Effect of Atorvastatin to RANKL/OPG System in Patients with Acute Coronary Syndrome

J. L. Pérez-Castrillon, MD, PhD*
L. Abad, MD*
G. Vega, MD*
A. Sanz-Cantalapiedra, MD†
A. San Miguel, MD, PhD*
A. Mazón, MD*
S. Sanchez, MD‡
G. Hernandez, MD‡
A. Dueñas-Laita, MD, PhD*

†Departamento de Medicina Interna, Hospital Universitario Río Hortega, Facultad de Medicina, Valladolid, Spain
‡Laboratorio de Pediatría, Departamento de Pediatría-Instituto de Biología y Genética Molecular, Facultad de Medicina, Valladolid, Spain
§Unidad Médica, Pfizer, Madrid, Spain

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ABSTRACT
Background: The prominent effect of statin therapy is the inhibition of hydroxymethyl glutaryl-Coenzyme A (HMG-CoA) reductase. This enzyme decreases the mevalonate level and prevents the synthesis of isoprenoids. This inhibition alters osteoclast activity. Osteoprotegerin (OPG) acts as a decoy receptor of the receptor activator of nuclear factor kB (NF-kB) ligand (RANKL), which is a key regulator of osteoclastogenesis.

Objective: The aim of this study is to evaluate the effect of atorvastatin on the RANKL/OPG system in patients with acute coronary syndrome.

Material and Methods: We studied 22 patients (17 men and 5 women, age 61 ± 8 years) referred to Hospital Río Hortega with a diagnosis of acute coronary syndrome. The following parameters were determined: osteocalcin, paratohormone, 25-vitamin D, RANKL, and osteoprotegerin. Ðexopyridinoline was determined in urine samples. The same parameters were determined after 12 months. The patients were treated with atorvastatin.

Results: Atorvastatin resulted in plasmatic level decreases of osteocalcin (3.8 ± 2 pmol/L vs 2 ± 1 pmol/L; P=0.022), RANKL (0.17 ± 0.13 vs 0.01 ± 0.02; P=0.02), and RANKL/OPG (0.02 ± 0.02; P=0.04).

Conclusions: Atorvastatin decreases RANKL and osteocalcin levels in patients with acute myocardial infarction. Atorvastatin may increase the bone mineral density by inhibiting the
INTRODUCTION
Statins are drugs used for the treatment of hyperlipidemia. They act on the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, inhibiting the synthesis of mevalonic acid and inducing the upregulation of liver low-density lipoprotein (LDL) receptor. In addition to their action on a decrease in cholesterol levels and a reduction of mortality in patients with ischemic heart disease, statins can have a beneficial effect on other chronic diseases, including osteoporosis.

Mundy and colleagues published the first in vitro study with animals and demonstrated an anabolic effect of the statins on bone. This group was looking for small molecules that activated the promoter of the morphogenetic protein (2BMP-2) gene. This protein can stimulate osteoblast differentiation. After examining over 30,000 components, they found that lovastatin stimulated the luciferase activity of this gene. The effect appeared with other statins (simvastatin, mevastatin, and fluvastatin) and was inhibited by the addition of mevalonic acid, suggesting that the effect was caused by inhibition of the HMG-CoA reductase enzyme.

The same group reported that the subcutaneous administration of statins over the murine calvaria increased bone formation by 50%. Another possible mechanism was the action of the statin on the multipotential cells of the bone marrow. These cells can be differentiated to osteoblasts or adipocytes in relation to the existing relationship between 2 mediators: core binding fa1 (Cbfa1/Runx) and PPRR[2]. The first mediator enhances the differentiation of osteoblasts, while the second makes it to adipocytes.

Statins increase Cbfa1/Run X and reduce PPRR[2]. The final result is an increase in the anabolic activity through osteoblast differentiation and maturation. These effects are suppressed by the addition to the acidic medium of mevalonic acid, suggesting that the effects occur through the inhibition of HMG-CoA reductase.

Statins will mainly cause an anabolic effect through the differentiation of osteoblasts, though statins also have an antiresorptive effect. Statins act in the metabolic path of mevalonic acid, preventing the prenylation of small glutaminyl transeptidases (GTP)—Ras, Rho, Rai, Rab—enhancing the apoptosis of osteoclasts. Moreover, osteoprotegerin (OPG) acts as a decoy receptor of the receptor activator of nuclear factor (NF-B) ligand (RANKL), which is a key regulator of osteoclastogenesis.

The effect of statins in the system OPG/RANKL has not been evaluated previously. The aim of this study is to evaluate the effect of atorvastatin on the RANKL/OPG system in patients with acute myocardial infarction.

MATERIALS AND METHODS
Subjects and Study Protocol
Patients were eligible for inclusion if they had been hospitalized for an acute coronary syndrome (ACS), which was defined as high-risk unstable angina, non-ST-elevated myocardial infarction, or ST-elevated myocardial infarction. Twenty-four patients (19 men and 5 women) with acute myocardial infarction were included in the study. Exclusion criteria were alcoholism, neoplasm, hyper- and hypocalcemia, hyperparathyroidism, and the use of drugs that affect bone mass. Patients were prescribed atorvastatin (40 mg average dose) and were monitored for 1 year. Dose of atorvastatin was adjusted based on lipid levels and cardiovascular risk profile.
Table 1. General characteristics of the subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Men</td>
<td>17</td>
</tr>
<tr>
<td>Women</td>
<td>5</td>
</tr>
<tr>
<td>Age (Median)</td>
<td>61 ± 8 years</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72 ± 10</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163 ± 7</td>
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<tr>
<td>Hypertension</td>
<td>33%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>29%</td>
</tr>
<tr>
<td>Use of ACEIs</td>
<td>33%</td>
</tr>
<tr>
<td>Use of angiotensin receptor antagonist</td>
<td>10%</td>
</tr>
<tr>
<td>Use of thiazides</td>
<td>0</td>
</tr>
</tbody>
</table>

Use of β-blockers 62%
Use of nitrates 29%
ACEIs= Antagonist converting enzyme inhibitor

Measurements
Blood samples were obtained after 8 and 9 hours of fasting. Total calcium, phosphorous, magnesium, and alkaline phosphatase were measured using a Hitachi 917 autoanalyzer (Tokyo, Japan). Osteocalcin was measured using a commercial radioimmunoassay (RIA) test (Schering, Germany) with a 6.7% interassay variation coefficient. Parathormone intact (PTHi) levels were determined by chemoluminiscence (Immulite DPC, Los Angeles, CA) with a CV of 6%. The 25-hydroxyvitamin D levels were determined by high-performance liquid chromatography (HPLC) with a CV of 12%. Osteoprotegerin and RANKLs were measured using a commercial ELISA test (Biomedica Gruppe, Viena, Austria) with a coefficient of variation (CV) of 7% to osteoprotegerin and 9% to RANKLs.

Urinary deoxypyridinoline levels were determined by chemoluminiscence after 24 hours (Immulite DPC, Dipesa, Los Angeles, CA). The results were expressed in relation to the excretion of creatinine with a CV of 14%.

Statistical Analysis
Descriptive statistical analysis was performed, including measures of central tendency and scattering for quantitative variables, and the absolute and relative frequency for categorical variables. The Mann-Whitney U-test was used for non-parametric tests. Results are expressed as mean ± standard deviation; significance was set at a P value of 0.05.

Ethics
The study was approved by the clinical research committee of the hospital, and all patients signed an informed consent form to participate in the study.

RESULTS
General Characteristics
Twenty-four patients with ACS were recruited. Mean age was 61 ± 8 years; 19 patients (79%) were men. Of these patients, 57% had AMI and 43% unstable angina. Demographic characteristics including number of diabetics and hypertensive patients, use of β-blockers, ACE inhibitors, angiotensin antagonists, thiazides, and anticoagulants (Table 1).

Bone Markers of Bone Metabolism
At baseline, biochemical markers of bone metabolism (25-vitamin D, PTH, osteocalcin, and deoxypyridinoline) did not vary from normal values. There was a statistically significant decrease in

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serum osteocalcin at 12 months after atorvastatin treatment. The other parameters were not altered (Table 2).

### RANKL/OPG System

The RANKL showed a statistically significant decrease at 12 months after atorvastatin treatment. The OPG did not change (Table 2). Serum levels of OPG and RANKL showed no association with serum concentration of bone markers remodeling (osteocalcin and deoxypiridinoline).

### DISCUSSION

The study results show that atorvastatin reduced the osteoblastic activity markers osteocalcin and RANKL, reflecting a bone turnover decrease. Similar results had been obtained by other studies. Stein and colleagues, when comparing in 846 hypercholesterolemic patients treated for 12 weeks the effects of simvastatin (40 mg and 80 mg) and atorvastatin (20 and 40 mg), showed that simvastatin decreased the alkaline bone phosphatase in the total population, for both women and men. The effect on resorption markers (CTX) was not significant. Although atorvastatin reduced the levels of markers, this was not statistically significant.

In another study conducted in older patients with hypercholesterolemia with a longer follow-up (6 months), atorvastatin significantly reduced resorption markers (NTx), with no effect on formation markers. No relationship was found between the reduction of cholesterol levels (total cholesterol and LDL cholesterol) and of NTx. A recently published randomized clinical trial of 49 postmenopausal women with hypercholesterolemia analyzed the effect of atorvastatin at doses of 20 mg and with a short follow-up (6 weeks). Considering the total population, no effects on markers were seen, but a statistically significant reduction in CTx was seen in the study performed in subjects over 63 years of age. Rejnmark and colleagues studied 140 postmenopausal women with hypercholesterolemia, comparing them to a control group. They found a reduction in bone remodeling, a reduction in formation and resorption markers, with increased PTH levels in the treated group. However, there were no bone mass differences. A weakness of the study was that the two groups were
not strictly comparable. Sixty percent of the women treated with statins also received other drugs with cardiovascular effect vs 5.7% in the control group, which could show a worse health condition in the first group. Because physical activity and quality of life were not assessed, the effect of statins on bone mass could have a bias, considering that in this population statins acted as antiresorption agents.

Other authors obtained different results. Breatveld and colleagues recently assessed the effect of atorvastatin on remodeling markers in diabetics through a randomized, cross-over clinical trial in a small population (25 patients) and observed no drug effect.13 The selected population of diabetics has its own characteristics. Patients with type 2 diabetes, unlike those with type 1 diabetes, show a higher risk of hip fracture, but their bone mass is greater with no increase in bone remodelling.14

Mostaza and colleagues, in a group of women with hypercholesterolemia treated with pravastatin, found an increase in PINP (bone formation marker), though no changes occurred in other markers of formation (bone alkaline phosphatase) and resorption (CTx).15 Pravastatin, because it is hydrophilic, has little effect on the bone. Quesada and colleagues assessed the effect of atorvastatin on the bone mass density (BMD) of lumbar spine and femoral neck in 36 postmenopausal women with hypercholesterolemia followed for 1 year.16 Statistically significant increases were found in the bone mass of both sites, with no changes in the markers. Rejnmark and colleagues determined the effect of simvastatin in 41 postmenopausal women without hypercholesterolemia.7 They did not find changes in the markers, but an increase in the bone mass in the forearm. There were no changes in other sites.

Lupatelli and colleagues assessed the effect of simvastatin 40 mg on 40 postmenopausal women with high cholesterol levels and a follow-up time of 2 years.18 A BMD increase of 3.3% was seen in the lumbar spine and 2.7% in the hip, with no changes in the markers.

RANKL is a marker of osteoblastic activation and function and, moreover, is an essential cytokine for the formation and activation of osteoclast and promote bone resorption, while OPG, as its endogenous counterpart, antagonizes these effects.19 Atherosclerosis is now recognized to have a notable inflammatory component and, in parallel, statins appear to inhibit inflammatory processes directly.20 In the immune system, RANKL is expressed and secreted by activated T-cells. Cytokines such as IL-1, IL-11, IL-17, and TNF-α increase RANKL and the ensuing enhanced RANKL/OPG and favor osteoclastogenesis.21 Statins inhibit the increase in cell-surface proteins of major histocompatibility complex class II induced by interferon.23 Such proteins are central in presenting antigens and activating T-cells. Statins would have a double beneficial effect in heart ischemic patients: the inhibition of inflammatory activity, which contributes to atherosclerotic plaque rupture and the reduction of osteoclastogenesis with beneficial effect on bone mass. An elevated RANKL/OPG ratio within the skeleton promotes bone loss, while restoring a balanced RANKL/OPG ratio or blunting RANK responsiveness prevents osteoclast activation and bone resorption.24

REFERENCES


