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Effect of the TNFα-308 G/A Polymorphism on the Changes Produced by Atorvastatin in Bone Mineral Density in Patients with Acute Coronary Syndrome

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Key Words
TNFα · 308 G/A polymorphism · Bone mineral density · Atorvastatin

Abstract
Aims: To evaluate the effect of atorvastatin on bone mass and markers of bone remodeling in patients with acute coronary syndrome depending on the tumor necrosis factor-α (TNFα)-308 G/A polymorphism. ***Methods:*** Sixty-two patients with acute coronary syndrome (35 males and 27 females), average age 60 ± 10 years, were included. Patients were given low (10–20 mg) and high doses (40–80 mg) atorvastatin according to their baseline levels of cholesterol and triglycerides and their index of vascular risk. Patients were studied during hospital admission (baseline) and at 12 months of follow-up. Cholesterol, triglycerides, total calcium, phosphorus, magnesium, osteocalcin and urinary deoxypyridinoline were determined in all patients at baseline and at 12 months of follow-up. Densitometric studies were conducted in the lumbar spine (L_2–L_4), femoral neck and trochanter using an X-ray densitometer. The TNFα-308 G/A polymorphism was determined by the polymerase chain reaction. ***Results:*** Forty-five patients were homozygous for G/G (72.5%) and 17 were heterozygous for G/A (27.5%). The prevalence of osteoporosis (T score ≤2.5 in the lumbar spine and/or hip) was 33% for the G/G genotype and 35% for the G/A genotype, with no statistically significant differences between groups. There was a statistically significant increase in bone mineral density (BMD) in the lumbar spine (1.107 ± 0.32 vs. 1.129 ± 0.23; p = 0.0001) in patients with the G/G genotype. No changes were observed in patients with the G/A genotype. ***Conclusion:*** In patients with acute coronary syndrome, atorvastatin increases lumbar spine BMD solely in patients with the G/G genotype of the TNFα-308 G/A polymorphism.

Introduction

The financial and social costs of atherosclerosis and osteoporosis are determined by the consequences of these clinically silent diseases, i.e. vascular disease and fractures. The relationship between these disorders has not been clearly established although clinical studies have shown an association [1, 2] with subjects with reduced bone mass or fracture presenting increased global mortality, especially cardiovascular mortality. Marcovitz et al. [3] recently reported that bone mass loss in nonvertebral sites is a predictive factor for coronary disease, with an odds ratio superior to traditional risk factors. Other

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studies have shown an association between osteoporosis and coronary calcification, subrogate markers of atherosclerosis and predictors of future cardiovascular events [4, 5]. Barengolts et al. [6], using electron beam computed tomography, found an inverse relationship between bone mass and coronary calcification. Other authors have not confirmed these data and suggest that age may be the nexus, since both pathologies predominate in the elderly [7].

Atherosclerosis and osteoporosis share etiopathogenic mechanisms modulated by the effect of various inflammatory mediators, with proinflammatory cytokines being key elements. Inflammation is implicated both in the formation of an atheroma plaque and its rupture, which causes acute coronary syndrome [8]. Inflammatory cytokines play an important role in the imbalance between bone formation and resorption that leads to the reduced bone mass seen in osteoporosis [9]. In addition, drugs like statins are effective in both diseases: they diminish the number of vascular events in patients with atherosclerosis by reducing the atheroma plaque and increasing bone mass in patients with high levels of cholesterol [10].

Tumor necrosis factor-α (TNFα) is a cytokine that plays a key role in the inflammatory cascade which has been implicated in coronary disease [11]. It is also implicated in the etiopathogenesis of osteoporosis by stimulating bone resorption, either directly by increasing the differentiation of osteoclasts from their precursors [12] or indirectly by stimulating the production of other cytokines (IL-11, IL-6) [13]. Likewise, it may inhibit bone formation by blocking the wingless signaling pathway (Wnt) and increasing levels of Dickkopf-1 (DKK-1) [14]. The gene that codifies this cytokine is located on chromosome 6 (p21.1-p21.3), with various polymorphisms being described. One is located at position 308 and results from the substitution of alanine (A) for guanine (G). It is located in the promoter region of the gene and is a functional polymorphism [15]. Blood cells of individuals with allele A express more TNFα in vitro after stimulation with lipopolysaccharides than cells of individuals with allele G [16]. It is not clear whether the TNFα promoter 308 A/G polymorphism has a functional significance; however, there may be a small but significant effect, with the A allele being associated with higher levels of TNF transcription [17].

The objective of this study was to evaluate the effect of a statin, atorvastatin, on bone mass and markers of bone remodeling in patients with acute coronary syndrome depending on the TNFα-308 G/A polymorphism.

Material and Methods

Subjects

Patients with acute coronary syndrome (acute myocardial infarction or unstable angina) diagnosed according to European Society of Cardiology criteria were included. During a hospital stay, a medical history was obtained using a standard questionnaire. Exclusion criteria were chronic alcohol abuse, neoplasia, chronic renal insufficiency, hyper- and hypocalcemia, hyperparathyroidism and the use of drugs modifying bone mineral density (BMD) (calcium, vitamin D, estrogens, calcitonin, bisphosphonates, fluorine). Patients were given low (10–20 mg) or high doses (40–80 mg) of atorvastatin according to their index of vascular risk, but there was no adjustment for body weight. Based on the presence of one or no cardiovascular risk factors (smoking, hypertension, diabetes, family history or low HDL cholesterol), the patients were classified in the low- or high-risk group [18]. Patients were studied during their stay in hospital and at 12 months of follow-up. The study was approved by the hospital ethics committee, and written informed consent was obtained from all participants.

Measurements

Blood samples were obtained after 8 h fasting. Cholesterol, triglycerides, total calcium, phosphorus, magnesium, and alkaline phosphatase were measured using a Hitachi 917 autoanalyzer (Tokyo, Japan). Osteocalcin was measured by immunoassay (Immulite DPC, Los Angeles, Calif., USA) with a 6.7% interassay coefficient of variation (CV). Urinary deoxypyridinoline levels were determined by immunoassay after 24 h (Immulite DPC, Dipesa, Los Angeles, Calif., USA). The results were expressed with respect to creatinine excretion with a 14% interassay CV.

Densitometric studies were conducted in the lumbar spine (L_2–L_4), femoral neck and trochanter using an X-ray densitometer (DXA, Lunar Corporation, Madison, Wisc., USA). BMD was expressed in g/cm^2 and as peak bone mass percentage in normal subjects (T-score), depending on the software used in the device. Patients with a T-score $\leq$2.5 were considered to be osteoporotic. The precision of the method (CV) was determined to be 1.5% at the lumbar spine, femoral neck and trochanter.

Genotyping of G308A Gene Polymorphism

Oligonucleotide primers and probes were designed using the Beacon Designer 4.0 (Premier Biosoft International®, Los Angeles, Calif., USA). The polymerase chain reaction (PCR) was carried out with 50 ng of genomic DNA, 0.5 μl of each oligonucleotide primer (primer forward: 5′-CTG TCT GGA AGT TAG AAG GAA AC-3′; primer reverse: 5′-TGT GTG TAG GAC CCT GGA G-3′), and 0.25 μl of each probe (wild probe: 5′-Fam-AAC CCC GTC CTC ATG CCC-Tamra-3′; mutant probe: 5′-Hex-ACC CCG TCT TCA TGC CCC-Tamra-3′) in a 25-μl final volume (Termociclador iCycler IQ (Bio-Rad®), Hercules, Calif., USA). DNA was denatured at 95°C for 3 min, followed by 50 cycles of denaturation at 95°C for 15 s, and annealing at 59.3°C for 45 s. PCRs were run in a 25-μl final volume containing 12.5 μl of IQTM Supermix (Bio-Rad®) with hot-start Taq DNA polymerase.

Statistical Analyses

The results are expressed as mean ± standard deviation. All variables were analyzed using descriptive statistics, including measurement of the central trend and dispersion for quantitative

Table 1. Analytical parameters at study entry (baseline) and 12 months after treatment with atorvastatin according to genotype

	GG homozygotes (n = 45)		GA heterozygotes (n = 17)	
	baseline	12 months	baseline	12 months
Cholesterol, mg/dl	184 ± 48	160 ± 30[a]	186 ± 56	163 ± 30[g]
HDL cholesterol, mg/dl	40 ± 14	50 ± 12[b]	38 ± 6	48 ± 7[h]
LDL cholesterol, mg/dl	116 ± 42	87 ± 34[c]	113 ± 47	95 ± 30[i]
Triglycerides, mg/dl	151 ± 100	126 ± 69[d]	168 ± 74	130 ± 62[j]
Calcium, mg/day	9.6 ± 0.5	9.6 ± 0.4	9.6 ± 0.5	9.7 ± 0.6
Phosphorus, mg/dl	3.7 ± 0.6	3.5 ± 0.6	3.5 ± 0.5	3.4 ± 0.5
Magnesium, mg/dl	2.2 ± 0.2	1.9 ± 0.3[e]	2.3 ± 0.3	2 ± 0.2[k]
Osteocalcin, nmol/l	2.9 ± 2	1.5 ± 1.5[f]	3.2 ± 1.1	0.95 ± 0.6[l]
Deoxypyridinoline, nmol/mmol creatinine	6.1 ± 2.0	6.3 ± 2.5	6.3 ± 3.5	5.2 ± 3.9

[a] p = 0.001; [b] p = 0.001; [c] p = 0.0001; [d] p = 0.0001; [e] p = 0.001; [f] p = 0.0001; [g] p = 0.022; [h] p = 0.0001; [i] p = 0.0001; [j] p = 0.022; [k] p = 0.022; [l] p = 0.001.

Table 2. Densitometric parameters at study entry (baseline) and at 12 months after treatment with atorvastatin according to genotype

	GG homozygotes (n = 45)		GA heterozygotes (n = 17)	
	baseline	12 months	baseline	12 months
BMD L2–L4, g/cm^2	1.107 ± 0.23	1.129 ± 0.21[a]	1.180 ± 0.24	1.187 ± 0.24
BMD femoral neck, g/cm^2	0.902 ± 0.15	0.897 ± 0.15	0.972 ± 0.12	0.966 ± 0.13
BMD femoral trochanter, g/cm^2	0.757 ± 0.22	0.761 ± 0.22	0.863 ± 0.14	0.813 ± 0.26

[a] p= 0.0001.

variables and absolute and relative frequencies for qualitative variables. Means were compared using the paired t test and the Mann-Whitney nonparametric U test. Correlations between variables were made using Pearson's r test and Spearman's test. A multivariate logistic regression analysis was performed to evaluate the effects of osteocalcin, magnesium, parathormone and 308 G/A polymorphism on spine BMD. The statistical analysis used SPSS software (SPSS, Chicago, Ill., USA; Base 11.4 for Windows) and SAS (SAS Institute, Carg, N.C., USA; Version 8.2). All statistical tests were two-tailed with $p < 0.05$ considered to be significant.

Results

Sixty-two patients (35 males and 27 females) with acute coronary syndrome (54 patients with acute myocardial infarction and 8 with unstable angina) with an average age 60 ± 10 years were included. Patients were divided into two groups according to 308 G/A polymorphism. Forty-five patients were homozygous for G/G (72.5%) and 17 heterozygous for G/A (27.5%). The prevalence of osteoporosis (T score ≤2.5 in the lumbar spine and/or hip) was 33% in the G/G genotype and 35% in the G/A genotype, with no statistically significant differences between the two groups (p = 0.556). Baseline parameters (tables 1, 2) showed no differences between groups. Analysis of the response to atorvastatin showed reduced cholesterol and triglyceride levels in both groups. There was a similar reduction in osteocalcin and magnesium (table 1).

There were differences in the response of bone mass to avortastatin according to genotype. In the lumbar spine (L2–L4) there was a statistically significant increase in BMD (1.107 ± 0.32 vs. 1.129 ± 0.23, p = 0.0001) in patients with the G/G genotype, but not in those with the G/A genotype. No changes in BMD were found in either

Table 3. BMD L2–L4 (g/cm^2) at study entry (baseline) and 12 months after treatment with atorvastatin according to genotype and atorvastatin doses

Atorvastatin doses	GG homozygotes (n = 45)		GA heterozygotes (n = 17)	
	baseline	12 months	baseline	12-months
Low doses (n = 30)	1.168 ± 0.23	1.201 ± 0.23[a]	1.216 ± 0.23	1.231 ± 0.22
High doses (n = 32)	1.127 ± 0.22	1.141 ± 0.22[b]	1.173 ± 0.25	1.162 ± 0.28

[a] p = 0.002; [b] p = 0.035.

Table 4. Results of the multiple linear regression analysis predicting changes in spine BMD

	β	p
308 G/A polymorphism	–0.392	0.011
Magnesium	–0.008	0.954
Osteocalcin	–0.081	0.579
PTH	–0.298	0.046

group in the femoral neck or trochanter (table 2). There were no differences in the response of bone mass to atorvastatin according to the drug doses (table 3). When a multivariate logistic regression analysis was performed to evaluate the effects of osteocalcin, magnesium, parathormone and the 308 G/A polymorphism on spine BMD, only the latter two were found to be significantly associated with BMD (table 4).

Discussion

Our results show that the TNFα-308 G/A polymorphism does not influence BMD in the lumbar spine and hip in patients with acute coronary syndrome. The incidence of osteoporosis was similar in the two genotypes analyzed. However, we found a different response to atorvastatin according to genotype. Patients with the G/G genotype showed increased BMD in the lumbar spine in response to treatment while those with the G/A genotype did not. The genotype distribution of our patients was similar to the European distribution, with more than 70% having the G/G genotype, although different from Asia, where the AA genotype predominates [15, 19, 20].

The reduction in serum magnesium observed in both groups is remarkable. Haenni et al. [21] demonstrated that the administration of simvastatin for 6 weeks caused a statistically significant reduction in magnesium levels in a group of 23 diabetic patients. These results are comparable to our findings. We observed a reduction in osteocalcin, a turnover marker. Atorvastatin is anticatabolic and reduces bone remodeling.

High circulating levels of TNFα have been associated with unstable angina and myocardial infarction and may predict a second infarction [22]. However, no significant association was found between the TNFα-308 G/A polymorphism and the incidence of coronary disease [15] or osteoporosis (BMD) either in Europeans or Asians [19, 20]. Nor does the polymorphism influence the peak bone mass although another polymorphism located in the promoter region of the gene, –863 CA [19], does. Only Fontova et al. [23], in a study on 104 postmenopausal women with osteoporosis and 51 without, found a higher bone mass in a group of patients with nonsevere osteoporosis and the G allele.

Few studies have evaluated the response to statins as a function of the TNFα-308 G/A polymorphism. In the Lipoprotein and Coronary Atherosclerosis Study (LCAS), no association was found between the 308 G/A polymorphism and the biochemical, angiographic and clinical response to fluvastatin [24] and no baseline differences were observed. No previous studies have evaluated the response of bone mass to statins according to the selected genotype. We found a favorable response in patients with the G/G genotype, comparable to the response observed in patients with rheumatoid arthritis treated with anti-TNF antibodies. The number of responders was greater in subjects with the G allele [25]. The worse response obtained by our patients with the G/A genotype may be due to the fact that these patients produce more TNFα and that, possibly, atorvastatin cannot reduce it below a threshold level. Another possibility is that disease severity is greater in these patients. However, this is unlikely as we found no baseline differences in BMD or the prevalence of osteoporosis. The role of other polymorphisms

close to the polymorphism we analyzed cannot be excluded and should be further studied. The response was only observed in the lumbar spine and not in the hip. This may be because the hip bone is metabolically less active, with a poorer response to anticatabolic drugs, meaning that greater antiresorptive power would be needed and that the effect of atorvastatin is small.

In conclusion, in patients with acute coronary syndrome, atorvastatin increases lumbar spine BMD only in patients with the G/G genotype of the TNFα-308 G/A polymorphism.

The main limitation of our study is the sample size even though the population was uniform. Moreover, we have not measured the TNF levels. Another limitation is the absence of an objective method for assessing therapeutic compliance. This was performed using the information provided by the patient at the last visit. In addition, initial triglyceride and cholesterol levels were not too high. These facts can explain the absence of differences between high and low drug doses.

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