earlier studies [21], rats administered pamidronate and prednisolone in combination displayed a decreased area of the marrow cavity transverse cross-section, increased periosteal and endosteal growth, and increased width of osteoid, in comparison with the group of rats with prednisolone-induced osteoporosis. Rats that were administered pamidronate and prednisolone together were noted to have an increased mass of long bones as well as increased content of mineral substances in bones in comparison with the animals in which experimental osteoporosis was induced by prednisolone. Additionally, the width of the epiphysial cartilage and the thickness of bone trabeculae were greater than those in the animals which were administered prednisolone alone. Pamidronate increased the force necessary to break the bones and their deformability in animals with prednisolone-induced osteoporosis.

It should also be noted that young rats, when administered pamidronate (3 mg/kg, *sc*, for the period of 3 or 6 weeks), displayed osteopetrosis. The experiments demonstrated that rats with pamidronate-induced osteopetrosis displayed increased blood pressure in the marrow cavity. In addition, a disorder in the effect of catecholamines on blood pressure in the marrow cavity of osteopetrotic bone was observed [11]. Pamidronate is accumulated in the aortas of rabbits [25], which indicates that it may also cumulate in other types of blood vessels, e.g. bone vessels, and thus may affect their function.

The results obtained in this study with respect to the effects of pamidronate on the action of catecholamines on blood pressure in rats with prednisolone-induced osteoporosis constitute another proof for in-

terrelationship between the bone nervous system, blood circulation and remodeling processes in bones.

References:

1. Artico M, Bosco S, Cavallotti C, Agostinelli E, Giuliani-Piccari G, Sciorio S, Cocco L et al.: Noradrenergic and cholinergic innervation of the bone marrow. Int J Mol Med, 2002, 10, 77–80.
2. Conrad TJ, Chandler DB, Corless JM, Klintworth GK: *In vivo* measurement of corneal angiogenesis with video data acquisition and computerized image analysis. Lab Invest, 1994, 70, 426–434.
3. Ficat RP, Arlet J: Ischemia and Necrosis of Bone. Williams & Wilkins, Baltimore 1980, 196.
4. Frediani U, Becherini L, Lasagni L, Tanini A, Brandi ML: Catecholamines modulate growth and differentiation of human preosteoclastic cells. Osteoporos Int, 1996, 6, 14–21.
5. Hall JE, Morse CL, Smith MJ Jr, Young DB, Guyton AC: Control of arterial pressure and renal function during glucocorticoid excess in dogs. Hypertension, 1980, 2, 139–148.
6. Hohmann EL, Elde RP, Rysavy JA, Einzig S, Gebhard RL: Innervation of periosteum and bone by sympathetic vasoactive intestinal peptide-containing nerve fibers. Science, 1986, 232, 868–871.
7. Ivarsen P, Jensen LW, Pedersen EB: Circadian blood pressure rhythm and atrial natriuretic peptide in prednisolone-induced blood pressure elevation. Scand J Clin Lab Invest, 1995, 55, 655–662.
8. Jackson SH, Beevers DG, Myers K: Does long-term low-dose corticosteroid therapy cause hypertension? Clin Sci (Lond), 1981, 61, 381S–383S.
9. Jones KB, Mollano AV, Morcuende JA, Cooper RR, Saltzman CL: Bone and brain: a review of neural, hormonal, and musculoskeletal connections. Iowa Orthop J, 2004, 24, 123–132.
10. Kaczmarczyk-Sedlak I, Cegieła U, Nowińska B, Folwarczna J: The effects of catecholamines on blood pressure in the long marrow cavity in rats with prednisolone-induced osteoporosis. Pharmacol Rep, 2006, 58, 540–550.
11. Kaczmarczyk-Sedlak I, Janiec W: The effects of catecholamines on the intramedullary pressure in the femur in rats with bisphosphonate-induced osteopetrosis. Pharmacol Rep, 2005, 57, 623–634.
12. Nishimura H, Yoshikawa T, Kobayashi N, Anzai T, Nagami K, Handa S, Ogawa S: Effects of methylprednisolone on hemodynamics and beta-adrenergic receptor signaling in rabbits with acute left ventricular failure. Heart Vessels, 1997, 12, 84–91.
13. Pethig K, Heublein B, Wahlers T, Dannenberg O, Oppelt P, Haverich A: Mycophenolate mofetil for secondary prevention of cardiac allograft vasculopathy: infuence on inflammation and progression of intimal hyperplasia. J Heart Lung Transplant, 2004, 23, 61–66.
14. Pongratz G, Zietz B, Gluck T, Scholmerich J, Straub RH: Corticotropin-releasing factor modulates cardiovascular and pupillary autonomic reflexes in man: is there a link to inflammation-induced autonomic nervous hyperreflexia? Ann N Y Acad Sci, 2002, 966, 373–383.
15. Prasad K, Szabo L: Cardiovascular effects of orciprenaline (Alupent) inhalation and prednisolone. Adv Myocardiol, 1980, 1, 437–452.
16. Ribeiro AB, Franco RJ, Kohlmann O Jr, Marson O, Ramos OL: Etiopathogenesis of excess methylprednisolone arterial hypertension in the rat. Clin Exp Hypertens, 1981, 3, 1219–1237.
17. Sack FU, Reidenbach B, Dollner R, Schledt A, Gebhard MM, Hagl S: Influence of steroids on microvascular perfusion injury of the bowel induced by extracorporeal circulation. Ann Thorac Surg, 2001, 72, 1321–1326.
18. Saito T, Takanashi M, Gallagher E, Fuse A, Suzaki S, Inagaki O, Yamada K, Ogawa R: Corticosteroid effect on early beta-adrenergic down-regulation during circulatory shock: hemodynamic study and beta-adrenergic receptor assay. Intensive Care Med, 1995, 21, 204–210.
19. Sato A, Funder JW, Okubo M, Kubota E, Saruta T: Glucocorticoid-induced hypertension in the elderly. Relation to serum calcium and family history of essential hypertension. Am J Hypertens, 1995, 8, 823–828.
20. Scherrer D, Lach E, Landry Y, Gies JP: Glucocorticoid modulation of muscarinic and beta-adrenergic receptors in guinea pig lung. Fundam Clin Pharmacol, 1997, 11, 111–116.
21. Tkocz-Kwiatkowska J, Kaczmarczyk-Sedlak I, Folwarczna J: Effects of pamidronate on the development of changes in bone mechanical properties and bone structure caused by the administration of prednisolone in rats. Pol J Pharmacol, 1998, 50, 253–258.
22. Ton FN, Gunawardene SC, Lee H, Neer RM: Effects of low-dose prednisone on bone metabolism. J Bone Miner Res, 2005, 20, 464–470.
23. Whitworth JA: Adrenocorticotrophin and steroid-induced hypertension in humans. Kidney Int Suppl, 1992, 37, S34–37.
24. Whitworth JA, Gordon D, Andrews J, Scoggins BA: The hypertensive effect of synthetic glucocorticoids in man: role of sodium and volume. J Hypertens, 1989, 7, 537–549.
25. Ylitalo R, Monkkonen J, Urtti A, Ylitalo P: Accumulation of bisphosphonates in the aorta and some other tissues of healthy and atherosclerotic rabbits. J Lab Clin Med, 1996, 127, 200–206.

Received:
November 3, 2005, in revised form: November 13, 2006.