

Influences of Treatment with Amlodipine and Valsartan on Bone Turnover Markers and OPG/RANKL/RANK System in Newly Diagnosed Hypertensive Adults; Which is more Beneficial?

Yeni Tanı Almış Yetişkin Hipertansif Hastalarda, Amlodipin ve Valsartan Tedavisinin Kemik Yapım-Yıkım Belirteçleri ve OPG/RANKL/RANK Sistemi Üzerine Etkisi; Hangisi daha Faydalı?

ABSTRACT

We aimed to investigate the effects of treatment with amlodipine and valsartan, on markers of bone remodeling in newly diagnosed hypertensive adults. Forty-three subjects with newly diagnosed were included in the study. Patients were also randomly divided into two groups, and each group received monotherapy with amlodipine or valsartan. Blood levels of bone turnover markers and osteoprotegerin (OPG) / receptor activator of nuclear factor- κ B ligand (RANKL) / RANK system were measured. Amlodipine reduced sRANKL levels and sRANKL/OPG ratio more than valsartan, and this decrease was statistically significant ($p<0.001$, $p=0.002$, respectively). Although blood OPG concentration did not change after treatment in both groups, sRANKL/OPG ratio decreased significantly ($p<0.001$). Amlodipine also caused some reduction in CTx blood levels compared to valsartan. So we can suggest that amlodipine may be a better option than valsartan in patients with osteoporosis or terms of prevention of bone loss in hypertensive adults.

KEY WORDS: Hypertension, Amlodipine, Valsartan, Osteoporosis, Bone remodeling

ÖZ

Bu çalışmada amacımız; yeni tanı almış, yetişkin hipertansif hastalarda Amlodipin ve Valsartan tedavisinin kemik yeniden şekillendirilmesi (remodeling) üzerine olan etkisini araştırmaktır. Çalışmaya daha önce tedavi almamış 43 yeni tanı hasta alındı. Hastalar rastgele iki gruba ayrıldı. Her bir gruba monoterapi şeklinde Amlodipin ve Valsartan verildi. Kemik yapım-yıkım belirteçleri yanında osteoprotegerin (OPG) / nükleer faktör kappa-B reseptör aktivatörü ve ligand(sRANKL) / RANK sistemi çalışıldı. Amlodipin kolunda sRANKL düzeyinin ve sRANKL/OPG oranınının valsartandan daha fazla azaldığı ve bunun istatistiki açıdan anlamlı olduğu görüldü ($p<0.001$, $p=0.002$, sırasıyla). Tedavi sonrası her iki grupta da OPG konsantrasyonu değişmemekle birlikte sRANKL/OPG oranının anlamlı şekilde azaldığı görüldü ($p<0.001$). Yine Amlodipinin C-telopeptide of type I collagen(CTx) düzeyini Valsartana göre daha fazla azalttığı görüldü. Bu veriler ışığında, osteoprotik hastalarda veya kemik kaybı yönünden risk taşıyan hastalarda amlodipin tedavisinin valsartan tedavisine göre daha iyi bir seçim olabileceğini söyleyebiliriz.

ANAHTAR SÖZCÜKLER: Hipertansiyon, Amlodipin, Valsartan, Osteoporoz, Kemik yeniden şekillendirme

INTRODUCTION

Hypertension and osteoporosis are highly prevalent and represent ongoing major public health problems of aging populations worldwide (1). It has been suggested that hypertension is linked to disturbances of

calcium metabolism, leading to an increase in calcium loss, subsequent activation of the parathyroid gland and increased calcium release from the bone, eventually increasing the risk of bone mineral density reduction (2-5).

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Calcium-channel blockers (CCB) are effective antihypertensive agents acting by blocking the initial calcium influx into vascular smooth muscle cells. Increasing evidence showed that CCB use is linked to decreased risk of falls and fractures (6). CCBs were found to stimulate osteoblast differentiation or inhibit osteoclast functions (7,8). Recent experimental studies also suggested that amlodipine exerts favorable effects on bone by decreasing bone turnover (9,10).

Angiotensin II type 1 receptor blockers (ARBs) reduce cardiovascular morbidity and mortality mainly through their inhibitory effect on renin-angiotensin-aldosterone system (RAS) (11). Beyond RAS inhibition, identification of accompanying mechanisms has motivated studies evaluating the relationship between blockade of this system and modification of bone density (12-14). Valsartan, a well known and widely used ARB, displayed no effect on bone transforming growth factor-beta1 which is known as osteoporosis tendency factor (15). However, the effects of ARBs on bone structure are controversial (12,16). Because, again in animals, angiotensin II was demonstrated to accelerate osteoporosis by activating osteoclasts, while this effect was abolished by an ARB administration (13). Moreover, increase in bone mass was demonstrated after treatment with ARBs, which was linked both to enhanced osteoblastic and suppressed osteoclastic activity (14). Nevertheless, angiotensin II was also shown to cause proliferation of osteoblasts (16), suggesting that blockage of this enzyme may result in impaired bone formation in vivo.

The process of bone remodeling requires a balance between formation and resorption, where osteoprotegerin (OPG)/RANKL (receptor activator of nuclear factor- κ B ligand)/RANK signaling pathway stands as the natural key regulator between the activity of osteoblasts and osteoclasts (17). RANKL, a protein that binds to its receptor RANK expressed on osteoclasts, induces osteoclast differentiation and activation, and improves their survival (17). OPG is a decoy receptor that binds to RANKL and prevents it from binding to its receptor RANK, thereby inhibiting osteoclastogenesis (17).

Favorable effects of antihypertensive interventions on bone mineral density and fracture risk were previously demonstrated by several authors (4,5). The primary aim of the present study was to search the influences of effective blood pressure lowering on blood levels of bone remodeling markers in subjects with newly diagnosed hypertension. As a secondary target, individual influences of two classes of medications were compared for their effects on the primary tested variables.

MATERIAL and METHODS

Forty-three subjects with newly diagnosed and never treated hypertension who attended the outpatient clinics of Internal Medicine and Geriatrics at Gulhane Medical Faculty Training Hospital were enrolled. They were selected from mild to moderate essential hypertensive patients who were free of any

other chronic diseases or medications. The study was approved by the local ethics committee (Ref. no=1491-49-11/1539-1547), and all patients gave informed, written consent.

Hypertension was defined as systolic blood pressure (SBP) \geq 140 mmHg or diastolic blood pressure (DBP) \geq 90 mmHg according to the JNC 7 guideline (18). Arterial blood pressures were measured in the right arm by mercury sphygmomanometer (Korotkoff I and V) three times in a resting condition in the morning, and mean values were calculated for diastolic and systolic pressures. Exclusion criteria were presence of secondary hypertension, malign hypertension, history of hypertensive encephalopathy or cerebrovascular accident, previous or current heart failure, myocardial infarction, angina pectoris, valvulopathy or clinically relevant arrhythmias, liver or kidney disease, clinically relevant hypo- or hyperthyroidism, primary and secondary metabolic bone disease, inability to ambulate independently, current use of herbal remedies and over-the-counter drugs, history of any fracture or >7 cm height shortening.

Weight and height of the participants were measured using a standard scale in light clothing. BMI was calculated as kg/m². Specifically designed questions were asked for each one of the following risk factors: current alcohol intake (no/yes), current smoking (no/yes), menopause age $<$ 40 years (no/yes), family history of hip fracture (no/yes), and height shortening (no/yes).

Patients were randomly assigned into two groups and received monotherapy with amlodipine 5 mg or valsartan 80 mg, with a doubled dose after two weeks in uncontrolled hypertensive subjects. All blood pressure measurements were performed in the morning before dosing. None of the prescription medications were changed during the study, and no medication was added.

Blood samples were drained from the subjects between 08.00 and 09.00 am after 12-h fast, and stored at -80°C until they were assayed. All samples collected at baseline and at the end of the study were run in the same assay.

Glucose, total cholesterol, HDL-cholesterol, triglyceride, creatinine, total calcium, magnesium, and phosphorus levels were measured by using Olympus AU2700 Chemistry Analyze (Olympus CO Ltd., Tokyo, Japan). LDL-cholesterol was calculated by Friedewald's formula. Fasting plasma 25-hydroxyvitamin D [25(OH)D] concentrations was measured by HPLC system (ClinRep, RECIPE Chemicals and Instruments GmbH, Munich/Germany). Intact serum parathormone (PTH) was measured using E-170 immunoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Serum osteocalcin (OC), C-telopeptide of type I collagen (CTx), soluble RANKL (sRANKL) and plasma osteoprotegerin (OPG) were measured by ELISA using quantitative kits (Human OC ELISA kit, hOST-EASIA, KAP1381, DIAsource ImmunoAssays S.A., Louvain-la-Neuve, Belgium; Human CTx ELISA kit, CSB-E10363h, Cusabio Biotech Co.,Ltd. Wuhan,

Hubei Province, China; Human sRANKL (Total) ELISA kit, RD 193004200R BioVendor Research and Diagnostics Products, Brno, Czech Republic Research; Human OPG Instant ELISA kit, BMS2021INST, eBioscience San Diego, CA, USA). Intra-assay coefficient of variation (CV) ranged from 3.1% to 4.7% for OC, from 7.25% to 11.51% for sRANKL, from 5.0% to 9.0% for OPG while inter-assay CV ranged from 3.5% to 5.6% for OC, from 11.21% to 12.77% for sRANKL, from 5.3% to 8.9% for OPG. The minimum detectable concentration for OC, CTx, sRANKL, and OPG were 0.08 ng/ml, 12.5 ng/ml, 0.4 pmol/l and 2.5 pg/ml, respectively. Measurements were carried out using ELISA plate reader Bio-Tek Synergy HT (Biotek Instruments Inc., Winooski, VT, USA).

The entire biochemical and hormonal measurements were repeated in both groups before and after a 12-week treatment period.

Statistical analysis

All data were recorded on a computer database and analyzed using SPSS 15.0 package program (SPSS Inc., Chicago, IL, USA). Results are expressed as mean± S.D. Distribution of normality was examined using Shapiro–Wilk test. Intra-group changes at two time points were analyzed by paired samples t-test or 2-related samples test. Inter-group differences were analyzed by Chi-square test, Mann–Whitney U test or Student’s t-test. Correlations between variables were evaluated by using Pearson’s or Spearman rho correlation analysis. Exact p values were presented, and p < 0.05 was considered significant.

RESULTS

Forty patients [age range: 40 to 80 years, mean age: 54.88 ± 10.56 years, body mass index (BMI) 30.86 ± 4.09 kg/m²] completed the study. Three patients (6.9%) were withdrawn from the study, 2 in the amlodipine group and 1 in the valsartan group.

The baseline demographic characteristics of the whole participants are given in Table I. Females comprised 72.5% of the study population. There were no significant differences in demographic and clinical characteristics at baseline between the

Table I: Baseline demographic characteristics of total study population (n=40).

| | |
|--|---------------|
| Age | 54.88 ± 10.56 |
| Gender (M/F) | 11/29 |
| Body mass index (kg/m ²) | 30.86 ± 4.09 |
| Current alcohol intake, no.(%) | 1 (2.5) |
| Current smoking, no.(%) | 9 (22.5) |
| Premature menopause | 2/29 |
| Family history of hip fracture, no.(%) | 2 (5.0) |
| Height shortening, no.(%) | 10 (25.0) |

subgroups allocated to amlodipine (mean age: 53.25±2.52 years, SBP: 155.80±2.14 mmHg and DBP: 92.95±1.93 mmHg, BMI: 30.60±7.90 kg/m²) or valsartan (mean age: 56.50±9.82 years, SBP: 156.80±2.79 mmHg and DBP: 93.75±1.79 mmHg, BMI: 31.12±1.04 kg/m²).

After the 12-week treatment period, SBP and DBP values reduced significantly (17.96% and 17.22%, respectively). Amlodipine and valsartan treatments caused similar systolic blood pressure declines (17.97% vs. 17.95%, NS). Corresponding reductions in DBP were 16.99% and 17.44%, respectively.

Biochemical parameters

Biochemical parameters before and after treatment were shown in Table II. After antihypertensive treatment, notable significant decreases in glucose, total and LDL cholesterol levels and increases in creatinine and 25(OH)D levels were detected in the whole study group (p=0.019, p=0.007, p=0.025, p=0.017, and p=0.007, respectively). There were no significant changes in HDL cholesterol, triglyceride, PTH, calcium, magnesium, and phosphorus levels.

Bone remodeling markers

Changes in bone remodeling markers after antihypertensive treatment are given in Table III. In the total group, antihypertensive treatment significantly decreased blood sRANKL level and sRANKL/OPG ratio (p=0.004 and p<0.001). There was no change in blood levels of OPG, OC, and CTx post-treatment (p=0.056, p=0.142, and p=0.493, respectively).

Regarding the individual effects of amlodipine and valsartan, amlodipine reduced sRANKL levels and sRANKL/OPG ratio more than valsartan, and this decrease was statistically significant (p<0.001, p=0.002, respectively). But, blood levels of OC, OPG and sRANKL were similar at baseline. The effects of antihypertensive treatment on circulating sRANKL level and sRANKL/OPG ratio were evident only in the amlodipine treated subjects. (Table III) Treatment of individuals either with amlodipine or valsartan did not result in any significant change in blood OC or CTx levels (Table III). Although amlodipine caused more reduction in blood CTx levels compared to valsartan, the difference between the two groups was not statistically significant (9.5% versus 1.0%, p=0.421). Blood OPG levels were similar in the two groups both before and after treatment (Table III).

In the whole study group, the decrease in blood sRANKL levels correlated negatively with circulating 25(OH)D concentration (r= -0.420, p=0.023). However, there was no significant correlation between 25(OH)D concentration and other blood parameters related to bone metabolism, including calcium (r=-0.157, p=0.560), magnesium (r=-0.108, p=0.739), phosphorus (r=-0.382, p=0.276), PTH (r=0.424, p=0.063), OC (r=-0.230, p=0.229), CTx (r=-0.067, p=0.732), OPG (r=-0.118, p=0.544), and sRANKL/OPG ratio (r=0.042, p=0.830).

Table II: Effects of antihypertensive treatment on biochemical parameters (n=40).

| | Before treatment | After treatment | P |
|------------------------------|------------------|-----------------|--------------|
| Glucose (mg/dL) | 99.49 ± 11.94 | 96.05 ± 8.70 | 0.019 |
| Creatinine (mg/dL) | 0.88 ± 0.13 | 0.91 ± 0.13 | 0.017 |
| Total cholesterol (mg/dL) | 226.90 ± 34.40 | 213.56 ± 34.89 | 0.007 |
| LDL cholesterol (mg/dL) | 146.87 ± 31.76 | 134.89 ± 30.24 | 0.025 |
| HDL cholesterol (mg/dL) | 51.19 ± 10.80 | 49.53 ± 9.67 | 0.072 |
| Triglyceride (mg/dL) | 141.28 ± 46.41 | 143.82 ± 57.11 | 0.708 |
| 25-hydroxy vitamin D (ng/mL) | 14.40 ± 5.16 | 19.68 ± 9.90 | 0.007 |
| Parathormone (pg/mL) | 50.11 ± 15.33 | 49.33 ± 12.53 | 0.762 |
| Calcium (mg/dL) | 9.69 ± 0.41 | 9.62 ± 0.56 | 0.435 |
| Magnesium (mg/dL) | 2.13 ± 0.17 | 2.09 ± 0.14 | 0.342 |
| Phosphorus (mg/dL) | 3.63 ± 0.37 | 3.42 ± 0.36 | 0.168 |

Results are mean ± SD.

A p value of less than 0.05 was accepted as statistically significant.

Table III: Circulating bone remodeling marker levels before and after treatment.

| Parameters | Total (n=40) | | Amlodipine (n=20) | | Valsartan (n=20) | | p1 | p2 | p3 |
|-------------------------|------------------|-----------------|-------------------|-----------------|------------------|-----------------|------------------|------------------|-------|
| | Before treatment | After treatment | Before treatment | After treatment | Before treatment | After treatment | | | |
| Osteocalcin (ng/mL) | 8.24±3.67 | 8.90±3.58 | 8.01±3.03 | 8.46±3.88 | 8.47±4.28 | 9.35±3.28 | 0.142 | 0.505 | 0.160 |
| CTX (ng/mL) | 18.16-617.34 | 16.73-598.75 | 18.16-617.34 | 16.73-560.93 | 23.44-544.20 | 25.56-598.75 | 0.493 | 0.263 | 0.881 |
| Osteoprotegerin (pg/mL) | 11.23-990.23 | 42.17-812.22 | 11.23-757.01 | 43.36-677.38 | 33.90-990.23 | 42.17-812.22 | 0.056 | 0.191 | 0.167 |
| sRANKL (pmol/L) | 60.41-2290.09 | 58.55-1113.37 | 97.90-2290.09 | 58.55-1113.37 | 60.41-331.16 | 103.28-320.16 | 0.004 | <0.001 | 0.502 |
| sRANKL/OPG ratio | 0.08-21.79 | 0.14-13.00 | 0.36-21.79 | 0.24-13.00 | 0.08-5.84 | 0.14-7.59 | <0.001 | 0.002 | 0.145 |

Results are mean ± SD or min.-max.

p1 Total subjects before vs. after treatment (paired simple t-test)

p2 Amlodipine group before vs. after treatment (paired simple t-test)

p3 Valsartan group before vs. after treatment (paired simple t-test)

Intergroup differences (amlodipine vs. valsartan) before treatment: p=0.043 for sRANKL/OPG ratio, others NS (student t test and Mann-Whitney U test as appropriate)

A p value of less than 0.05 was accepted as statistically significant.

CTx, C-telopeptide of type I collagen; sRANKL, soluble receptor activator of nuclear factor-κB ligand; OPG, osteoprotegerin.

DISCUSSION

With the aging boom, increased prevalence of the coupling of hypertension and osteoporosis is inevitable. Both of these conditions might have multiple physiological or pathological consequences, some of which are lethal. Currently, various

medications are available for these two major diseases. However, it is well known that osteoporosis medications have limited effects on bone resorption, formation and fracture. Therefore, a hypertensive drug with possible favorable effects on bone metabolism may provide further advantages.

The results of the present study showed that a 12-week antihypertensive treatment significantly decreased blood sRANKL levels, which may be indicative of reduced osteoclastogenesis. Interestingly, this was evident only in patients treated with amlodipine compared to those treated with valsartan. The results also showed that antihypertensive therapy did not affect circulating bone turnover markers, but there were tendencies as bone formation markers to increase and bone resorption markers to decrease. To the best of our knowledge, this study is the first to show, albeit small, the beneficial effect of amlodipine on bone OPG/RANKL/RANK system in hypertensive adults.

Experimental studies demonstrated that CCBs exert favorable effects on bone metabolism through several mechanisms. While verapamil displayed inhibitory actions on PTH induced bone resorption (19), benidipine blocked the L-type voltage dependent calcium channels on osteoblast more potently than nifedipine and amlodipine, and increased bone mineralization (7). Previously, amlodipine treatment in rats was found to improve orchidectomy-induced decrease in the whole bone mineral density, bone turnover markers and insulin-like growth factor I which potentiates bone formation (10). Accordingly, amlodipine and lacidipine prevented ovariectomy induced bone loss in osteopenic rat femur (9). Moreover, amlodipine alleviated the reduction in femur bone density in stroke-prone spontaneously hypertensive rats (20). However, in another study, amlodipine showed no action on bone metabolism in ovariectomized hypertensive rats (21). Despite the convincing evidence given above, there are hardly enough data about the effects of CCB use on bone physiology in humans.

The mechanisms of beneficial effects of CCBs have not been fully understood but they are mostly attributed to improvements in bone remodeling. The protective effects of CCBs might be based on the enhancement of osteoblastic activity and/or the suppression of osteoclastic activity (7,8). In the present study, after treatment with amlodipine, we observed a decrease in serum sRANKL level and sRANKL/OPG ratio, suggesting a potentially preventive effect on bone loss, although these changes were accompanied by no improvement in blood OPG level. Nevertheless, it may be speculated that the number of active and mature osteoclast decreases and bone resorption is reduced after antihypertensive treatment. The findings of Nishiya et al. that benidipine stimulated osteoblast differentiation and increased in vitro bone mineralization (7) are in correlation with the results in the present work. Moreover, benidipine was reported to regulate osteoblast growth and stimulate functions of these cells and $1,25(\text{OH})_2\text{D}_3$ (22). Thus, we hypothesized that one of the mechanisms of amlodipine induced improvement in bone metabolism might be derived from its effect on osteoblast function.

In our study, we found a non-significant trend for increase in OC (5.6%) and a decrease in CTx (9.5%) after amlodipine treatment.

Blocking the L-type calcium channel of osteoblast by CCB might come into play during the later osteogenic differentiation of mesenchymal stem cells from bone marrow (23). Lifespan of an osteoblast is approximately 3 months and OC is a protein product of mature osteoblasts (24). In this context, a 12-week treatment period might not be enough for an increase in OC due to stimulation of the osteoblast differentiation by amlodipine. On the other hand, the decrease in bone resorption markers might also be due to the CCB type or insufficient concentration of amlodipine in terms of osteoclast production. Ritchie et al. reported that calcium channel antagonists could reduce bone resorption via decreasing osteoclast functions through blockage of membrane calcium channels (8). In addition, cilnidipine but not amlodipine has been demonstrated to exert inhibitory effects on osteoclast functions (21).

Contradictory results have been reported about the effect of ARBs on bone mineralization. Telmisartan treatment was shown to improve rosiglitazone-induced bone loss in ovariectomized spontaneously hypertensive rats (25). Another ARB, losartan, enhanced bone mass in adult mice and this effect was based on the stimulation of bone formation and the inhibition of bone resorption (14). On the contrary, no effect of ARBs has been found on bone loss in several other animal studies (7,12,26). Clinically, it was reported that use of ARBs was not associated with bone loss in elderly men (27). According to our findings, valsartan does not seem to have a significant effect on circulating bone remodeling markers.

The present study has several limitations. The relatively shorter follow-up period and small sample size may have obscured the affects of both treatments on bone metabolism. Thus, larger studies with longer follow-up periods are warranted to clearly investigate the effects of antihypertensive medications on bone. Another limitation may be the lack of bone mineral density measurements, although it was not a primary outcome parameter in this observational study. Finally, we focused specifically on adults and our findings may not be applied to other specific groups such as young adults or postmenopausal women.

CONCLUSIONS

Physicians treating hypertensive patients who are also at risk for developing osteoporosis, fracture, and fall will need to make clinical judgment based upon current literature about the benefits and risks of antihypertensive therapy on bone mass. To the best of our knowledge, no human study published so far searched for the association of antihypertensive drugs and bone remodeling in humans, especially in the adult population. Amlodipine resulted in some decrease in blood sRANKL levels, suggesting that it may be a better treatment option than valsartan to prevent bone loss in hypertensive adults. Future studies with larger groups and longer follow-up periods are needed to examine these associations.

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