

Endothelial Dysfunction, Inflammation, and Statins: New Evidence

José Tuñón^a and Jesús Egido^b

^aServicio de Cardiología, Fundación Jiménez Díaz, Universidad Autónoma de Madrid, Madrid, Spain.

^bLaboratorio de Patología Vasculár, Fundación Jiménez Díaz, Universidad Autónoma de Madrid, Madrid, Spain.

Ever since statins have been shown to reduce the incidence of cardiovascular events, few other drugs have been as widely studied. As a result, almost all relevant biological processes implicated in atherogenesis and complicated plaque have been shown to be inhibited by these drugs. Statins can improve endothelial function.¹ They also have antiinflammatory and immunomodulatory properties, show antithrombotic and antiproliferative action, and lower the rate of apoptosis. It has even been shown that these actions are not secondary to hypolipemic effects but are independent of changes in cholesterol levels.

STATINS REDUCE ENDOTHELIAL DYSFUNCTION

Statins have been attributed a range of beneficial actions, but their capacity to inhibit endothelial dysfunction and their antiinflammatory effect were probably the ones that generated most interest. Already in 1994, treatment of patients with ischemic heart disease with 80 mg/day of lovastatin for 5 months was shown by Treasure et al² to improve coronary endothelial function as assessed by response to acetylcholine infusion. Later, several studies demonstrated that these drugs improve endothelial function over periods of time as short as 15 days.

There are several reasons for this beneficial effect. First, hypercholesterolemia induces endothelial dysfunction, and *in vitro* studies have shown that oxidized

low density lipoproteins (LDL) lower expression of endothelial nitric oxide synthase (eNOS).³ This enzyme is responsible for production of NO, an essential molecule for maintaining good endothelial function. The reduction in hypercholesterolemia achieved with statin therapy therefore helps preserve expression of eNOS. However, lower cholesterol is not the only reason why eNOS expression is maintained. In the same study, it was shown that addition of statins to the medium inhibited the decrease in expression of eNOS induced by oxidized LDL.² The fact that statins did not affect lipid concentrations in a cell culture—these were set by the investigators—suggests that the effect on the expression of eNOS is due to mechanisms that are independent of their lipid-lowering effect. Finally, statins not only favor synthesis of eNOS but also prevent degradation of this enzyme because they lower endothelial expression of caveolin, a protein able to deactivate eNOS.⁴

STATINS AS ANTIINFLAMMATORY DRUGS

The antiinflammatory property of statins is another of their important beneficial effects. Several groups, including our own, have demonstrated the antiinflammatory effect in experimental studies. In 1998, we reported how atorvastatin lowered macrophage infiltration in atherosclerotic lesions induced in rabbits by a combination of lipid-rich diet and endothelial damage.⁵ The expression of many types of proinflammatory molecules is induced by nuclear factor kappa B (NF- κ B). Therefore, the lower macrophage infiltration may be partly explained because atorvastatin decreases the activation of NF- κ B. In accordance with this observation, the expression of several of proinflammatory molecules, such as monocyte chemoattractant protein 1 (MCP-1) and cyclooxygenase 2, involved in chemotaxis of monocytes to the vascular wall were also reduced.^{5,6} *In vitro* studies with smooth muscle cells and monocytes by our group have confirmed that statins decrease activation of NF- κ B and MCP-1 expression,⁷ suggesting that antiinflammatory effects could be partially independent of decreases in choles-

SEE ARTICLES ON PAGES 909-15 AND 916-23

The authors of articles cited in the text received support from Pfizer, S.A., FIS (98/1269), the Spanish Cardiology Society, the Spanish Heart Foundation, the MAPFRE Foundation, the Foundation of Ramón Areces, and the Cardiovascular Research Network.

Correspondence: Prof. Jesús Egido.
Laboratorio de Patología Vasculár.
Fundación Jiménez Díaz.
Avda. Reyes Católicos, 2. 28040 Madrid. España.
E-mail: jegido@fjd.es

Full Spanish text available at: www.revvespcardiol.org

terol levels. Subsequent experiments confirmed this possibility by showing that simvastatin reduced inflammatory infiltration in the rabbit model of atherosclerosis more effectively than a low-lipid diet, even though the lipid levels decreased less in the animals who received the drug because they followed an atherogenic diet.⁸ The antiinflammatory effect of statins has also been demonstrated in humans. Among the studies carried out, those that investigated the effect of these drugs on plasma levels of proinflammatory molecules are particularly important because they showed that statins decrease the levels of C-reactive protein (CRP) and of several proinflammatory cytokines.

NEW DATA, NEW PERSPECTIVES

The present issue of the *REVISTA ESPAÑOLA DE CARDIOLOGÍA* contains 2 studies that have analyzed the action of statins on endothelial dysfunction and inflammatory markers. Tomás et al⁹ studied the effects of 3 months of treatment with 20 mg/day of atorvastatin on a population of 21 hypercholesterolemic patients. With noninvasive Doppler echocardiography, they assessed the effects of statins on carotid intima-media thickness, endothelial function of the brachial artery and coronary flow reserve (CFR) measured in the distal segment of the anterior descending coronary artery by transthoracic echocardiography. Although the carotid intima-media thickness did not change, endothelial function in the brachial artery tended to improve but the difference was not statistically significant. On the other hand, CFR increased significantly.

The lack of changes in neointimal thickness is not surprising given the short treatment period and the dose of atorvastatin used. The ASAP¹⁰ and REVERSAL¹¹ studies showed that atorvastatin induces partial regression of atherosclerosis in both the carotid and coronary artery, but the dose used was 80 mg/day and follow up lasted 18 to 24 months. Furthermore, disease progression was observed in patients randomized to less aggressive strategies, with less potent statins and lower doses. The lack of significant effects on endothelial function in the brachial artery was probably due to the small sample size. Importantly, studies in the biomedical literature show that less potent statins and those used at lower doses also have a consistent and beneficial effect, and that the effect is apparent after 2 to 4 weeks of treatment.

In our opinion, the improvement in CFR seen with dipyridamole is of particular interest. Dipyridamole-induced vasodilation provides an integrated assessment of relaxation of smooth muscle cells of the arterial intima-media under dipyridamole stress and flow induced NO-mediated vasodilation. Previous studies have reported similar findings, but they were conducted using invasive techniques or with positron emission

tomography—a technique that is not available in all hospitals. Analysis of flow in the left anterior descending coronary artery by transthoracic echocardiography offers the possibility of studying CFR by a noninvasive technique that is more widely available in daily clinical practice. Moreover, a noninvasive assessment of vasodilation of the coronary artery is of interest since determination of endothelial function in peripheral arteries has not been consistently found to be a reliable measure of coronary artery reactivity. The findings of the present study are similar to those published recently by other authors using the same technique,¹² though both studies are limited by the absence of a placebo treatment group which could control for the possibility that some of the observed changes may be related to spontaneous variation in CFR and not to statin treatment.

In this same issue, González et al¹³ compared the effect of 40 mg/day of pravastatin on plasma levels of CRP and interleukin 6 (IL-6) with that of placebo in 71 patients with acute ST segment elevation myocardial infarction (AMI). The levels of IL-6 were similar after 2 and 7 days, whereas those of CRP were lower in the pravastatin group after 7 days. After 2 months, the CRP levels were found to have remained low.

In the last 4 years, several prospective and retrospective studies have shown that statins decrease the incidence of cardiovascular events after an acute coronary syndrome. Furthermore, many authors have reported that statin treatment reduces CRP levels in different subpopulations, including patients with unstable angina or non-Q-wave AMI, as was seen recently in the MIRACL study.¹⁴ González et al¹³ confirm these findings in a population with ST segment elevation AMI. Levels of CRP increased from admission until 48 hours in response to AMI, and no significant differences were seen between treatment groups. From this moment on, levels of CRP started to decrease, and by 7 days the difference was significantly more evident in the pravastatin group. However, this treatment did not affect levels of IL-6. Given that IL-6 is considered the main inducer of hepatic synthesis of CRP, it might be expected that statins also lower the levels of this interleukin in parallel. However, to date this has not been proven. Although studies in the literature agree in the reduction of CRP levels, the results for IL-6 differ—some studies report an increase and others report no significant changes. The differences in the behavior of these two molecules may be partly due to differences in half-life—the half-life of IL-6 is only 2-4 hours, whereas CRP has a half-life of 20 hours. The half-life of IL-6 is also more variable, thus the greater stability of the concentrations of CRP probably contributes to the greater predictive value of this protein for cardiovascular risk.

The decrease in CRP did not, however, correlate with changes in the lipid profile induced by pravastatin.

tatin. This finding agrees with those reported when statins were administered to patients with hyperlipidemia or stable ischemic heart disease. Likewise, other beneficial actions of statins such as their antithrombotic action are not related to lower LDL levels. In unstable angina and non-Q-wave AMI, the MIRACL study showed that the decrease in CRP after treatment with atorvastatin in patients with initial LDL below the mean was similar to that seen in patients with higher levels of LDL.¹⁴ In fact, patients with low initial LDL had higher levels of CRP at the start of the study. The lack of correlation between lipid levels and CRP has already been reported, and increased levels of CRP were found to predict risk of cardiovascular events, even in patients with normal cholesterol levels. These findings support the idea that part of the action of statins is independent of decreases in cholesterol levels, largely because the mevalonate pathway produces molecules involved in processes necessary for correct cell function, such as isoprenylation of proteins.¹⁵ Statins inhibit this pathway in cells that participate in the pathophysiology of atherosclerosis, and therefore interfere with disease progression. Thus, statins have been reported to improve processes in which inflammation is present without hyperlipidemia, such as rejection of heart transplants or rheumatoid arthritis.

In conclusion, the evidence that statins inhibit several biological processes implicated in atherothrombosis is accumulating. Such findings have also extended our knowledge of the pathophysiology of this disease and have provided us with new potential therapeutic targets. Hopefully, this knowledge will lead to the future development of even more effective new drugs that act on these targets.

REFERENCES

- Iraculis E, Cequier A, Sabate M, Pinto X, Antoni Gómez-Hospital J, Mauri J, et al. Improvement of endothelial function in patients with hypercholesterolemia and normal coronary arteries with lipid-lowering therapy. *Rev Esp Cardiol* 2001;54:685-92.
- Treasure CB, Klein JL, Weintraub WS, Talley JD, Stillabower ME, Kosinski AS, et al. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. *N Engl J Med* 1995;332:481-7.
- Hernández-Perera O, Pérez-Sala D, Navarro-Antolín J, Sánchez-Pacuala R, Hernández G, Díaz C, et al. Effects of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, atorvastatin and simvastatin on the expression of endothelin-1 and endothelial nitric oxide synthase in vascular endothelial cells. *J Clin Invest* 1998;101:2711-9.
- Feron O, Dessy C, Desager JP, Balligand JL. Hydroxy-methylglutaryl-coenzyme A reductase inhibition promotes endothelial nitric oxide synthase activation through a decrease in caveolin abundance. *Circulation* 2001;103:113-8.
- Bustos C, Hernández-Presa MA, Ortego M, Tuñón J, Ortega L, Pérez F, et al. HMG-CoA reductase inhibition by atorvastatin reduces neointimal inflammation in a rabbit model of atherosclerosis. *J Am Coll Cardiol* 1998;32:2057-64.
- Hernández-Presa MA, Martín-Ventura JL, Ortego M, Gómez-Hernández A, Tuñón J, Hernández-Vargas P, et al. Atorvastatin reduces the expression of cyclooxygenase-2 in a rabbit model of atherosclerosis and in cultured vascular smooth muscle cells. *Atherosclerosis* 2002;160:49-58.
- Ortego M, Bustos C, Hernández-Presa MA, Tuñón J, Díaz C, Hernández G, et al. Atorvastatin reduces NF- κ B activation and chemokine expression in vascular smooth muscle cells and mononuclear cells. *Atherosclerosis* 1999;147:253-61.
- Hernández-Presa MA, Ortego M, Tuñón J, Mas S, Martín-Ventura JL, Blanco-Colio LM, et al. Simvastatin reduces inflammation in peripheral mononuclear cells and in rabbit atheroma more markedly than lipid lowering diet. *Cardiovasc Res* 2003;57:168-77.
- Tomás JP, Moya JL, Campuzano R, Barrios V, Megías A, Ruiz S, et al. Determinación no invasiva del efecto de atorvastatina en la microvasculatura coronaria y la función endotelial periférica de pacientes dislipémicos. *Rev Esp Cardiol* 2004;57: 909-15.
- Smilde TJ, van Wissen S, Wollersheim H, Trip MD, Kastelein JJ, Stalenhoef AF. Effect of aggressive versus conventional lipid lowering on atherosclerosis progression in familial hypercholesterolemia (ASAP): a prospective, randomised, double-blind trial. *Lancet* 2001;357:577-81.
- Nissen SE, Tuzcu EM, Schoenhagen P, Brown BG, Ganz P, Vogel RA, et al. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. *JAMA* 2004;291:1071-80.
- Fujimoto K, Hozumi T, Watanabe H, Shimada K, Takeuchi M, Sakanoue Y, et al. Effect of fluvastatin therapy on coronary flow reserve in patients with hypercholesterolemia. *Am J Cardiol* 2004;93:1419-21.
- González M, Ruiz Ros JA, Pérez-Paredes M, Lozano ML, Jiménez DM, Martínez-Corbalán F, et al. Efecto de la administración precoz de pravastatina en los valores de proteína C reactiva y de interleucina 6 en la fase aguda del infarto de miocardio con elevación del segmento ST. *Rev Esp Cardiol* 2004;57:916-23.
- Kinlay S, Schwartz GG, Olsson AG, Rifai N, Leslie SJ, Sasiela WJ, et al. High-dose atorvastatin enhances the decline in inflammatory markers in patients with acute coronary syndromes in the MIRACL study. *Circulation* 2003;108:1560-6.
- Blanco-Colio LM, Tuñón J, Martín-Ventura JL, Egido J. Anti-inflammatory and immunomodulatory effects of statins. *Kidney Int* 2003;63:12-23.