Early Anticoagulant Effect of Atorvastatin

To the Editor,

Undas et al\(^1\) reported the effect of short term treatment of 14 hypercholesterolemic patients with 40 mg/d simvastatin, showing that this statin can reduce thrombin generation independently of its lipid lowering effect; they also claim that “the anticoagulant effect of any statin within first days of its administration have not been reported.” However, the findings by Undas and colleagues confirm what we have already demonstrated in 30 hypercholesterolemic patients randomly allocated to 3 days treatment with diet or 10 mg/d atorvastatin.\(^2\) Thus, although patients allocated to only diet did not show any change of the prothrombin fragment F1\(\alpha\)2, a marker of thrombin generation, patients treated with statin had significant decrease of thrombin generation suggesting that statins may have an early anticoagulant effect. At least 2 mechanisms may account for this finding. One mechanism could be dependent on statin-induced downregulation of CD40L, a protein of the tumor-necrosis factor family that enhances the expression of tissue factor (TF) and the rate of thrombin generation.\(^3\) Another mechanism might involve a direct interference of the drug with other intracellular signaling responsible for TF expression. This suggestion is supported by a previous study demonstrating that statin inhibits in vitro the expression of TF and the generation of thrombin\(^4\) with a mechanism likely involving the activation of the nuclear factor NF-\(\kappa\)B, which has a key role in cellular expression of TF.\(^5\) As reactive oxidant species (ROS) are known to stimulate NF-\(\kappa\)B, the downregulation of CD40L induced by statin could interfere with cellular pathway implicated in the ROS formation. Accordingly with this hypothesis, statins have been shown to inhibit the activation of NADPH oxidase enzyme, which has a key role in the cellular formation of superoxide anion,\(^6\) but the mechanism has not been fully clarified and deserves further investigation. Therefore the findings by Undas et al reinforce our data showing that statins have an early anticoagulant effect likely in virtue of a direct interference with mechanisms involved in the expression of TF; these findings may help to explain recent data indicating that statins are of clinical usefulness in patients with acute coronary syndrome.

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In Response:

We recently reviewed the growing body of evidence which indicates that statins produce anticoagulant effects in vivo through incompletely understood mechanisms.\(^3\) Suppression of thrombin formation after statin administration for a few days has been shown independently by Sanguigni et al\(^6\) and by us.\(^7\) Our report was submitted for publication before the publication of Sanguigni et al. A larger randomized double-blind study by Undas et al\(^1\) showing early anticoagulant and antiplatelet effects of simvastatin versus fenofibrate has also been published.

It should be stressed that our findings show a rapid decrease in thrombin generation by statins using an in vivo challenge to the physiological coagulation system. These studies show that thombin-mediated reactions toward its physiological substrates such as fibrinogen and factor V are significantly depressed to an extent similar to that observed after 3 months of simvastatin therapy. Conversely, the protein C-thrombomodulin–thrombin dynamic anti-

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Monocyte activation by SA (SI)

\(\text{at}roz\)statin (microM )

Effect of scalar doses of atorvastatin on staphylococcus aureus induced monocyte O\(_2^-\) production (\(\text{IP}<0.05,\ ^{**}P<0.001\ vs\ control,\ n=5\)). Human peripheral blood mononuclear cells (monocytes and lymphocytes) were isolated from whole blood as described by Boyum\(^8\) using Ficoll. To isolate monocytes, we used MS Columns according to the manufacturer’s instructions (MACS; Biotec GmbH). Monocytes (10\(^6\) per mL) were incubated at 37\(^\circ\)C with dihydrorhodamine 123 (DHR 100 nmol/L) for 15 minutes and stimulated with staphylococcus aureus at a final concentration of 500 \(\mu\)g/mL. Quantitation of ROS production as marker for phagocytic activity was examined by flow cytometry (Cytometer EPICS XL Coulter) using 488 nm excitation and comparing the increase in fluorescence intensity with staphylococcus untreated controls (SI, stimulation index).

Demonstrating that statin inhibits in vitro the expression of TF and the generation of thrombin with a mechanism likely involving the activation of the nuclear factor NF-\(\kappa\)B, which has a key role in cellular expression of TF.\(^5\) As reactive oxidant species (ROS) are implicated in the activation of NF-\(\kappa\)B, it is possible that the downregulation of TF could interfere with cellular pathway implicated in the ROS formation. Accordingly with this hypothesis, statins have been shown to inhibit the activation of NADPH oxidase enzyme, which has a key role in the cellular formation of superoxide anion,\(^6\) but the mechanism has not been fully clarified and deserves further investigation. Therefore the findings by Undas et al reinforce our data showing that statins have an early anticoagulant effect likely in virtue of a direct interference with mechanisms involved in the expression of TF; these findings may help to explain recent data indicating that statins are of clinical usefulness in patients with acute coronary syndrome.

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<th>atorvastatin (microM)</th>
<th>Monocyte activation by SA (SI)</th>
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<tr>
<td>0.1</td>
<td>7</td>
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<tr>
<td>1</td>
<td>6</td>
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coagulant pathway is enhanced leading to increased factor Va inactivation by activated protein C (APC). Thus simvastatin has a dual role of depression of the procoagulant processes and enhancement of an anticoagulant reaction. In addition, our novel intriguing observation, not reported by Sanguigni et al, has been the association between a reduction in C-reactive protein levels in response to simvastatin and decrease in thrombin generation.

We agree with the prevailing view that the most likely mechanism of the thrombin-lowering action of statins represents suppressed TF expression but potentially also enhanced thrombomodulin expression/function. We cannot exclude indirect mechanisms, including involvement of the CD40 ligand pathway. Nevertheless, in 14 subjects analyzed in our report, soluble CD40 ligand levels were also determined and their values were significantly reduced by ~20%. However, there was no association with the magnitude of a decrease in soluble CD40 ligand levels and that of changes in thrombin formation or other coagulant reactions studied. Undoubtedly, a role of sCD40 ligand in statin-induced reduction in thrombin generation deserves further elucidation. Likewise, a relative contribution of the antioxidant properties of statins to the overall antithrombotic potential of this class of drugs, determined not only in vitro, but also in vivo, remains to be established.

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