

## Statins as Antithrombotic Drugs

Francesco Violi, MD; Camilla Calvieri, MD; Domenico Ferro, MD; Pasquale Pignatelli, MD, PhD

Statins are powerful lipid-lowering drugs that inhibit cholesterol biosynthesis via downregulation of hydroxymethylglutaryl coenzyme-A reductase.<sup>1</sup> They are largely used in patients with or at risk of cardiovascular disease inasmuch as randomized trials have consistently shown that statins lower the rate of myocardial infarction, stroke, and cardiovascular death.<sup>2</sup> The majority of trials with statins investigated patients with stable atherosclerotic disease, in whom the reduction in cardiovascular events appeared to be related to the cholesterol-lowering effect of statins and ultimately to plaque stabilization.<sup>3</sup> However, experimental data demonstrated that statins may also exert a direct antithrombotic effect in models of arterial and venous thrombosis via a mechanism unrelated to the cholesterol-lowering activity<sup>4,5</sup>; this may suggest that statins inhibit several pathways of hemostasis, including platelet activation and coagulation cascade. Consistent with these observations are the potential negative properties that have been observed, including the association between statin therapy and cerebral hemorrhage.

In this review, we present experimental data in support of the ability of statins to interfere directly with the clotting system and platelet activation, as well as the clinical settings that suggest that statins exert beneficial effects related to their antithrombotic properties.

### Statins and Coagulation

Colli et al<sup>6</sup> were the first to show that statins interfere with activation of the clotting system and the coagulation cascade. Specifically, they demonstrated that fluvastatin dose-dependently inhibits activity of tissue factor (TF), a glycoprotein that converts factor X to factor Xa,<sup>7</sup> and suggested that downexpression of geranylgeranylated protein is implicated in TF lowering. These data were confirmed in patients affected by polygenic hypercholesterolemia<sup>8</sup> in whom simvastatin 20 mg/d for 8 weeks resulted in downregulation of TF antigen and activity and thrombin generation. Of note, inhibition of clotting activation, as assessed by plasma values of prothrombin fragment F1+2, a marker of thrombin generation, appeared as early as 3 days after administration of atorvastatin 10 mg/d and was independent from its lipid-lowering effect.<sup>9</sup> Undas et al<sup>10</sup> supported these findings in subjects with low-density lipoprotein (LDL) cholesterol >130 mg/dL, in whom simvastatin 40 mg/d reduced thrombin and factor Va generation and accelerated activated protein C-mediated factor Va inactivation as early as 3 days after its administration.

In vitro studies confirmed that the prevention of isoprenoid intermediate synthesis by statins is crucial for TF inhibition. In fact, statins were shown to downregulate the expression of TF via inhibition of geranylgeranylation of the Rho/Rho kinase pathway, an enzyme that upregulates the expression of TF in cultured endothelial cells and monocytes via nuclear factor- $\kappa$ B activation.<sup>11–14</sup>

The inhibition of isoprenoid intermediates also explains the upregulation of thrombomodulin by statins, exerting anticoagulant activity by activating the protein C pathway.<sup>15</sup> Statins, in fact, increase thrombomodulin mRNA levels in a dose-dependent manner by inhibiting geranylgeranylation of Rho subfamily proteins, such as Rac1/Cdc42, and nuclear factor- $\kappa$ B activation.<sup>16,17</sup>

Statins can elicit downregulation of TF and overexpression of thrombomodulin by another mechanism characterized by upregulation of Kruppel-like factor 2 (KLF2). KLF2 is a transcriptional factor with anticoagulant and atheroprotective effects via upregulation of thrombomodulin and endothelial nitric oxide synthase and downregulation of TF and plasminogen activator inhibitor-1.<sup>18</sup> Changes in KLF2 have been observed in endothelial cells, in which statins dose-dependently increased KLF2 expression via inhibition of geranylgeranylated proteins.<sup>19</sup>

Another intriguing mechanism that potentially accounts for the antithrombotic activity of statins is related to the ability of statins to lower oxidized LDL, known to be internalized within macrophages by a CD36/TLR4/TLR6 heterotrimeric complex and to induce TF gene expression via nuclear factor- $\kappa$ B activation.<sup>20</sup> Owens et al<sup>20</sup> demonstrated both in vitro and in vivo that simvastatin hampers oxidized LDL interaction with monocytes and lowers oxidized LDL-induced TF expression without causing a change in cholesterol levels. This antithrombotic activity likely accounts for the prolongation in time to vascular occlusion observed in an in vivo model of arterial thrombosis.<sup>20</sup>

Other ways that statins interfere with the clotting system have been explored, including inhibition of factor V and XIII activation, although results need further confirmation.<sup>21</sup> A profibrinolytic activity has been also suggested because statins inhibit the expression of plasminogen activator inhibitor-1 and upregulate tissue-type plasminogen activator from vascular smooth muscle and endothelial cells via inhibition of Rho geranylgeranylation.<sup>22</sup>

Together, these findings suggest that inhibition of Rho family activation represents one of the key mechanisms through

From I Clinica Medica, Sapienza, University of Rome, Italy.

Correspondence to Francesco Violi, MD, I Clinica Medica, Sapienza, University of Rome, Viale del Policlinico 155, Roma 00161, Italy. E-mail francesco.violi@uniroma1.it

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which statins exert an anticoagulant effect. Thus, inhibition of Rho family activation by statins results in downexpression and overexpression of transcriptional factors such as nuclear factor- $\kappa$ B and KLF2, respectively, which ultimately slows down the clotting system via TF downregulation and thrombomodulin upregulation. Inhibition of oxidized LDL-induced TF expression is another mechanism likely related to the ability of statins to lower oxidized LDL by downregulating NADPH oxidase activation<sup>23</sup> (Figure 1).

### Statins and Platelet Activation

Inhibition of platelet activation is another mechanism by which statins likely exert an antithrombotic effect. The influence of statins on platelet function has been investigated in patients at risk for cardiovascular disease, such as those with hypercholesterolemia, diabetes mellitus, or metabolic syndrome, and in patients with established atherosclerosis, such as those with peripheral artery disease or coronary heart disease.<sup>24–29</sup> Studies that lasted  $\geq 30$  days demonstrated that statins inhibit platelet function, as assessed by *ex vivo* tests of platelet aggregation or by analysis of circulating molecules released by platelets on activation, such as soluble CD40L or P-selectin.<sup>25,26,29</sup> At least 2 mechanisms appear to be involved, including downregulation of cyclooxygenase-1 activation and upregulation of nitric oxide synthase.<sup>23,24</sup>

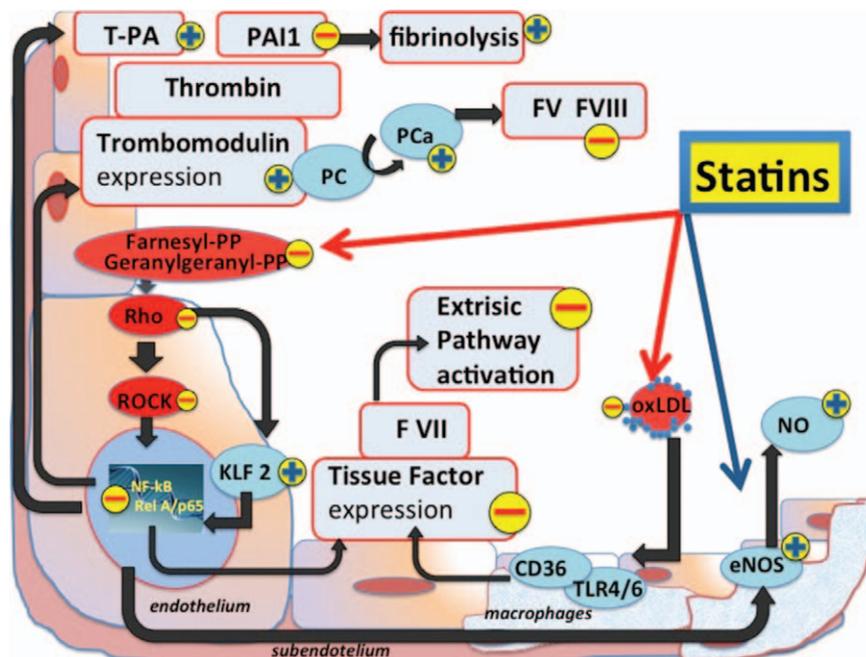
Experimental and clinical studies explored the hypothesis that statins may exert a direct antiplatelet effect independent from cholesterol lowering. In an experimental model of acute thrombosis induced in the carotid artery, Obi et al<sup>30</sup> demonstrated that intravenous infusion of lovastatin significantly reduced thrombosis by hampering platelet adhesion to the carotid lesion. Human studies confirmed these findings after 3 to 7 days of treatment with statins,<sup>31,32</sup> with the small inhibition of serum cholesterol that occurred after 3 days not fully supporting a direct antiplatelet effect by statins. To address this question, we measured several markers of platelet activation

as early as 2 hours after administration of 40 mg of atorvastatin in hypercholesterolemic patients.<sup>23</sup> This study showed an immediate downregulation of NOX2, the catalytic subunit of NADPH oxidase, along with inhibition of platelet isoprostanes,<sup>33</sup> a family of eicosanoids with proaggregatory properties. Further measurements performed after 24 hours showed inhibition of platelet thromboxane A<sub>2</sub> (TxA<sub>2</sub>) formation, an effect mediated by downregulation of phospholipase A<sub>2</sub>. Of note, platelet formation of TxA<sub>2</sub> continued to decrease in parallel with LDL lowering, which is consistent with previous studies that showed a coincident reduction in platelet TxA<sub>2</sub> formation and serum LDL after prolonged statin treatment.<sup>34</sup>

These findings led to a hypothesis of the existence of an early and late antiplatelet effect, which appears to be mediated by both lipid and lipid-lowering independent. The early antiplatelet effect appears to occur by NOX2 downregulation, with ensuing platelet isoprostane lowering, and by phospholipase A<sub>2</sub> downregulation, with ensuing platelet TxA<sub>2</sub> formation reduction; these changes appear to be independent of lipid lowering, because no decrease in serum cholesterol was detected up to 24 hours after statin administration. *In vitro* studies confirmed the hypothesis that atorvastatin may interfere directly with platelet activation, because it dose-dependently (0.1–10  $\mu$ mol/L) downregulated NOX2-derived oxidative stress, which ultimately led to impaired platelet isoprostane formation and phospholipase A<sub>2</sub> activation, which caused reduced production of platelet TxA<sub>2</sub>. The late antiplatelet effect appears to be closely associated with LDL lowering, because a progressive and parallel reduction in platelet TxA<sub>2</sub> and LDL was detected in our study and previous studies.<sup>23,34,35</sup>

Overgeneration of platelet nitric oxide is another mechanism that accounts for the antiplatelet effect of statins. Because of the negative effect of oxidative stress on nitric oxide biosynthesis and activity,<sup>36</sup> the impaired activation of NOX2-derived oxidative stress is likely to affect nitric oxide generation.

**Figure 1.** Statins as an anticoagulant drug. Statins act by (1) inhibiting tissue factor synthesis and increasing thrombomodulin formation via inhibition of geranylgeranylation of the Rho/Rho kinase pathway and, in turn, nuclear factor- $\kappa$ B (NF- $\kappa$ B) activity; (2) increasing Kruppel-like factor 2 (KLF2) expression and endothelial nitric oxide synthase (eNOS) activity; (3) inhibiting oxidized low-density lipoprotein (oxLDL)-mediated CD36 and Toll-like receptor 4/6 (TLR4/6) activation; and (4) enhancing fibrinolysis. FV indicates factor V; FVIII, factor VIII; NO, nitric oxide; PAI1, plasminogen activator inhibitor 1; PC, protein C; PCa, activated protein C; PP, pyrophosphate; Rho, Rho/Rho kinase; and T-PA, tissue-type plasminogen activator.



However, other studies demonstrated that statins enhance platelet cGMP, which indicates that they upregulate platelet endothelial nitric oxide synthase activity<sup>24,37,38</sup> (Figure 2).

### Clinical Studies

The possibility that the decrease in cardiovascular events with statin use is attributable to its antithrombotic properties has never been addressed directly in clinical trials; however, this can be inferred from data analysis of studies performed in the setting of acute coronary syndromes, in which the early reduction in cardiovascular events cannot be attributed solely to lipid-lowering activity. This argument is also corroborated by the antithrombotic effect observed in patients in whom statin administration has been associated with reduction in deep venous thrombosis. Another interesting aspect of statin therapy, which may be related to its antithrombotic activity, is its putative association with cerebral hemorrhage. The antithrombotic and more particularly the antiplatelet activity of statins may provide a new basis for discussing an issue that has been widely debated and not yet solved.

### Percutaneous Coronary Intervention

Coronary revascularization is the prevailing strategy for myocardial reperfusion. Even if severe complications are rare, an early myocardial infarction can occur in 5% to 40% of patients with stable or unstable coronary syndrome, depending on whether percutaneous coronary intervention (PCI) has been performed.<sup>39,40</sup> Periprocedural myocardial infarction usually occurs <24 hours from PCI and is a hallmark of poor outcomes.<sup>41</sup>

Two meta-analyses investigated the effects of preprocedural statin therapy on periprocedural cardiovascular events. Winchester et al<sup>42</sup> performed a meta-analysis of 21 trials

performed in patients undergoing PCI, coronary artery bypass grafting, and noncardiac surgery. Trials included 4805 patients who were randomly allocated to statin or control treatment. The latter could be placebo, usual care, or a low-dose statin. The statin treatment arm included pravastatin (40 mg), simvastatin (20–80 mg), atorvastatin (20–80 mg), fluvastatin (80 mg), or rosuvastatin (20–40 mg). The primary outcome of the meta-analysis was postprocedural nonfatal myocardial infarction. Beneficial effects of statins were observed regardless of the method used for diagnosis of myocardial infarction in patients undergoing PCI; conversely, no beneficial effects were detected in patients undergoing coronary artery bypass grafting.

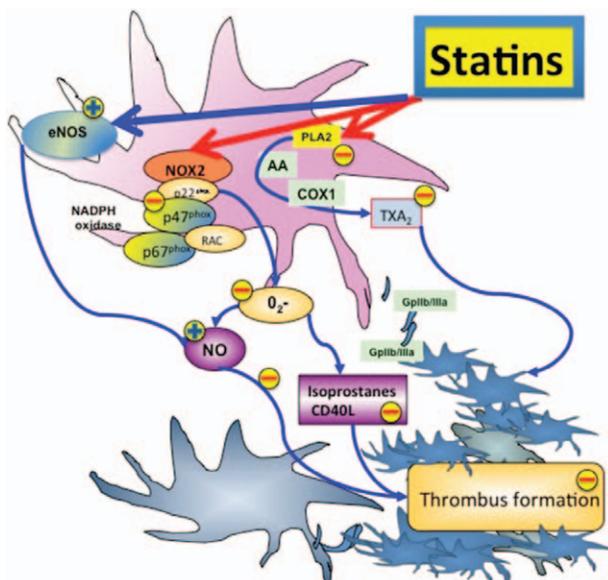
In the meta-analysis by Patti et al,<sup>40</sup> there were 13 interventional trials in which 3341 patients were randomly allocated to high-dose statins or control/low-dose statins before PCI. The incidence of periprocedural myocardial infarction was 11.9% in control patients and 7% in patients pretreated with high doses of statins, with a 44% risk reduction. This effect may be independent from clinical presentation, because pretreatment with high doses of statins was effective in patients with either stable or unstable coronary heart disease.

That the beneficial effect of statins after PCI may be explained by their antithrombotic effect is speculative, because no study explored whether statins inhibit clotting or platelet activation immediately after PCI. Of note, however, Patti et al<sup>43</sup> demonstrated that 24 hours after PCI, patients treated with atorvastatin 40 mg/d showed a significant reduction in endothelial activation as assessed by plasma levels of intercellular cell adhesion molecule-1 and E-selectin.

### Acute Coronary Syndrome

The clinical efficacy of the immediate administration of statins in patients with acute coronary syndrome was considered in a meta-analysis by Hulten et al,<sup>44</sup> who demonstrated a significant reduction in death and cardiovascular disease that became apparent after 4 months of treatment. However, in trials in which clinical outcomes were analyzed within 1 month of the acute episode, an earlier beneficial effect could be observed. In a large study that included >4000 patients with acute coronary syndromes, a significant reduction in vascular outcomes was observed with statin use, which suggests a potential role for its antithrombotic property. The PROVE-IT (Pravastatin or Atorvastatin Evaluation and Infection Therapy) trial investigated the effects of high doses of statins in 4162 patients with acute coronary syndromes, including myocardial infarction and high-risk unstable angina.<sup>45</sup> After 10 days of hospitalization, patients were randomized to 40 mg of pravastatin or 80 mg of atorvastatin. Follow-up was 30 days, and the primary end points of the study were myocardial infarction, stroke, revascularization, or unstable angina that required rehospitalization. The primary end points occurred in 15.1% of patients assigned to atorvastatin and 17.7% of patients assigned to pravastatin, with an absolute risk reduction of 2.6%. This effect appeared after as little as 15 days of follow-up, reached statistical significance at 30 days, and was independent of baseline LDL cholesterol.

In another study, Patti et al<sup>46</sup> randomized 171 patients with non-ST-segment acute coronary syndrome to pretreatment



**Figure 2.** Statins as an antiplatelet drug. Statins act by (1) inhibiting NADPH oxidase–mediated isoprostane formation, (2) modulating phospholipase A2 (PLA2)–mediated thromboxane A2 (TXA<sub>2</sub>) formation, and (3) upregulating endothelial nitric oxide synthase (eNOS). AA indicates arachidonic acid; COX1, cyclooxygenase 1; and GpIb/IIIa, glycoprotein IIb/IIIa.

with 80 mg of atorvastatin 12 hours before PCI and a further 40-mg preprocedural dose versus placebo. In that study, too, curve divergence was detected as early as 15 days after treatment began, with a significant reduction in myocardial infarctions at 30 days.<sup>46</sup>

The results of a retrospective study of 1616 patients in the Platelet Receptor Inhibition in Ischemic Syndrome Management (PRISM) study<sup>47</sup> are consistent with these findings. The study stratified the clinical outcomes of death and nonfatal myocardial infarction during 30-day follow-up according to the concomitant use of statins in patients with acute coronary syndrome. Statin therapy was associated with a 51% risk reduction in clinical outcomes compared with patients who did not undergo therapy with statins. Furthermore, a significant increase in risk of cardiac events was detected among patients who discontinued statin treatment after hospital discharge.

The early beneficial effect of high doses of statins in acute coronary syndrome is of particular interest when compared with the effect observed in patients at risk for or with stable atherosclerosis, in whom the reduction in major cardiovascular events appeared within 1 year of treatment and was greater in the subsequent years of follow-up.<sup>48</sup> It is, therefore, arguable that the earlier reduction in vascular outcomes in patients with acute coronary syndrome may not be related solely to plaque stabilization, a process thought to require at least 9 months,<sup>49</sup> but also to the antithrombotic effect of statins. This hypothesis may be supported by an experimental study that showed significant reductions in TF antigen and activity by 29% and 56%, respectively, in human atherosclerotic plaque after 4 to 6 months of atorvastatin 20 mg/d.<sup>50</sup>

## Stroke

Several studies have established that in patients at risk for atherosclerotic complications, treatment with statins can prevent stroke.<sup>45,51–53</sup> Conversely, the potential beneficial effects of statins in patients with acute cerebral ischemia have been the object of debate. The SPARCL trial (Stroke Prevention by Aggressive Reduction in Cholesterol Levels), in which statin treatment was given within 1 to 6 months of the acute episode of stroke or transient ischemic attack, demonstrated a significant reduction in fatal and nonfatal stroke in patients receiving atorvastatin 80 mg/d (11.2%) compared with those receiving placebo (13.1%)<sup>54</sup>; however, in a post hoc analysis, an excess rate of hemorrhagic stroke was detected in the atorvastatin-treated patients.<sup>55</sup> In a retrospective analysis of the Heart Protection Study, similar results were observed; patients with previous cerebrovascular disease who were undergoing treatment with simvastatin were at higher risk of cerebral hemorrhage.<sup>56</sup> This issue has been addressed recently by Hackam et al,<sup>57</sup> who retrospectively analyzed the rate of intracranial hemorrhage in patients with acute cerebral ischemia treated with a statin within 120 days of the index event. Among 17 872 patients, 213 episodes of intracranial cerebral hemorrhage were recorded; no relationship between cerebral hemorrhage and statin therapy was observed. However, although low-dose statin use was associated with a trend toward a reduced risk of cerebral hemorrhage, an opposite finding was observed with high statin dosages. Such uncertainty led to a recommendation

for careful control when statins are administered to patients with cerebral ischemia until high-level evidence on the relationship between statin and cerebral hemorrhage can be provided.<sup>58</sup>

Despite such inconclusive data, clinical and experimental research supporting the antiplatelet effect of statins may provide interesting material for this debate, because the relationship between antiplatelet drugs and cerebral hemorrhage has been firmly established.<sup>59</sup>

## Venous Thromboembolism

The relationship between venous thrombosis and statins was addressed by the JUPITER study (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin),<sup>60</sup> which analyzed the rate of thrombosis in 17 802 apparently healthy subjects with LDL cholesterol <130 mg/dL and C-reactive protein of 2 mg/mL who were randomly allocated to placebo or rosuvastatin 20 mg/d. The end point of the study was the occurrence of pulmonary embolism and deep vein thrombosis. Venous thromboembolism was classified as provoked if it occurred after cancer, trauma, hospitalization, or surgery and unprovoked if patients were free from these events 3 months before the thrombotic events. After a median follow-up of 1.9 years, 60 subjects in the placebo group and 34 undergoing rosuvastatin treatment experienced the event, with a significant 63% reduction in the rosuvastatin-treated group. This finding was similar whether patients were divided according to spontaneous or provoked thromboembolism. A recent meta-analysis on this topic confirmed the beneficial effect of statins in the prevention of venous thrombosis.<sup>61</sup>

Consistent with the antithrombotic effect of statins in the venous circulation are experimental studies in animals in which venous thrombosis was experimentally provoked. In animals treated with atorvastatin, venous thrombosis elicited by ligation of the inferior vena cava was significantly reduced compared with placebo and recurred after 4-day discontinuation of the statin treatment.<sup>4</sup>

## Future Perspectives and Conclusion

The data reported in the present review suggest that statins may exert a beneficial effect with a mechanism related to their antithrombotic properties. In support of this observation, clinical pharmacological studies have consistently shown an immediate anticoagulant and antiplatelet property that may contribute to the early reduction in thrombosis-related events in some clinical settings. The early reduction in post-PCI periprocedural myocardial infarction and in vascular events after acute coronary syndrome favors this hypothesis, even though the positive effect of statins on vascular endothelium via enhanced endothelial nitric oxide synthase activation<sup>62</sup> (Figures 1 and 2) is likely to also play a role. The data showing a reduction in venous thrombosis in apparently healthy subjects are also consistent with a direct antithrombotic property.

The antithrombotic activity of statins is peculiar because it differs from that of commonly used anticoagulants or antiplatelet drugs. The anticoagulant property of statins occurs via downregulation of TF or upregulation of thrombomodulin,

which results in impaired thrombin generation. With respect to the antiplatelet activity, the peculiarity of statins lies in their ability to inhibit not only platelet TxA<sub>2</sub> but also platelet isoprostane formation. Even if experimental and clinical studies indicated a direct antithrombotic effect, the parallel reduction in serum LDL and biomarkers of clotting and platelet activation after long-term treatment would suggest that a lipid-lowering effect is also implicated.

The inhibitory effect of clotting and platelet activation provides a plausible explanation for the early clinical efficacy elicited by statins in clinical settings associated with acute thrombosis, such as PCI, acute coronary syndromes, and deep venous thrombosis.<sup>40,51,61</sup> Of note, the clinical efficacy of statins was often achieved by comparison of high doses of statins, such as simvastatin or atorvastatin 40 to 80 mg/d or rosuvastatin 20 to 40 mg/d, versus lower statin doses. This was consistent with a study performed in 383 patients with stable and unstable coronary heart disease undergoing chronic statin treatment who were randomized to atorvastatin reload or placebo<sup>63</sup>; the study showed a significant reduction in periprocedural myocardial infarction in patients on atorvastatin reload (3.7%) compared with placebo (9.4%). On this basis, it would be tempting to speculate about the existence of a dose-related antithrombotic effect by statins, but this hypothesis must be evaluated.

These observations suggest novel antithrombotic approaches in other settings characterized by acute artery and venous thrombosis. In this context, statins could potentiate the antiplatelet efficacy of aspirin, because unlike aspirin, they downregulate platelet isoprostanes.<sup>23</sup> The association of statins with aspirin could be investigated in clinical settings in which the clinical usefulness of statins has never been explored, such as acute ischemic stroke, or in settings at risk of atherosclerotic complication, such as diabetes mellitus, in which the clinical efficacy of aspirin is less than expected.<sup>36</sup> An association between statins and aspirin would be of particular interest because statins could counteract the upregulation of isoprostanes elicited by aspirin in patients with type 2 diabetes mellitus.<sup>36</sup>

Treatment of acute venous thrombosis or prevention of venous thrombosis recurrence in patients at risk may be another attractive setting in which the antithrombotic property of statins may become useful clinically.<sup>23,61</sup> Given that inhibition of platelet TxA<sub>2</sub> prevents thrombosis recurrence in patients with previous deep venous thrombosis<sup>61</sup> and that statins inhibit not only platelet TxA<sub>2</sub> but also thrombin generation, interventional trials exploring the association between statins and aspirin to prevent thrombosis recurrence in deep venous thrombosis may be of interest.

In conclusion, these studies and observations support the consideration of statins not only as lipid-lowering but also as antithrombotic drugs, potentially useful in settings characterized by acute thrombosis. Statins possess a potentially unique antithrombotic mechanism that alters both coagulation and platelet activation, an ability that is not shared by the anticoagulant and antiplatelet drugs currently in use. These properties may offer a new therapeutic strategy to improve antithrombotic treatment and to further reduce vascular outcomes.

## Disclosures

None.

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