Atorvastatin and BMD in Coronary Syndrome. Role of Lys656Asn Polymorphism of Leptin Receptor Gene

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Abstract. Objectives. To evaluate the effect of atorvastatin on bone mass and markers of bone remodeling in patients with acute coronary syndrome according to the Lys656Asn leptin receptor gene polymorphism. Methods. Sixty-two patients with acute coronary syndrome were included. Patients were allocated to low and high doses of atorvastatin according to baseline levels of cholesterol and triglycerides and the index of vascular risk and were studied at hospital admission and at 12 months. 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 and hip. Patients with a T-score < -2.5 were considered osteoporotic. The Lys656Asn leptin receptor gene polymorphism was determined by PCR. Results. Forty-two patients were Lys/Lys homozygotic and 20 Lys/Asn heterozygotic. The prevalence of osteoporosis was 31% for the Lys/Lys genotype and 27% for the Lys/Asn genotype with no significant differences between groups. There was a significant increase in bone mineral density in the lumbar spine (1.117 ± 0.24 versus 1.135 ± 0.24 , $P = 0.008$) in patients with the Lys/Lys genotype. Conclusion. Atorvastatin increases lumbar spine bone mineral density only in patients with the Lys/Lys genotype of the Lys656Asn polymorphism.

Key words: Atorvastatin, Leptin, Lys658Asn polymorphism, Coronary syndrome

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ATHEROSCLEROSIS and osteoporosis share etio-pathogenic mechanisms modulated by the effect of inflammatory mediators, with proinflammatory cytokines being key elements [1]. Inflammation intervenes in both the formation and rupture of atheroma plaque, leading to acute coronary syndrome [2]. Inflammatory cytokines play an important role in the imbalance between bone formation and resorption that leads to the reduced bone mass seen in osteoporosis [3]. In addition, drugs such as statins are effective in both diseases, diminishing vascular events in patients with atherosclerosis and increasing bone mass in those

with high levels of cholesterol [4].

Human leptin is a protein of 167 amino acids manufactured in fat cells which is involved in regulating the ingestion and consumption of energy. It intervenes in the regulation of bone mass by means of a central hypothalamic mechanism, exerting a double antagonistic effect on bone formation and resorption through activation of the sympathetic nervous system and the Cocaine Amphetamine Regulated Transcript (CART) [5]. In addition, studies have shown that hyperleptinemia can exert atherogenic effects [6]. In North American patients, elevated leptin levels have been associated with an increased risk of myocardial infarction in both males (3.16, 95% CI 1.40–7.37) and females (3.95, 95% CI 1.29–12.72) [7].

Leptin exerts its biological effect by bonding with a receptor from the cytokine-receptor family. The leptin

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receptor has been cloned, with 7 SNPs being described in the coding region of the OB-RB receptor. The variability in circulating levels of leptin and its dependency on ingestion may explain the discrepancies found between bone mass and leptin [8, 9]. Therefore, analysis of genetic polymorphisms of the leptin system may aid understanding of the association between bone mass, leptin and the response to atorvastatin, independently of the biological variability of leptin levels.

The objective of this study was to evaluate the effect of atorvastatin on bone mass and markers of bone remodeling in patients with acute coronary syndrome according to the Lys656Asn polymorphism of the leptin receptor gene.

Material and Methods

Patients with acute coronary syndrome diagnosed according to European Society of Cardiology criteria were included. Exclusion criteria were chronic alcohol abuse, neoplasia, chronic renal failure hyper- and hypocalcaemia, hyperparathyroidism and use of drugs modifying bone mineral density (BMD). Patients were allocated to low (10–20 mg) and high doses (40–80 mg) of atorvastatin according to baseline levels of cholesterol and triglycerides and the index of vascular risk. Patients were classified as low or high risk according to the presence of no or one or more cardiovascular risk factors (smoking, hypertension, diabetes, family history and low HDL-cholesterol) [10]. Measurements were made at baseline 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.

Cholesterol, triglycerides, total calcium, phosphorus, magnesium, and alkaline phosphatase were measured using a Hitachi 917 autoanalyzer (Tokyo, Japan). Serum osteocalcin and urinary deoxypyridinoline levels were determined by immunoassay (Immulate DPC, Dipesa, Los Angeles, USA). Densitometric studies were conducted in the lumbar spine (L2–L4) and femoral neck and trochanter using an X-ray densitometer (DXA, Lunar Corporation, Madison, Wisconsin, USA). Patients with a T-score ≤ -2.5 were considered osteoporotic.

Genotyping of *LEPR* gene polymorphism

Oligonucleotide primers and probes were designed with the Beacon Designer 4.0 (Premier Biosoft International®, LA, CA). Polymerase chain reaction (PCR) was carried out with 250 ng of genomic DNA, 0.5 μ L of each oligonucleotide primer (primer forward: 5'-GCA GTT CCT ATG AGA GGA CC-3'; primer reverse: 5'-AAA TTG GGA ATA CCT TCC AAA GT-3'), and 0.25 μ L of each probe (wild probe: 5'-Fam-AGT GAC ATT TTT CTC CTT TTT CAT AGT ATC-Tamra-3') and (mutant probe: 5'-Hex-AGT GAC ATT TTT CTC GTT TTT CAT AGT AT-Tamra-3') in a 25 μ L final volume (Termociclador iCycler IQ (Bio-Rad®), Hercules, CA). DNA was denaturated at 95°C for 3 min; this was followed by 50 cycles of denaturation at 95°C for 15 s, and annealing at 59.3° for 45 s. PCR were run in a 25 μ L final volume containing 12.5 μ L of IQTM Supermix (Bio-Rad®, Hercules, CA) with hot start Taq DNA polymerase.

The results are expressed as mean $\pm$ standard deviation. Comparisons of the mean were made using the paired t-test (for sample sizes >30 , homozygotic Lys/Lys polymorphism patients) and the Mann-Whitney nonparametric U test (for sample sizes <30 , heterozygotic Lys/Asn polymorphism patients). Correlations between variables were made using Pearson's r test and Spearman's test.

Results

Sixty-two patients (35 males and 27 females) with acute coronary syndrome (54 with acute myocardial infarction and 8 with unstable angina) with a mean age of 61 ± 10 years were included. Patients were divided into two groups according to the Lys656Asn polymorphism of the leptin receptor gene. Forty-two patients were homozygotic Lys/Lys (69%) and 20 heterozygotic Lys/Asn (31%). The prevalence of osteoporosis was 31% in the Lys/Lys genotype and 27% in the Lys/Asn genotype, with no significant differences between the two groups ($p = 0.578$). Baseline parameters (Tables 1 and 2) showed no differences between the two groups. Age (61 ± 10 years vs 62 ± 9 years, $p = 0.7$), height (162 ± 9 cm vs 163 ± 8 cm, $p = 0.7$), weight (74 ± 12 vs 75 ± 9 , $p = 0.7$) and BMI (28 ± 4 vs 28 ± 4 , $p = 0.7$) were similar in the two groups. No patient had limitations in the activities of daily living

Table 1. Baseline and 12-months analytical parameters after treatment with atorvastatin according to the genotype expressed.

	Homozygote Lys/Lys		Heterozygote Lys/Asn	
	Baseline	12-month	Baseline	12-month
Cholesterol mg/dl	173 ± 41	160 ± 35 ^a	200 ± 56	160 ± 30 ^e
Triglycerides mg/dl	142 ± 70	116 ± 44 ^b	174 ± 129	145 ± 94
Calcium mg/dl	9.5 ± 0.5	9.6 ± 0.5	9.6 ± 0.6	9.7 ± 0.4
Phosphorus mg/dl	3.7 ± 0.6	3.5 ± 0.6	3.4 ± 0.6	3.4 ± 0.5
Magnesium mg/dl	2.2 ± 0.3	2 ± 0.3 ^c	2.2 ± 0.2	2 ± 0.3
Osteocalcin nmol/l	3.2 ± 1.8	1.6 ± 1.5 ^d	2.8 ± 1.8	0.89 ± 0.5 ^f
Deoxypyridinoline nmol/mmol creatinine	6.6 ± 2.7	7.2 ± 4.7	6.4 ± 1.9	5.5 ± 0.82

a) $p = 0.028$, b) $p = 0.024$, c) $p = 0.001$, d) $p = 0.0001$, e) $p = 0.001$, f) $p = 0.0001$

Table 2. Baseline and 12-month densitometric parameters after treatment with atorvastatin according to the genotype expressed.

	Homozygote Lys/Lys		Heterozygote Lys/Asn	
	Baseline	12-month	Baseline	12-month
BMD L2–L4 g/cm ²	1.117 ± 0.24	1.135 ± 0.24 ^a	1.167 ± 0.21	1.174 ± 0.23
BMD femoral neck g/cm ²	0.903 ± 0.15	0.904 ± 0.15	0.916 ± 0.15	0.924 ± 0.15
BMD femoral trochanter g/cm ²	0.743 ± 0.25	0.753 ± 0.22	0.726 ± 0.31	0.755 ± 0.27

a) $p = 0.008$

during the 12-month follow up. Only one patient had a vertebral fracture. There were no differences between the two groups in the drugs received. Analysis of the response to atorvastatin showed reduced cholesterol and triglyceride levels in the Lys/Lys group and only reduced cholesterol in the Lys/Asn group. The reduction in osteocalcin was similar in the two groups (Table 1).

There were differences in the response of bone mass to atorvastatin according to the genotype. In the lumbar spine (L2–L4), there was a statistically significant increase in BMD (1.117 ± 0.24 versus 1.135 ± 0.24 , $p = 0.008$) in patients with the Lys/Lys genotype, but not in those with the Lys/Asn genotype. After adjustment for age and sex (z-score— 0.062 ± 1.89 versus 0.149 ± 1.51 , $p = 0.001$) an increase in BMD of $1.9\% \pm 4.2\%$ in Lys/Lys patients and a reduction of $0.1\% \pm 0.1\%$ in Lys/Ans patients was observed, with the difference being significant ($p = 0.005$).

Fig. 1 shows the changes in BMD for each patient according to the polymorphism expressed. No changes in BMD were found in either 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 dose (initial, 1.192 ± 0.22 vs 1.144 ± 0.23 , $p = 0.4$ and final, 1.213 ± 0.22 vs 1.153 ± 0.23 , $p = 0.4$).

Discussion

Our results show that the Lys656Asn polymorphism of the leptin receptor gene does not influence BMD 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 the genotype. Patients with the Lys/Lys genotype showed increased BMD in the lumbar spine, while those with the Lys/Asn genotype did not. In the polymorphism analysed, lysine was replaced by arginine in codon 656 of exon 14. This modification in the extracellular domain of the receptor can change its charge, with functional consequences affecting bonding of the receptor with leptin.

Few studies have analyzed variations in bone mass between the polymorphisms of the leptin receptor. The results differ, with some studies finding an association with the substitution of adenine by guanine in position 668 of exon 6 (Gln223Arg) and changes in bone mass and an increase in vertebral fractures [11, 12], but no modifications of bone density with the Lys656Asn polymorphism. These results are similar to those of our study. We found no baseline differences between the two groups studied. A reduction in osteocalcin was observed in both polymorphisms. Osteocalcin is a small non-collagenous protein synthe-

Fig. 1. Individual changes of BMD in homozygote lys/lys and heterozygote lys/asn patients.

sized by matrix osteoblasts. It is considered a sensitive index of osteoblastic activity and a marker of bone formation. However, the proteolytic fragments of this peptide could be released during bone matrix resorption [13] and thus it can be considered a marker of bone turnover. A reduction in remodeling increases BMD. However, the treatment response was different. It should be remembered that the polymorphism analysed is not associated with changes in leptin levels but rather causes leptin resistance [14]. Atorvastatin may modify this situation and the increase in BMD may be related to an anabolic action of leptin.

This differing response may be explained by the interaction between leptin and the enzyme, HMG-CoA reductase. Atorvastatin acts at the level of HMG-CoA reductase, a hepatic enzyme which limits endogenous cholesterol synthesis. Experimental models have shown that a defective leptin signal causes a loss of the circadian rhythm of the enzyme with an increase in hepatic enzyme activity and a reduction in 27-hydroxylase cholesterol and 7- α -reductase cholesterol [15]. The mechanism which produces this increase in activity is a reduction of the activity of the enzyme, AMP-protein kinase [16, 17]. This difference could explain modification of the response to the statin.

Few studies have evaluated the response to statins according to leptin polymorphisms. Takahashi-Yasuno *et al.* [18] analysed a different statin, simvastatin, and a different polymorphism. The role of other polymorphisms adjacent to that analysed cannot be discarded 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.

The main limitation of our study was the sample size, even though the study population was uniform. In addition, we did not measure leptin levels. Another limitation is the absence of an objective method for assessing therapeutic compliance, which was assessed using the information provided by the patient at the last visit.

In conclusion, in patients with acute coronary syndrome, atorvastatin increases lumbar spine bone mineral density only in patients with the Lys/Lys genotype of the Lys/656/Asn polymorphism of the leptin receptor gene.

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