Effect of Maximum Dose of Atorvastatin on Inflammation, Thrombogenesis, and Fibrinolysis in High-Risk Patients With Ischemic Heart Disease

Antonio Tello, Francisco Marín, Vanessa Roldán, Ana García-Herola, Sofía Lorenzo, Vicente E. Climent, Luis de Teresa, and Francisco Sogorb

Introduction and objectives. It has been suggested that high doses of statins can be more effective in reducing the incidence of new cardiovascular events than conventional doses. The present study analyzed the effect of increasing the atorvastatin dose to 80 mg/day on indices of inflammation (C-reactive protein or CRP), thrombogenesis (prothrombin fragment F1+2) and fibrinolysis (tissue-type plasminogen activator antigen, t-PA, and its inhibitor PAI-1) in high-risk patients with ischemic heart disease.

Patients and method. We studied 27 patients with high-risk coronary heart disease who had lipid levels above those recommended despite treatment with atorvastatin at 40 mg/day. At baseline, patients were compared with 21 normocholesterolemic subjects without arteriosclerotic disease. Twenty-four patients were reevaluated 3 months after the atorvastatin dose was increased to 80 mg/day.

Results. The CRP, F1+2, t-PA and PAI-1 levels were significantly higher in patients than control subjects (all P < 0.05). After the atorvastatin dose was increased, significant reductions in CRP, F1+2, and PAI-1 levels were observed (P < 0.05). There was a significant positive correlation between the reduction in cholesterol level and that in F1+2 (r = 0.46; P = 0.023). No other significant correlations were found.

Conclusions. In a group of patient with high-risk heart disease and elevated lipid levels, increasing the atorvastatin dose led to significant improvements in inflammatory, thrombogenic, and hypofibrinolytic states.

Key words: Hypercholesterolemia. C-reactive protein. Fibrinolysis. Thrombosis.

Efecto de dosis máximas de atorvastatina en la inflamación, la trombogénesis y la función fibrinolítica en pacientes con cardiopatía isquémica de alto riesgo

Introducción y objetivos. Se ha indicado que la utilización de dosis altas de estatinas podría reducir la aparición de nuevos eventos cardiovasculares en comparación con las dosis convencionales. Nuestro objetivo fue estudiar el efecto del incremento de la dosis de atorvastatina (80 mg/día) en diferentes marcadores del sistema inflamatorio (proteína C reactiva [PCR]), hemostático (fragmento F 1+2 de la protrombina [F1+2]) y fibrinolítico (activador tisular del plasminógeno antígenico [t-PA] y su inhibidor [PAI-1]).

Pacientes y método. Se estudió a 27 pacientes con cardiopatía isquémica de alto riesgo que presentaban cifras lipídicas superiores a las recomendadas a pesar del tratamiento con 40 mg/día de atorvastatina. Se compararon con 21 sujetos normocolesterolémicos sin enfermedad arteriosclerótica conocida. Se revaluó a 24 pacientes a los 3 meses del incremento de la dosis.

Resultados. Los pacientes presentaron cifras elevadas de PCR, F1+2, t-PA, y PAI-1 en comparación con el grupo control (en todos, las variables tuvieron un valor de p < 0.05). Tras incrementar la dosis de atorvastatina se observó una reducción de las cifras de PCR, F1+2 y PAI-1 (p < 0.05). Se observó una correlación positiva entre los porcentajes de reducción de colesterol y del F1+2 (r = 0.46; p = 0.023), sin que se hallara otra correlación significativa entre los demás parámetros.

Conclusiones. Al incrementar las dosis de atorvastatina a 80 mg/día se consigue una reducción de los estados inflamatorios, trombogénicos e hipofibrinolíticos en un grupo de pacientes con cardiopatía isquémica de alto riesgo y cifras elevadas de lípidos a pesar del tratamiento con dosis de 40 mg/día de atorvastatina.

Palabras clave: Hipercolesterolemia. Proteína C reactiva. Fibrinólisis. Trombosis.
INTRODUCTION

The use of HMG-CoA reductase inhibitors (statins) in the treatment of dyslipidemia has been shown to improve survival and significantly reduce the appearance of cardiac events, both in primary and secondary prevention. Given these clinical findings, there are clear guidelines on control of lipid levels with statins, but their beneficial effects seem to go beyond lowering cholesterol. Statins also have so-called pleiotropic effects, which improve endothelial function, reduce inflammation, enhance angiogenesis and vasculogenesis, limit oxidative processes, stabilize atherosclerotic plaques, and inhibit the thrombogenic response.

Atherosclerotic disease has been redefined as a chronic inflammatory disease in which other abnormalities besides lipid deposition occur. These abnormalities range from endothelial cell dysfunction to the formation of plaques and, above all, loss of plaque stability, which may ultimately lead to an acute coronary syndrome.

Evidence of a close relationship between the hemostatic and inflammatory systems is mounting. Use of high doses of statins has recently been suggested to help reduce low-density lipoprotein cholesterol (LDL-C) levels, and so further limit the appearance of cardiovascular events. However, apart from lipid levels, and so further limit the appearance of cardiovascular events, little information is available on the pleiotropic effects of high doses of statins.

The objective of this study was to analyze the effect of an increase in dose of atorvastatin to 80 mg/day on inflammatory markers (C-reactive protein [CRP], prothrombin fragment 1+2 [F1+2], and fibrinogen) and endothelial dysfunction (tissue-type plasminogen activator antigen, t-PA, and its inhibitor [PAI-1]) in high-risk patients with stable ischemic heart disease.

PATIENTS AND METHOD

Patients

A total of 27 high-risk patients with ischemic heart disease from the Secondary Prevention Clinic of the Hospital General Universitario in Alicante, Spain, were included. Patients were enrolled if they met any of the following inclusion criteria: a) diffuse coronary artery disease (2 or more diseased vessels) with coronary bypass surgery ruled out because of poor state of the distal beds; b) exercise-limiting angina after bypass surgery; or c) premature coronary artery disease (age ≤45 years) with 3 or more cardiovascular risk factors, in particular, patients who still smoked. In addition, all patients had lipid levels above recommended values (LDL-C ≤50 mg/dL). Despite treatment with atorvastatin at doses of 40 mg/day, lipid-lowering treatment had not been altered in any of the patients in the 3 months prior to inclusion in the study and patients had also been insistently advised to follow a low-fat diet.

Exclusion criteria were as follows: a) hemodynamic instability or deterioration in functional class in the last 3 months; b) acute coronary syndrome or coronary revascularization in the year before the study; c) chronic or paroxysmal atrial fibrillation; d) valve disease of at least moderate severity; e) renal or hepatic impairment; f) neoplastic or inflammatory disease; g) thyroid dysfunction; and h) anticoagulant treatment.

Patients were examined on study entry and 3 months after increasing the atorvastatin dose to 80 mg/day. In all cases, close clinical and analytical monitoring was done (with particular attention paid to aspartate aminotransferase, alanine aminotransferase, and creatine kinase levels at 4 and 12 weeks after increasing the atorvastatin dose).

Liver enzymes 3 times greater than the upper limit of normal of our laboratory were considered as an adverse drug reaction. Creatinine kinase levels greater than 3 times the upper limit of normal were also considered as an adverse drug reaction if accompanied by myalgia. Patients with such reactions were withdrawn from the study. Any patient could voluntarily withdraw from the study at any time. The control group comprised 21 age- and sex-matched subjects with normal cholesterol levels and no known atherosclerotic disease. All patients and control patients were informed of the aims of the study and signed the informed consent before entering the study. The study was approved by the Institutional Review Board/Independent Ethics Committee of the Hospital General Universitario in Alicante, Spain, and was designed in accordance with the tenets of the Declaration of Helsinki.

Analysis of Blood Samples

Blood samples were taken first thing in the morning after 12 hours of fasting and after the patient had been resting for at least 20 minutes. The samples were taken by qualified personnel without causing bruising or ecchymosis. Citrated plasma was obtained with syringes pre-

ABBREVIATIONS

- CRP: C-reactive protein.
- F1+2: prothrombin fragment 1+2.
- HDL-C: high-density lipoprotein cholesterol.
- LDL-C: low-density lipoprotein cholesterol.
- PAI-1: inhibitor of plasminogen tissue activator.
- t-PA: plasminogen tissue activator.
filled with trisodium citrate to give a final concentra-
tion of 0.011 mol/L. Serum was also obtained. The
plasma and serum were centrifuged at 4°C and 2200 g
for 15 minutes and stored at –80°C until subsequent
processing.

Serum samples were analyzed for total cholesterol,
LDL-C, high-density lipoprotein cholesterol (HDL-C),
and triglycerides with a colorimetric enzymatic
method (Hitachi® 917). Serum CRP was quantitated
by kinetic nephelometry with an immunochemical sys-
tem (IMMAGE®, Beckman).

Prothrombin fragment 1+2 was measured in citrated
plasma as a marker of thrombogenesis by ELISA
(Dade Behring®). Fibrinolysis was assessed by mea-
suring t-PA and PAI-1 antigen levels in citrated plasma
by ELISA (American Diagnostica®).

**Statistical Analysis**

Variables were analyzed for a normal distribution
with the Kolmogorov-Smirnov test. Normally distri-
buted variables were presented as means (SD). Varia-
tables that did not follow a normal distribution were
expressed as medians (interquartile range) and were
log-transformed prior to statistical analysis. Qualita-
tive variables were expressed as percentages. The χ²
test was used for analysis of the association between
qualitative variables, whereas the Student’s t test was
used for the association between a qualitative variable and another quantitative one. The Student’s t test was also used for analysis of paired
variables. For analysis of the correlation between 2
quantitative variables, the Pearson test was used. A
multivariate analysis with a linear regression model
(Enter technique) was used to study the influence of
possible confounding variables and independent vari-
rables on the quantitative study variables. The statis-
tical analyses were done using the SPSS program,
version 11.0. A statistically significant association
was defined as when the level of significance was
greater than 95%.

**RESULTS**

The clinical characteristics of the treated patients
and control patients, as well as the lipid profile at the
time of inclusion in the study, are shown in Table 1.

The high-risk group of patients had higher levels of
CRP and F1+2, as well as higher t-PA and PAI-1 antigen
levels, compared to the control group (Table 1). A sig-
nificant inverse correlation was observed between F1+2
and HDL-C levels ($r=-0.52; P=.007$) (Figure 1). In
contrast, CRP levels did not correlate with the remai-
ning variables analyzed (age, lipid profile, and t-PA
and PAI-1 antigen levels).

In the linear regression analysis, significant associa-
tion was only found between CRP levels and age and
hypertension ($r^2=0.245; P=.001$) (Table 2). No other
association was found with the remaining variables
(markers studied with sex, age, and cardiovascular risk
factors).

**Increase in Atorvastatin Dose**

Two patients withdrew voluntarily from the study
after increasing the atorvastatin dose and 1 patient had
no samples at 3 months. Follow-up was therefore com-
pleted in a total of 24 patients. No patients presented

<table>
<thead>
<tr>
<th>TABLE 1. Clinical Characteristics, Lipid Profile, and Biological Markers of Treated Patients and Healthy Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients (n=27)</strong></td>
</tr>
<tr>
<td>Men, %</td>
</tr>
<tr>
<td>Age, Years</td>
</tr>
<tr>
<td>Hypertension, %</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
</tr>
<tr>
<td>Smoking, %</td>
</tr>
<tr>
<td>Active</td>
</tr>
<tr>
<td>Ex smoker</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
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<tr>
<td>HDL-C, mg/dL</td>
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<tr>
<td>LDL-C, mg/dL</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
</tr>
<tr>
<td>CRP, mg/dL</td>
</tr>
<tr>
<td>t-PAag, ng/mL</td>
</tr>
<tr>
<td>PAI-1ag, ng/mL</td>
</tr>
</tbody>
</table>

*HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; F1+2, prothrombin fragment 1+2; t-PAag, tissue-type plasmino-
gen activator antigen; PAI-1ag, tissue-type plasminogen activator inhibitor antigen; CRP, C-reactive protein.*

with cardiovascular events during the course of the study. Treatment was well tolerated by all patients and no adverse drug reactions occurred during the 3 months of follow-up. The pharmacological treatment of the patients remained unchanged during the study and no significant change in blood pressure or weight of the patients was found.

The results of the measurements at 3 months are shown in Table 3. A significant reduction in cholesterol and LDL-C levels were observed, although only 7 patients (37.5%) attained the recommended treatment goals for LDL-C. The concentration of HDL-C did not change significantly.

After increasing the dose of atorvastatin, a decrease in CRP levels was detected among treated patients. An improvement in the remaining biological markers was also observed—thrombogenesis was reduced in our patients and fibrinolytic function improved, with a decrease in PAI-1 antigen levels close to the level of significance of t-PA antigen levels ($P = .056$). A significant correlation was also observed between the decrease in cholesterol levels and the decrease in F1+2 levels ($r = 0.46; P = .023$) (Figure 2). No correlation with the decrease in CRP levels or with the change in the lipid profile was observed.

**DISCUSSION**

According to the present study of high-risk patients with ischemic heart disease and lipid levels above recommended values, inflammatory processes are present and thrombogenesis and fibrinolytic dysfunction are increased, despite treatment with 40 mg/day of atorvastatin. It should be emphasized that treatment with 40 mg/day of atorvastatin had been unable to lower lipid levels to recommended target values at the start of the study. After increasing the dose to 80 mg/day, significant improvement was

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**TABLE 2. Multivariate Analysis by Linear Regression of Variables Associated with C-Reactive Protein Levels ($r^2 = 0.245$; $P < .001$)**

<table>
<thead>
<tr>
<th>Variables</th>
<th>$b$ Coefficient</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.047</td>
<td>.047</td>
</tr>
<tr>
<td>Hypertension</td>
<td>$-0.245$</td>
<td>.066</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.052</td>
<td>.766</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.205</td>
<td>.239</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.209</td>
<td>.065</td>
</tr>
</tbody>
</table>

*LDL-C indicates low-density lipoprotein cholesterol.

Only variables with $P < .150$ in the univariate analysis were included in the model.

**TABLE 3. Lipid Profile and Biological Markers of the Patients When Treated with 40 mg of Atorvastatin and 3 Months After Dose Increase to 80 mg (n=24)*

<table>
<thead>
<tr>
<th></th>
<th>40 mg</th>
<th>80 mg</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mg/dL</td>
<td>234.5±51.2</td>
<td>193.4±44.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>42 (38-49)</td>
<td>48 (42-55)</td>
<td>.897</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>159.8±50.8</td>
<td>121.3±46.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>177.0±118.9</td>
<td>150.6±79.7</td>
<td>.095</td>
</tr>
<tr>
<td>CRP, mg/dL</td>
<td>0.02 (0.01-0.03)</td>
<td>0.04 (0.03-0.06)</td>
<td>.009</td>
</tr>
<tr>
<td>F1+2, nmol/dL</td>
<td>0.50 (0.42-0.64)</td>
<td>0.42 (0.30-0.45)</td>
<td>.011</td>
</tr>
<tr>
<td>t-PA ag, ng/mL</td>
<td>14.5±7.9</td>
<td>10.2±5.8</td>
<td>.056</td>
</tr>
<tr>
<td>PAI-1 ag, ng/mL</td>
<td>65.8±22.4</td>
<td>45.6±22.2</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein.
seen, both in the lipid profile and in the state of inflammation, hypercoagulation, and hypofibrinolysis. However, only a third of our patients attained the treatment goals recommended for LDL-C, possibly because of the inclusion criteria applied. Treatment was very well tolerated by all patients, and no adverse reaction was reported.

Interest in the pleiotropic properties of statins has been growing in recent years. The most studied of these properties is probably the effect on the inflammatory system and significant decreases in CRP levels have been shown.17-19 This marker is a consistent independent predictor of future cardiovascular events.20-21 Interestingly, the reduction in risk of cardiovascular events in patients treated with statins is greater in groups of patients with higher CRP levels at the start of the study compared to subgroups of patients with lower levels. We also observed that high-risk patients maintained high CRP levels despite treatment with statins at normal doses. A significant reduction was obtained after increasing the dose. In our study and in previous ones,23-25 CRP levels decreased regardless of changes in the lipid profile.

The hemostatic system is also related to the pathogenesis of arteriosclerosis and the triggering of cardiovascular events.22 The polypeptide, F1+2, is derived from prothrombin during conversion to thrombin. Therefore, F1+2 is produced at the end of the process of thrombin formation and, as a result, is a sensitive marker of activation of the thrombin system and of thrombus formation. We observed a significant decrease in the levels of this marker after increasing the atorvastatin dose as far as permissible. The inhibition of the increase in thrombin formation mediated by statins is another effect that has been widely reported in previous studies.23-29 It is interesting to note that this effect seems to be due to the decrease in lipid levels rather than direct action on the hemostatic system. In fact, fenofibrate, which belongs to another class of lipid-lowering drugs, has also shown such effects, decreasing F1+2 in hyperlipidemic patients when the lipid levels return to normal.25 In agreement with these studies, we also found a significant and weak correlation between the decrease in cholesterol levels and F1+2 levels. The elevated levels of this marker of thrombin formation might be explained by higher than recommended lipid levels, despite treatment with standard doses of atorvastatin.

The fibrinolytic system plays a fundamental role in the development of intravascular thrombosis. Fibrinolytic dysfunction has been demonstrated both in subjects with cardiovascular risk factors, in particular, dyslipidemia and hypertension,20-28 and in patients with acute coronary syndrome.29 Analysis of t-PA antigen levels determines the amount of functionally active t-PA and t-PA complexes bound to PAI-1. Slow clearance of these complexes means that t-PA antigen levels are elevated when fibrinolytic dysfunction is present.30 On the other hand, t-PA and PAI-1 could even be considered as markers of endothelial damage, given that endothelial cells release both these substances. Several studies have suggested that statins cause an improvement in fibrinolytic function31-33; however, low or medium doses were almost always used (only occasionally have studies reached a dose of 40 mg/day). In the present study, we found that doses of 80 mg/day of atorvastatin decrease PAI-1 and t-PA antigen levels, which is indicative of an improvement in fibrinolytic function. This reduction did not correlate with lipid levels or with the decrease in lipid levels, and so the two effects appear to be independent, in agreement with most of the studies that have been published.

Aggressive treatment with statins is more effective for controlling lipid levels and can also prevent future ischemic events.2-14,24 New studies published recently have suggested that a stricter control of lipid levels is important.35-37 Current therapeutic goals might even have to be revised37 and it might be necessary to consider using statins at high doses. Moreover, some authors have suggested not only changing treatment goals but also basing them on CRP levels38,39 and not just on the lipid profile. Such an approach would be a confirmation of previous findings.2 On the other hand, evidence for the class effect of statins could be based on their differences rather than their ability to decrease CRP levels.39 Our study and others have shown that statin therapy at these doses has pleiotropic effects,40-42 providing further support for beneficial effects of these drugs in addition to their lipid-lowering properties.41-42

CONCLUSIONS

Treatment with atorvastatin at a dose of 80 mg reduces inflammation and thrombin formation, and improves fibrinolytic function compared to the 40 mg dose in high-risk patients with ischemic heart disease. This treatment regimen also improves the lipid profile and is well tolerated.

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