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Simvastatin given for 3 days can inhibit thrombin generation and activation of factor V, and enhance factor Va inactivation in hypercholesterolemic patients

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The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins have been reported to exert multiple cholesterol-independent actions, including inhibition of blood coagulation, most likely due to downregulation of tissue factor (TF) expression.¹ Previously, we have demonstrated that simvastatin inhibits blood clotting following a 3-month simvastatin therapy.² It is unknown how rapidly statin administration can influence thrombin formation, factor (F)V activation and the proteolytic degradation of FVa. The aim of the current study was to assess early effects of statins on coagulant reactions.

Fourteen men (mean age, 54.6 years) with LDL cholesterol levels >3.4 mmol/L (130 mg/dL), who were treated with low-dose aspirin (75 mg/d), participated in the study after giving informed written consent. At baseline and after 3 days of simvastatin administration (40 mg/d), coagulant reactions were assessed in blood collected every 30 seconds at the site of microvascular injury, as previously described.^{2,3} Using quantitative Western blotting, we evaluated the time-courses of FVa generation and FVa inactivation, along with thrombin formation. We also determined lipid profiles, C-reactive protein (CRP) levels, fibrinogen and thrombin-antithrombin complexes (TAT) in peripheral venous blood (Dade Behring) with data expressed as mean \pm SEM. Paired *t* test and Wilcoxon's signed ranks test were used when appropriate to compare the differences before and after simvastatin treatment. Spearman's rank correlation coefficients were calculated to test the association between two variables.

A 3-day simvastatin administration significantly retarded prothrombin activation ($p=0.002$; Figure, panel A) and FVa heavy ($p=0.027$; Figure, panel B) and light chains ($p=0.033$) at the site of hemostatic plug formation. Plasma TAT levels showed a tendency toward lower posttreatment values ($p=0.1$), and fibrinogen was unchanged. Following simvastatin use, thrombin generation at the site of injury slowed from 0.258 ± 0.017 to 0.176 ± 0.014 nmol/L/s ($p=0.006$), while the maximum rate of FVa heavy chain formation decreased by 21.4% to 0.023 ± 0.002 nmol/L/s ($p=0.01$), with similar 20.2% reductions for FVa light chain ($p=0.008$). Simvastatin accelerated FVa inactivation by 60 seconds ($p=0.005$; Figure, panel B) and the activated protein C (APC)-mediated release of the 30 kD fragment of FVa heavy chain (residues 307 to 506) proceeded at a higher rate (0.026 ± 0.002 versus 0.032 ± 0.002 nmol/L/s; $p=0.021$). Simvastatin did not affect the generation of the 97 kD fragment of FVa heavy chain (Figure 1, panel B).

None of these effects were associated with lipid variables (data not shown). There were significant correlations between CRP reductions (from 2.71 ± 0.55 to 1.98 ± 0.48 mg/L; $p=0.0003$) and the magnitude of changes in thrombin B-chain ($r=0.6$; $p=0.001$) and FVa formation ($r=0.44$; $p=0.008$), along with those in FVa inactivation ($r=-0.54$; $p=0.004$).

The major finding of this current study is that simvastatin at 40 mg daily given for only 3 days resulted in significant reductions in thrombin formation, associated with delayed FVa generation, and accelerated APC-mediated FVa inactivation. The magnitude of these effects was similar to that observed following 90 days of therapy with this statin.² The anticoagulant effects of any statin within first days of its administration have not been reported nor has it been observed that statin-induced CRP reductions correlated with alterations in the rates of coagulant reactions. These data are consistent with the concept that inflammation is closely linked to coagulation.

The rapid enhancement of FVa proteolytic degradation by APC in response to simvastatin is likely due to increased thrombomodulin expression on endothelial cells by statins, as shown by Masamura et al.⁴ Moreover, our data suggest that since aspirin alone can inhibit blood clotting,³ the combination of simvastatin with aspirin exerts additional anticoagulant effects.

In conclusion, inhibition of blood coagulation at the site of vascular injury occurs as early as following a 3-day simvastatin administration, which might help explain early clinical benefits from statin use.

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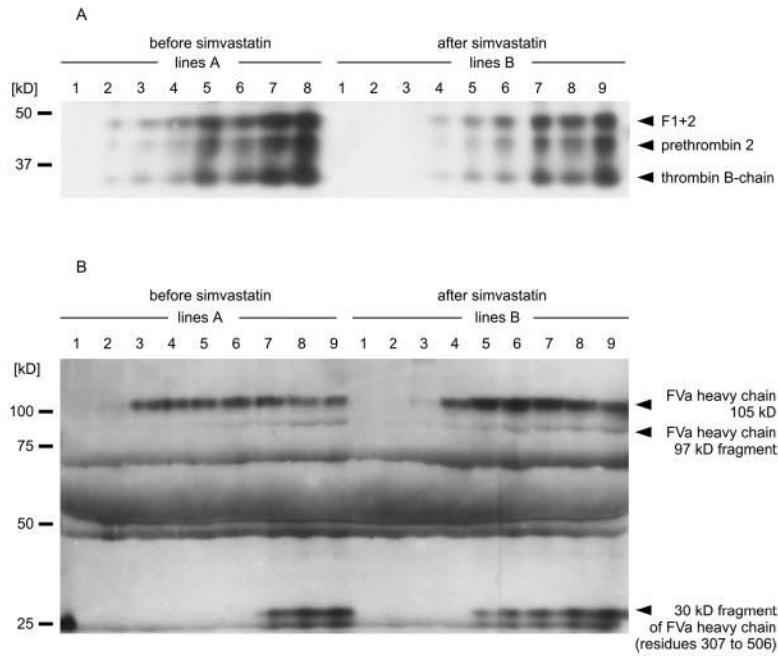


Figure 1.

Panel A. Representative immunoblots showing the formation of thrombin B-chain, prethrombin 2 and prothrombin fragment 1+2 (F1+2) in panel A, and in panel B, the formation of factor Va heavy chain (FVaHC) and FVa inactivation. Consecutive 30-second blood samples, collected from standardized skin incisions, were separated on a 4 to 12% linear gradient SDS-PAGE gel under reducing conditions. Three products, seen in panel A, were probed with a burro polyclonal antibody, which recognizes prethrombin 2, fragment 2, and thrombin B-chain. The bands in panel B were stained with a murine monoclonal antibody against FVaHC (residues 307 to 506). Molecular standards were marked on the left. Lines A – samples collected prior to simvastatin administration; lines B – samples taken after 3 doses of simvastatin (40 mg/d).